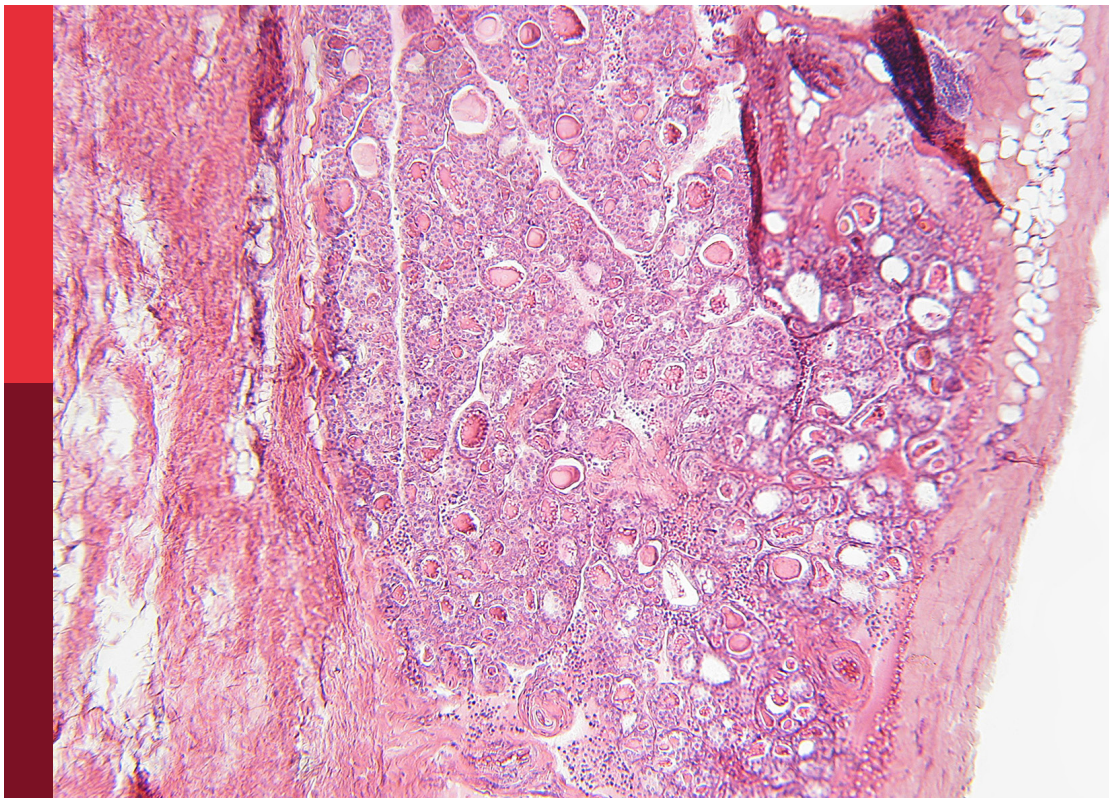
frontiers | Research Topics

Hormonal imbalance-associated oxidative stress and protective benefits of nutritional antioxidants

Edited by
Dipak Kumar Sahoo, Luna Samanta, Kavindra Kumar Kesari and Sutapa Mukherjee

Published in
Frontiers in Endocrinology



ISSN 1664-8714
ISBN 978-2-8325-4494-5
DOI 10.3389/978-2-8325-4494-5

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Hormonal imbalance-associated oxidative stress and protective benefits of nutritional antioxidants

Topic editors

Dipak Kumar Sahoo — Iowa State University, United States
Luna Samanta — Ravenshaw University, India
Kavindra Kumar Kesari — Lovely Professional University, India
Sutapa Mukherjee — Visva-Bharati University, India

Citation

Sahoo, D. K., Samanta, L., Kesari, K. K., Mukherjee, S., eds. (2024). *Hormonal imbalance-associated oxidative stress and protective benefits of nutritional antioxidants*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-4494-5

Table of contents

05 Editorial: Hormonal imbalance-associated oxidative stress and protective benefits of nutritional antioxidants
Dipak Kumar Sahoo, Luna Samanta, Kavindra Kumar Kesari and Sutapa Mukherjee

08 A review on phytochemical and pharmacological facets of tropical ethnomedicinal plants as reformed DPP-IV inhibitors to regulate incretin activity
Srishti Chhabria, Shivangi Mathur, Sebastian Vadakan, Dipak Kumar Sahoo, Pragnyashree Mishra and Biswaranjan Paital

32 The protective role of nutritional antioxidants against oxidative stress in thyroid disorders
Mirjana T. Macvanin, Zoran Gluvic, Sonja Zafirovic, Xin Gao, Magbubah Essack and Esma R. Isenovic

51 Protective effects of melatonin against the toxic effects of environmental pollutants and heavy metals on testicular tissue: A systematic review and meta-analysis of animal studies
Niloofar Dehdari Ebrahimi, Shima Parsa, Farnoosh Nozari, Mohammad Amin Shahlaee, Amirhossein Maktabi, Mehrab Sayadi, Alireza Sadeghi and Negar Azarpira

66 Protective effects of melatonin against physical injuries to testicular tissue: A systematic review and meta-analysis of animal models
Niloofar Dehdari Ebrahimi, Sara Shojaei-Zarghani, Ehsan Taherifard, Sanaz Dastghaib, Shima Parsa, Nasim Mohammadi, Fatemeh Sabet Sarvestani, Zahra Moayedfard, Nima Hosseini, Heidar Safarpour, Alireza Sadeghi, Negar Azarpira and Ali Reza Safarpour

81 Is beta-carotene consumption associated with thyroid hormone levels?
Bahareh Farasati Far, Nima Broomand Lomer, Hossein Gharedaghi, Hadi Sahrai, Golnaz Mahmoudvand and Arian Karimi Rouzbahani

96 Mating modifies the expression of crucial oxidative-reductive transcripts in the pig oviductal sperm reservoir: is the female ensuring sperm survival?
Manuel Álvarez-Rodríguez, Jordi Roca, Emilio A. Martínez and Heriberto Rodríguez-Martínez

109 Reactive oxygen species mediated apoptotic death of colon cancer cells: therapeutic potential of plant derived alkaloids
Vinod K. Nelson, Mohana Vamsi Nuli, Juturu Mastanaiah, Mohamed Saleem T. S., Geetha Birudala, Yahya F. Jamous, Omar Alshargi, Kranthi Kumar Kotha, Hari Hara Sudhan, Ravishankar Ram Mani, Alagusundaram Muthumanickam, Divya Niranjan, Nem Kumar Jain, Ankur Agrawal, Arvind Singh Jadon, Vinyas Mayasa, Niraj Kumar Jha, Adriana Kolesarova, Petr Slama and Shubhadeep Roychoudhury

130 Protective effects of exogenous melatonin therapy against oxidative stress to male reproductive tissue caused by anti-cancer chemical and radiation therapy: a systematic review and meta-analysis of animal studies
Niloofar Dehdari Ebrahimi, Alireza Sadeghi, Sara Shojaei-Zarghani, Mohammad Amin Shahlaee, Erfan Taherifard, Zahra Rahimian, Zahra Eghlidos, Negar Azarpira and Ali Reza Safarpour

146 Sea buckthorn, its bioactive constituents, and mechanism of action: potential application in female reproduction
Michal Mihal, Shubhadeep Roychoudhury, Alexander V. Sirotkin and Adriana Kolesarova

160 Modulatory effect of pomegranate peel extract on key regulators of ovarian cellular processes *in vitro*
Adriana Kolesarova, Simona Baldovska, Ladislav Kohut, Jaromir Vasicek, Eva Ivanisova, Julius Arvay, Michal Duracka and Shubhadeep Roychoudhury

171 Harnessing the power of nutritional antioxidants against adrenal hormone imbalance-associated oxidative stress
Anil Patani, Deepak Balram, Virendra Kumar Yadav, Kuang-Yow Lian, Ashish Patel and Dipak Kumar Sahoo

191 The multiple actions of grape and its polyphenols on female reproductive processes with an emphasis on cell signalling
Ladislav Kohut, Simona Baldovska, Michal Mihal, Lubomir Belej, Alexander V. Sirotkin, Shubhadeep Roychoudhury and Adriana Kolesarova

frontiers | Frontiers in Endocrinology

TYPE Editorial
PUBLISHED 09 February 2024
DOI 10.3389/fendo.2024.1368580

OPEN ACCESS

EDITED AND REVIEWED BY
Ralf Jockers,
Université Paris Cité, France

*CORRESPONDENCE
Dipak Kumar Sahoo
dsahoo@iastate.edu
dipaksahoo11@gmail.com

RECEIVED 10 January 2024
ACCEPTED 31 January 2024
PUBLISHED 09 February 2024

CITATION
Sahoo DK, Samanta L, Kesari KK and Mukherjee S (2024) Editorial: Hormonal imbalance-associated oxidative stress and protective benefits of nutritional antioxidants. *Front. Endocrinol.* 15:1368580. doi: 10.3389/fendo.2024.1368580

Editorial: Hormonal imbalance-associated oxidative stress and protective benefits of nutritional antioxidants

Dipak Kumar Sahoo[1*], Luna Samanta[2], Kavindra Kumar Kesari[3,4] and Sutapa Mukherjee[5]

[1]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, United States, [2]Department of Zoology, Ravenshaw University, Cuttack, Odisha, India, [3]Research and Development Cell, Lovely Professional University, Phagwara, Punjab, India, [4]Department of Applied Physics, Aalto University, Espoo, Finland, [5]Department of Zoology, Visva-Bharati University, Bolpur, West Bengal, India

KEYWORDS

endocrine disorders, oxidative stress, redox imbalance/homeostasis, hormone receptors, mitochondrial dysfunction, antioxidants, ROS

Editorial on the Research Topic
Hormonal imbalance-associated oxidative stress and protective benefits of nutritional antioxidants

The intricate interplay exists among the endocrine system, redox equilibrium, and oxidative stress (OS) in various vital biological processes encompassing fertilization, embryonic development, somatic growth, aging, and pathophysiological conditions (Figure 1). Understanding the relationship between hormonal conditions, redox state, and OS in living systems is an intricate challenge (1, 2). Reaching a unified conclusion becomes more challenging when certain hormones display oxidant capabilities while others possess antioxidant characteristics. In comparison to the antioxidative properties exhibited by hormones such as melatonin, estrogen, progesterone, and insulin, hormones such as thyroid hormone, catecholamines, and corticosteroids have the ability to augment the production of free radicals and OS as a consequence of disequilibrium in redox homeostasis (1, 2). Aerobic cells possess a proficient antioxidant defense mechanism that serves to counteract the deleterious impact of reactive oxygen species (ROS) through the maintenance of redox homeostasis (3). The elucidation of hormonal fluctuations and the examination of the mechanisms underlying the effects of antioxidants hold promise for the advancement of novel therapeutic approaches targeting disorders associated with hormonal dysregulation (Figure 1).

Various plant-based compounds, such as alkaloids, flavonoids, phenolic acid, and coumarin, have been shown to possess anti-diabetic and antioxidative effects (Figure 1) (4, 5). The review article by Chhabria et al. explores the potential of plant-based Dipeptidyl peptidase (DPP)-IV inhibitors in combating OS in diabetes-related pathological conditions and also delves into the study of the creation of polyherbal formulations and nanophytomedicines to regulate incretin activity. The review by Nelson et al. presents multiple preclinical and clinical studies demonstrating the strong anti-colon cancer effects

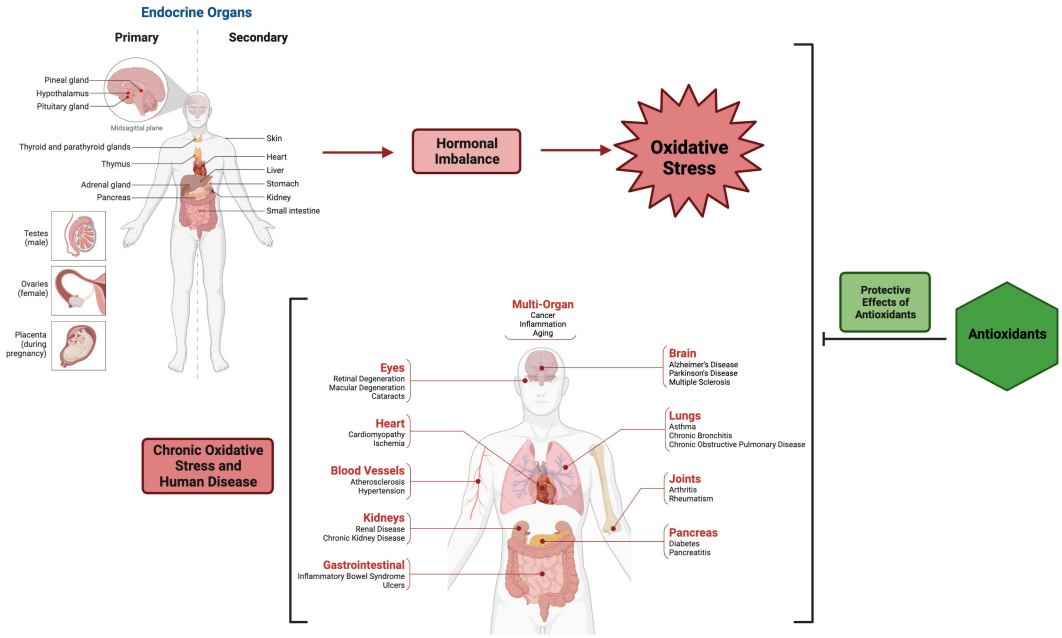


FIGURE 1
Role of antioxidants in hormonal imbalance-associated oxidative stress and various pathological conditions. The figure was generated using BioRender (www.biorender.com; accessed on 10th Jan 2024).

of alkaloids derived from medicinal plants and phytocompounds. These alkaloids have shown minimal toxicity towards normal cells. Additionally, the studies indicate that various alkaloids can induce apoptosis in colon cancer cells by targeting different cellular components, such as hormones and growth factors, which are involved in metastasis, angiogenesis, proliferation, and invasion. This review offers a detailed account of each alkaloid that has undergone clinical trials, either alone or in combination with other drugs, and also discusses different classes of phytochemicals that can induce cell death in various types of cancers, including colon cancer. Sea buckthorn and its bioactive ingredients show promise in addressing gynecological issues like uterine inflammation and endometriosis and alleviating symptoms of vulvovaginal atrophy in postmenopausal women (Mihal et al.). The polyphenolic flavonoids found in sea buckthorn have various health benefits and exhibit antioxidant, anti-inflammatory, and anti-cancer properties. They also play a role in promoting healthy ovarian cell proliferation, regulating cell death, and hormone release. Additionally, sea buckthorn may help reduce the risk of ovarian cancer by promoting apoptosis and regulating estrogen release.

The study by Kolesarova et al. explores the promising potential of pomegranate peel extract (PPE) in the prevention and/or therapy of ovarian cancer. A brief treatment with PPE suppressed human adenocarcinoma cell line metabolic activity and elevated the expression of cyclin-dependent kinase 1. Also, the administration of PPE resulted in a reduction in the secretion of growth factors, specifically TGF-β2 and EGF. Additionally, the expression of their respective receptors, TGFBR2 and EGFR, was also diminished. The research review by Kohut et al. delves into the effects of grapeseed extract and grape polyphenols on female reproductive processes. Research has shown that grape extract and its polyphenols, like resveratrol, proanthocyanidin B2, and delphinidin, have the potential to impact female reproductive health. These compounds can regulate various signaling pathways involved in reproductive hormones, steroid hormone receptors, oxidative stress, inflammation, apoptosis, and cell growth. The significance of these compounds in the treatment of ovarian cancer, ovarian ischemia, polycystic ovary syndrome, age-related reproductive insufficiency, or menopausal syndrome has been suggested. Grapeseed extracts and/or proanthocyanidin B2 and delphinidin may have an impact on developmental capacity, ovarian steroidogenesis, and oocyte maturation, although these effects may occur at varying regulatory levels.

Utilizing the potency of dietary antioxidants has promise in mitigating OS resulting from adrenal hormone imbalance (Patani et al.). This review explores the benefits of different nutritional antioxidants, including selenium, zinc, polyphenols, coenzyme Q10, vitamin C, vitamin E, carotenoids, and probiotics, in reducing the negative impacts of OS caused by abnormalities in adrenal hormone levels. Various studies have reported the importance of nutritional antioxidants in preserving a healthy balance of redox homeostasis in various thyroid pathologies. Several research studies have indicated a favorable correlation between beta-carotene levels and thyroid function. However, other investigations have not observed a notable impact. The review article by Far et al. explores the interactions between beta-carotene/retinol and thyroid hormones, as well as the results of clinical trials investigating the relationship between beta-carotene intake and thyroid hormone levels. The review article by Macvanin et al. offers insights into the role of nutritional antioxidants in maintaining a healthy balance of redox homeostasis in different thyroid pathologies. These pathologies

include the development of diseases like goiter, thyroid cancer, or thyroiditis. New findings regarding the link between the thyroid gland and gut microbiome were also explored, and the impact of probiotics with antioxidant properties on thyroid diseases was also assessed.

Natural mating can trigger alterations in the activity of female genes that control antioxidant enzymes crucial for the survival of sperm during their transport, primarily influenced by estrogen present in the bloodstream and semen. The study conducted by Álvarez-Rodríguez et al. reveals interesting findings regarding changes in the reproductive tract following mating, including a decrease in the expression of estrogen and progesterone receptors, as well as an increase in superoxide dismutase 1, glutaredoxin 3, and peroxiredoxin 1 and 3 that may play a role in preventing OS in the region near the sperm reservoir at the utero-tubal junction. However, Multiple studies highlight the significance of hormones, ROS generation, and their impact on male fertility. A systematic review and meta-analysis study conducted by Ebrahimi et al. analyzed 20 toxic materials and found that melatonin therapy improved reproductive hormonal panel, testicular histopathological characteristics, and tissue markers of oxidative stress. Research suggests that melatonin has antioxidant properties and could potentially safeguard testicular tissue against the harmful effects of toxic substances. Melatonin therapy also had positive effects on testicular health in male rodents with various types of testicular injuries (Ebrahimi et al.). Another systematic review and meta-analysis found evidence supporting the protective effects of melatonin against anti-cancer stressors in rodent testicular tissue. The meta-analysis revealed significant improvements in various outcomes with melatonin therapy, including enhancements in sperm quantity and quality, as well as improvements in the serum levels of reproductive hormones (testosterone and Follicle-Stimulating Hormone). Additionally, melatonin therapy decreases tissue markers of oxidative stress, such as testicular tissue malondialdehyde and caspase-3 activity. At the same time, it increases glutathione and total antioxidant capacity along with superoxide dismutase, catalase, and glutathione peroxidase activities (Ebrahimi et al.).

Henceforth, antioxidant supplements (5, 6) have emerged as a subject of discourse in contemporary times, particularly in light of their purported efficacy in addressing various pathological conditions, including endocrine disorders, and serving as adjunctive modalities to enhance conventional therapeutic interventions.

Author contributions

DS: Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. LS: Conceptualization, Writing – review & editing. KK: Writing – review & editing. SM: Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. Chainy GBN, Sahoo DK. Hormones and oxidative stress: an overview. *Free Radic Res* (2020) 54(1):1-26. doi: 10.1080/10715762.2019.1702656

2. Sahoo DK, Chainy GBN. Hormone-linked redox status and its modulation by antioxidants. *Vitam Horm* (2023) 121:197–246. doi: 10.1104/PP.106.077073

3. Halliwell B. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiol* (2006) 141:312. doi: 10.1104/PP.106.077073

4. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *Int J BioMed Sci* (2008) 4:1217165/BIBTEX. doi: 10.3389/FENDO.2023.1217165/BIBTEX

5. Sahoo DK, Heilmann RM, Paital B, Patel A, Yadav VK, Wong D, et al. Oxidative stress, hormones, and effects of natural antioxidants on intestinal inflammation in inflammatory bowel disease. *Front Endocrinol (Lausanne)* (2023) 14:1217165/BIBTEX. doi: 10.3389/FENDO.2023.1217165/BIBTEX

6. Ilango S, Sahoo DK, Paital B, Kathirvel K, Gabriel JI, Subramaniam K, et al. A review on annona muricata and its anticancer activity. *Cancers 2022* (2022) 14:4539. doi: 10.3390/CANCERS14184539

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 11 November 2022
DOI 10.3389/fendo.2022.1027237

OPEN ACCESS

EDITED BY
Brian Yee Hong Lam,
University of Cambridge,
United Kingdom

REVIEWED BY
Sudhanshu Kumar Bharti,
Patna University, India
Gopal L. Khatik,
National Institute of Pharmaceutical Education and Research, India

*CORRESPONDENCE
Biswaranjan Paital
biswaranjanpaital@gmail.com
Dipak Kumar Sahoo
dsahoo@iastate.edu
dipaksahoo11@gmail.com

SPECIALTY SECTION
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 24 August 2022
ACCEPTED 18 October 2022
PUBLISHED 11 November 2022

CITATION
Chhabria S, Mathur S, Vadakan S, Sahoo DK, Mishra P and Paital B (2022) A review on phytochemical and pharmacological facets of tropical ethnomedicinal plants as reformed DPP-IV inhibitors to regulate incretin activity.
Front. Endocrinol. 13:1027237.
doi: 10.3389/fendo.2022.1027237

A review on phytochemical and pharmacological facets of tropical ethnomedicinal plants as reformed DPP-IV inhibitors to regulate incretin activity

Srishti Chhabria[1,2], Shivangi Mathur[2,3], Sebastian Vadakan[1,2], Dipak Kumar Sahoo[4]*, Pragnyashree Mishra[5] and Biswaranjan Paital[6]*

[1]Department of Biochemistry and Biotechnology, St Xavier's College, Ahmedabad, India, [2]Department of Biotechnology, Gujarat University, Ahmedabad, India, [3]Department of Biotechnology, President Science College, Ahmedabad, India, [4]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, United States, [5]Department of Horticulture, College of Agriculture, Odisha University of Agriculture and Technology, Chipilima, Sambalpur, India, [6]Redox Regulation Laboratory, Department of Zoology, College of Basic Science and Humanities, Odisha University of Agriculture and Technology, Bhubaneswar, India

Type 2 diabetes mellitus is a metabolic disorder resulting from impaired insulin secretion and resistance. Dipeptidyl peptidase (DPP)-IV is an enzyme known to trigger the catalysis of insulinotropic hormones, further abating the endogenous insulin levels and elevating the glucose levels in blood plasma. In the field of drug development, DPP-IV inhibitors have opened up numerous opportunities for leveraging this target to generate compounds as hypoglycemic agents by regulating incretin activity and subsequently decreasing blood glucose levels. However, the practice of synthetic drugs is an apparent choice but poses a great pharmacovigilance issue due to their incessant undesirable effects. The ideology was set to inventively look upon different ethnomedicinal plants for their anti-diabetic properties to address these issues. To date, myriads of phytochemicals are characterized, eliciting an anti-diabetic response by targeting various enzymes and augmenting glucose homeostasis. Antioxidants have played a crucial role in alleviating the symptoms of diabetes by scavenging free radicals or treating the underlying causes of metabolic disorders and reducing free radical formation. Plant-based DPP-IV inhibitors, including alkaloids, phenolic acid, flavonoids, quercetin, and coumarin, also possess antioxidant capabilities, providing anti-diabetic and antioxidative protection. This review article provides a new gateway for exploring the ability of plant-based DPP-IV inhibitors to withstand oxidative stress under pathological conditions related to diabetes and for reforming the strategic role of ethnomedicinal plants as potent DPP-IV inhibitors through the development of polyherbal formulations and nanophytomedicines to regulate incretin activity.

KEYWORDS

dipeptidyl peptidase-IV, insulin resistance, incretin, polyherbal formulations, antioxidants, hormonal disorder

Introduction

Diabetes mellitus (DM) is a chronic hyperglycemic metabolic condition caused by decreased insulin production, peripheral insulin resistance, or both. According to a WHO study, diabetes was the ninth biggest cause of death in 2019, directly responsible for almost 1.5 million fatalities. Type 2 diabetes mellitus (T2DM) affects the majority of people with diabetes, accounting for more than 90% of those with diabetes, and is characterized by insulin secretion defects in pancreatic β-cells and insulin resistance, whereas type 1 DM is caused by autoreactive T cell-mediated destruction of β-cells (Figure 1) (1, 2). In reaction to food consumption, pancreatic β-cells secrete the hormone insulin. The primary function of insulin is to regulate blood glucose levels by inducing muscles, liver, and fat cells to absorb accumulated glucose from the bloodstream and store it as an energy source. T2DM, also called non-insulin DM, plays a cardinal role in the humongous populace and is triggered by an interplay of environmental and genetic factors (3). It is hallmarked by hyperglycemia and characterized by the impairment of the inositol triphosphate kinase (PI3K-Akt) pathway (metabolic arm) of insulin signaling, failing to transport glucose and synthesize glycogen and thus further leading to compensatory hyperinsulinemia to maintain euglycemia which ultimately causes insulin resistance (4, 5).

Consequently, this results in the redundant stimulation of the unaffected RAS-MAPK pathway (mitogenic arm) of insulin signaling, contributing to cardiovascular dysfunction and endothelial injury and advancing myriads of chronic diabetic complications by significantly compromising the quality of life (5). The previous studies show a growing prevalence of DM in India, triggering a shift in the onset age of DM from adult to adolescence (6). This invokes a constant need for a promising treatment to mitigate DM and the progression of chronic symptoms.

Role of incretin hormones

The insulinotropic effects of incretin hormones are the reason why the body produces more insulin in response to meals when glucose is ingested orally rather than when it is administered intravenously, even when the plasma glucose levels are the same. This phenomenon is referred to as the incretin effect (7). The previous studies demonstrated that gut extracts possess a hormone that tightly regulates the secretion of pancreas and was named as glucose-lowering element or

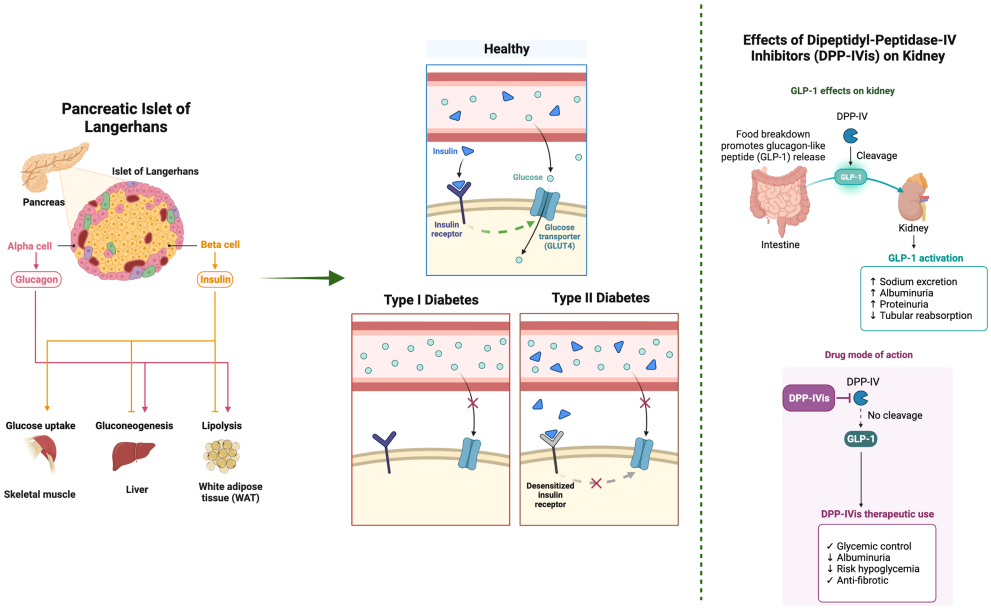


FIGURE 1
An overview of the impact of dipeptidyl peptidase (DPP)-IV inhibitors on the kidney and the insulin signaling pathways. The key aspects of the insulin signaling pathway, such as insulin binding, signal cascade, exocytosis, and glucose entry, are presented. DPP-IV inhibitors (DPP-IVis) are non-insulin glucose-lowering agents that are members of the incretin family. They are able to improve glycemic control while maintaining an acceptable level of safety. In individuals with chronic kidney disease, these medications are associated with a minimal risk of hypoglycemia, no weight gain, and good overall tolerability. The results of clinical trials are still up for debate, even though several experimental and clinical research point to the possibility that DPP-IVis may exert considerable pleiotropic effects, particularly on the kidneys. The figure was created with BioRender.com.

incretin (INtestine seCRETtion Insulin). It was verified that diabetic patients unveil a total loss of the incretin effect. Henceforth, a hypothesis suggesting that an impaired incretin function contributes to the pathogenesis of T2DM was formulated. The two most potent incretin hormones released in response to oral glucose are glucagon-like peptide-1 (GLP-1) (Figure 1) and gastric inhibitory peptide (GIP) (7–9).

GIP is a peptide of 42 amino acids belonging to the glucagon secretin family of peptides secreted from the K-cells of the upper intestine was earlier known to inhibit gastric acid secretion, so it was called gastric inhibitory peptide, but then later, it also showcased its efficacy on the pancreas by stimulating insulin secretion glucose dependently and was renamed as glucose-dependent insulinotropic peptide (7–9). GLP1 is the second most potent incretin hormone, a peptide of 31 amino acids known to be one of the enteroglucagons synthesized from proglucagon genes while secreted from both pancreatic alpha cells as well as L cells of the lower intestine and colon, which are known to stimulate the islets of the pancreas and secrete insulin (8).

Both GLP-1 and GIP validate their functional role by binding to their specific receptors GLP-1R and GIPR, which belong to the G protein-coupled receptor family triggering adenylate cyclase activity and elevating the levels of intracellular cyclic adenosine monophosphate (C-AMP) in pancreatic β-cells with the activation of protein kinase A (PKA) and exchange protein activated by C-AMP2 (EPAC2) involved in a broad range of intracellular actions such as altered ion channel activity, elevated cytosolic calcium levels which facilitate the fusion of insulin granules to the plasma membrane, and enhanced exocytosis of insulin-containing granules, contributing to the enhancement of insulin secretion in a glucose-dependent manner (8–10). Both GLP-1 and GIP induce insulin secretion in response to oral glucose consistently with their functional role as incretins. Based on data from several studies previously, both incretins share a few common insulinotropic actions, like increasing insulin secretion, enhancing resistance to apoptosis, and increased β-cell proliferation, but also differ in a few biological attributes, like GIP stimulates the pancreas by increasing glucagon secretion and acts on the gastric tract by inhibiting gastric acid secretion, whereas GLP 1 decreases glucagon secretion and acts on the gastrointestinal (GI) tract by decreasing gastric emptying and postprandial blood glucose (8, 10).

Crux of the matter: Dipeptidyl peptidase-IV

The human gene dipeptidyl peptidase (DPP)-IV is located on chromosome 2 and encodes dipeptidyl peptidase IV, a serine protease, an enzyme located on epithelial as well as endothelial cells found to be expressed in varied tissues including the liver, gut, placenta, and kidney, causing the catalytic degradation and reduction in the half-lives of GLP-1 and GIP levels and further ensuing the perturbation of glucose homeostasis (Figure 2). From the recent investigations done, it was reported that 75% of GLP-1 metabolism by DPP-IV and elimination occurs in the gut and liver, respectively, permitting only 10–15% of GLP-1 circulation in the blood (8, 10). In order to augment the insulinotropic activity in blood plasma, various synthetic and herbal DPP-IV inhibitors have gained much focus as oral hypoglycemic agents with minimal side effects (7, 11, 12).

Status of the naturally available DPP-IV inhibitors

Various naturally available plant-based compounds are investigated as DPP-IV inhibitors. Different plant parts such as leaves, seeds, flowers, buds, and bulbs are analyzed to identify their inhibitory capacity against DPP-IV activity. Parts of the plants were found to have different IC_{50} values against DPP-IV. The distinction arises from the fact that diverse phytochemicals, such as glycosides, flavonoids and phenols, terpenoids, and stilbenoids, are located in different parts of various plants. Numerous bioactive peptides derived from various plant components are likewise highly efficient against DPP-IV activity (Figure 3).

DPP-IV inhibitors in the leaves of plants

Many phytochemicals, including some that specifically target the DPP-IV enzyme, can be found in the leaves of plants. The leaves contain the most DPP-IV inhibitory compounds compared with other parts of the plant. To identify DPP-IV inhibitory compounds, the leaves of various plant orders, including Myrtales, Fabales, Sapindales, Gentianales, Urticales, Ericales, and Liliales, have been studied (Figure 3)—for example, the leaves of different plants from the order Myrtales, such as *Psidium guajava* (29), *Eucalyptus globulus*, *Eucalyptus citriodora*, and *Eucalyptus camaldulensis* (30), have IC_{50} values against DPP-IV activity of 380, 3.098, 6.138, and 3.99 μg ml^{-1}, respectively. Similarly, the IC_{50} value against DPP-IV activity in the leaves of *Cicer arietinum*, *Pterocarpus marsupium*, and *Punica Granatum* belonging to order Fabales are 0.09 (31), 273.73 (29), and 0.19 μg ml^{-1} (32), respectively. *Aegle marmelos*, *Mangifera indica*, and *Melicope glabra*, which belong to the order Sapindales, have an IC_{50} value against DPP-IV activity of 790, 182.7, and 169.40 μg ml^{-1} (33–35), respectively. The IC_{50} value was 96.8 μM (36) and 773.22 μg ml^{-1} (16) in a few other plants such as *Apocynum cannabinum* and *Gymnema sylvestre* from the order Gentianales. Similarly,

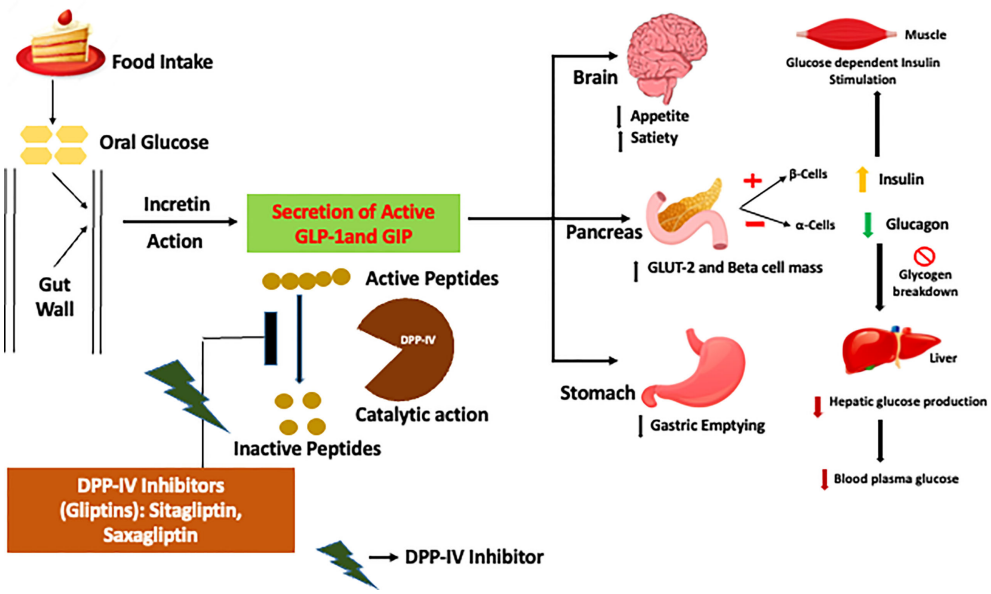


FIGURE 2
Model of the mode of action of dipeptidyl peptidase (DPP)-IV inhibitors for augmenting incretin activity. In healthy individuals, food intake aids the secretion of incretin hormones from the alimentary canal, which further favors the synthesis and release of insulin and additionally arrests the synthesis and release of glucagon, subsequently maintaining the glucose levels in blood plasma. However, type 2 diabetes mellitus patients incur a loss of incretin activity by the catalytic action of the DPP-IV enzyme, which is abated by DPP-IV inhibitors in order to escalate the incretin levels for regulating glucose homeostasis. .

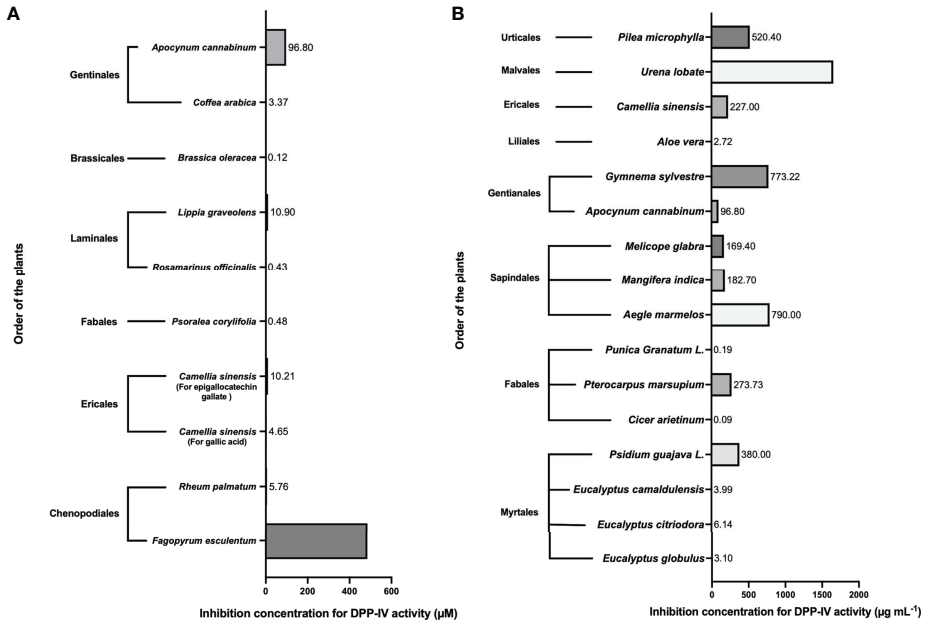


FIGURE 3
Levels of dipeptidyl peptidase (DPP)-IV activity inhibition concentration of bioactive compounds in certain plants studied. Different inhibitory concentrations of the plant products are found for the inhibition of DPP-IV (13–28). In **(A)**, the inhibitory concentration is evaluated in μM, while in **(B)** the inhibition concentrations are emulated in μg/ml.

for several other plants such as *Morus alba* and *Pilea microphylla* (order Urticales), *Aloe vera* (order Liliales), *Camellia sinensis* (order: Ericales), and *Urena lobate* (order: Malvales), the recorded IC_{50} value against DPP-IV activity was 480 (37), 520.4, 2.716, 227, and 1,654.64 μg ml^{-1}, respectively (37–39). These values show that the leaves of many plants have a very important role in inhibiting DPP-IV activity.

The leaves of *Fagopyrum esculentum* and *Rheum palmatum* (order: Chenopodiales) containing rutin and emodin were found to have IC_{50} values of 485 μM (27) and 5.76 μM (40), respectively. Fan et al. (2013) (41) documented that the leaves of *Camellia sinensis* from the order Ericales have gallic acid and epigallocatechin gallate having IC_{50} values of 4.65 and 10.21 μM, respectively. Against DPP-IV activity, the leaves of *Psoralea corylifolia* (order: Fabales) contain genistein, *Coffea arabica* (order: Gentinales) contains caffeic acid, and *Brassica oleracea* (order: Brassicales) contains luteolin with IC_{50} values of 0.48, 3.37, and 0.12 M, respectively (41). Similarly, Bower et al. (2014) (14) observed that the leaves of *Rosmarinus officinalis* and *Lippia graveolens* (order: Lamiales) have cirsimaritin with IC_{50} value of 0.43 and 10.9 μM, respectively, which can be used for inhibition of DPP-IV activity.

DPP-IV inhibitors in plant seeds

The seeds of various plants belonging to different orders, such as *Castanospermum austral*, *Cicer arietinum*, *Trigonella foenum graceum* (order: Fabales), *Avena sativa*, *Hordeum vulgare* var. trifurcatum (order: Graminales), *Amaranthus hypochondriacus* (Order: Chenopodiales) *Eugenia jambolana* (order: Myrtales), *Fagopyrum esculentum* (Order: Polygonales), *Ferula assa-foetida* (order: Apiales), and *Prunus amygdalus* (order: Rosales), were documented to have IC_{50} concentration against DPP-IV activity of 13.96 (42), 0.09, 0.03 (31), 0.99, 1.83 (43), 1.1 (44), 278.94 (32), 1.98 (43), 24.5 (45), and 162.9 μg ml^{-1} (46), respectively. Kim et al. (2018) (47) also examined that the seeds of *Lens culinaris* (order: Fabales) have different compounds such as kaempferol-3-O-βgulcopyranosyl-(1→2)-βgalactopyranosyl-7-Oαrhamnopyranoside, kaempferol-3-O-βgulcopyranosyl-(1→2)-[αrhamnopyranosyl(1→6)]-βgalactopyranosyl-7-O-αrhamnopyranoside, robinin, and kaempferol with IC_{50} value against DPP-IV activity of 27.89, 36.52, 37.01, and 51.9 μM, respectively.

DPP-IV inhibitors in other parts of plants

Several additional plant parts have been identified to have compounds with DPP-IV activity-inhibiting properties. The aerial parts of *Hedera nepalensis* (order: Apiales), *Fagonia cretica* (order: Zygophyllales), and *Desmodium gangeticum* (order: Fabales) have IC_{50} value against DPP-IV activity of 17.2 (19), 38.1 (19), and 255.5 μg ml^{-1}, respectively. Similarly, the IC_{50} values for *Helichrysum arenarium* (order: Asterales) flowers, *Berberis aristate* (order: Ranunculales) bark, and *Anogeissus latifolia* (order: Myrtales) were reported as 41.2, 14.4, and 754 μg ml^{-1} (34, 48), respectively, for DPP-IV inhibition. The bulb of *Allium sativum* (Order: Asparagales) was likewise documented with an IC_{50} value of 70.9 μg ml^{-1} (49), while the fruits of *Schisandra chinensis* (Order: Austrobaileyales) and *Punica granatum* (Order: Fabales) were recorded with an IC_{50} value of about 10.8 μg ml^{-1} (31, 32) against DPP-IV activity.

Saleem et al. (2014) and Kalhotra et al. (2018) (19, 49) noticed that lupeol, present in the aerial parts of *Hedera nepalensis* (Order: Apiales), has an IC_{50} value of 31.6 μM, but malvidin, found in the aerial parts of *Anagallis monellin* (Order: Ericales), has an IC_{50} value of 1.41 μM against DPP-IV activity. The stems and roots of *Coptis chinensis* (Order: Ranunculales) contain berberine, which exhibits DPP-IV activity with an IC_{50} value of 14.4 μg ml^{-1} (13). The whole *Pilea microphylla* (Order: Rosales) plant contains the active ingredient isoquercetin, which has an IC_{50} value of 96.8 μM against DPP-IV activity (50). Lin et al. (2015) (28) identified hopeaphenol, vitisin A, and vitisin B in the stems and leaves of *Vitis thunbergii* that inhibited DPP-IV activity with IC_{50} values of 401, 90.75, and 15.3 μM, respectively. Similarly, Fan et al. (2013) (41) found apigenin in the stems and pods of *Acacia auriculiformis* (order: Fabales), which had an IC_{50} value of 0.14 μM, in the fruits of *Citrus aurantium*, *Citrus limon*, and *Citrus maxima* belonging to order Sapindales having hesperetin (with IC_{50} value of 0.28 μM), eriocitrin (IC_{50} value of 10.36 μM), and naringenin (IC_{50} value of 0.24 μM), respectively. They also recorded that the fruits of *Rubus fruticosus* belonging to order Rosales have cyanidin (IC_{50} value 1.41 μM) that acts as a DPP-IV inhibitor. The compound cyanidin-3-glucoside present in *Vaccinum corymbosum* (order: Ericales) has an IC_{50} value of 125.1 μM against DPP-IV activity (26).

DPP-IV inhibitor peptides

It has been demonstrated that several bioactive peptide sequences found in a wide variety of plants are effective against DPP-IV activity. In *Phaseolus vulgaris*, Mojica et al. (2017) (18) identified peptide sequences such as KTYGL, KKSSG, GGGLHK, and CPGNK, each of which had an IC_{50} value of 0.03, 0.64, 0.61, and 0.87 mg DW ml^{-1}, respectively. Similarly, Lammi et al. (2016) (17) reported that the IC_{50} value for the peptide AVPTGVA in *Glycine max* and LTFPGSAED in *Lupinus albus* was 106 and 228 μM, respectively. Wang et al. (2015) (43) determined that the peptide LQAFEPLR present in *Avena sativa* has an IC_{50} value of 103.5 μM. Harnedy et al.

(2015) (15) found that ILAP and LLAP present in *Palmaria palmata* had IC_{50} values of 43.40 and 53.67 μM, respectively. The details about these plants are specified in Section 9.

Pharmacovigilance status of DPP-IV inhibitors

With an ideology to work on the crux of the matter, DPP-IV to manage diabetes, orally active small molecules called DPP-IV inhibitors have already been introduced in the market since 2006 (51). From previous investigations, a therapeutic dosage of different authorized DPP-IV inhibitors led to a two- to threefold raise in endogenous GLP-1 concentration without any inherent hypoglycemia risk and a favorable safety profile (Figure 2). Synthetic authorized drugs in the market as DPP-IV inhibitors are known as gliptins such as sitagliptin, linagliptin, and saxagliptin, usually substantiated to be efficient competitive inhibitors. In order to address the crisis imparted by DPP-IV, sitagliptin (Merck) was the initial lead to embark on the journey towards the upregulation of GLUT-4 expressions in the skeletal muscles of spontaneously hypertensive rats by controlling the postprandial glucose concentration and glycated hemoglobin (52). With an appropriate drug administration, gliptins can prove their efficacy for 24 h (53). Moreover, from the pharmacovigilance studies done priorly, gliptins were able to curtail the risk of hypoglycemia and weight loss and additionally also had the potential to proliferate β-cell mass.

Variously targeted (GLP-1) and off-targeted substrates lead to DPP-IV inhibition, contributing to managing incretin activity and maintaining the basal blood glucose level (54). Lately, these oral hypoglycemic agents, because of their insulinotropic effect, have progressively replaced sulfonylurea as second-line therapy and are also endorsed in the guidelines in triple therapies along with metformin and SGLT-2 inhibitors (55–57). For attaining the benchmark among all the other synthetic drugs, structural backbones ranging from cyanopyrrolidines, triazopiperazine amides, and pyrrolidines highly influenced the pharmacodynamics of each commercialized gliptins (58). The pharmacodynamics of each commercially authorized gliptin has been summarized in Table 1 (54, 59).

Data are partially retrieved from the approved list of DPP inhibitors by the US Food and Drug Administration (FDA) (60). FDA has underlined the adverse effects of DPP-IV inhibitor drugs such as Januvia, Janumet, Janumet XR, Onglyza, Kombiglyze XR, Tradjenta, Glyxambi, Jentadueto, Nesina, Kazano, and Oseni with active compounds sitagliptin, sitagliptin and metformin, sitagliptin and metformin extended release, saxagliptin, saxagliptin and metformin extended release, linagliptin, linagliptin and empagliflozin, linagliptin and metformin, alogliptin, alogliptin and metformin andalogliptin, and pioglitazone, respectively (60).

Shortcomings associated with DPP-IV inhibitors

The previous studies revealed the most common adverse effects in patients, including infections in the respiratory passage, nasopharyngitis, headache, and bladder infections associated with sitagliptin and saxagliptin, respectively (61, 62). Moreover, cases of hypoglycemia were also encountered with the synergistic intake of sitagliptin and saxagliptin. From analyzing the pharmacovigilance report and data supported by FDA, it was proclaimed that, on intake of DPP-IV inhibitors, several patients were diagnosed with hypersensitivity and joint pain issues, and in some cases, these drugs have proven to be fatal for patients with a history of pancreatitis (7, 56, 61, 63).

On a wider scale, strong aversions were laid against incretin-based therapy regarding its cost-effectiveness and the increasing risk of morbidity in patients with cardiovascular issues (64). With an ideology to surpass the discredits, investigators have inclined toward plant origin to discover innovative therapies to combat glycemia and the emergence of severe morbidities associated with the same.

Pharmacognosy: A strategy to alleviate the glycemic load

Since antiquity, many traditional practitioners across the globe have assertively exploited natural flora, which has proven superlatively therapeutic over synthetic agents to regulate several pathological conditions. Botanical leads, being a repository of bioactive compounds, fostered the strategic role of pharmacognosy (65). Metformin, a commercially effective anti-diabetic medicine, was recently identified in the field of drug discovery by adapting the concept of pharmacognosy and establishing the foundation for formulating herbal products to inhibit DPP-IV and regulate incretin activity (7, 66, 67).

Potential benefits of DPP-IV inhibitors with antioxidant properties in treating diabetes

Oxidative stress (OS) has been linked to the onset and progression of diabetes and its many consequences resulting from insulin insufficiency or insulin resistance (68). Several clinical studies have demonstrated that T2DM reduces antioxidant status and free radical scavenging activity due to the decreased activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and ascorbate and vitamin E levels, increasing the likelihood of diabetic patients acquiring chronic OS (68). Several different molecular

TABLE 1 Commercial dipeptidyl peptidase-IV inhibitors.

Attributes of marketed drugs	Sitagliptins	Vildagliptins	Saxagliptins	Alogliptins	Linagliptin
DPP-IV inhibition (%)	More than 80%–90%	More than 90%	75%–80%	More than 80%	More than 80%
Specificity for DPP-IV and type of inhibition	Very high, competitive, and dose-dependent inhibitor	High affinity (but not as sitagliptin) as compared with sitagliptin; it acts as a substrate blocker	Moderate affinity, selective, reversible, competitive inhibitor	High affinity, competitive, and dose-dependent inhibitor	High affinity, high specificity, and dose dependent inhibitor
HbA1C reduction (%)	0.6% only sitagliptin 0.89% with metformin	0.7%	0.45–0.65%	0.6% with sitagliptin 0.7% with vildagliptin	0.53%
Hypoglycemia risk	No risk detected	No risk detected	No risk detected	No risk detected	No risk detected
Mean half life	8–14 h	1.3–2.4 h	2.5 h	12.4–21.4 h	12 h
Bioavailability	±87%	±85%	±67%	100%	±30%
Metabolism/ elimination	Primary route: kidneys (only 16%) About 74% has been accounted for as parental drug	Primary route: kidneys About 85.04% fraction of the drug is absorbed and recovered in urine, wherein 27.14% (22.60 % recovered in urine and 4.54% recovered in feces) is unchanged parental drug and 57.90% is after hydrolysis	Primary route: kidneys and liver Liver: metabolizes the drug by cytochrome P450 enzymes and forming an active metabolite Kidney: as renal circulation 22.1% as an unchanged parent compound and 44.1% as a metabolite	About 60% to 80% of the administered dose tends to be unchanged in the urine after 24 to 72 h and 10%–20% of the dose is hepatically metabolized by cytochrome enzymes Primary excretion: kidneys (76%) Secondary: fecal (13%) Two minor metabolites explored were N-demethylated alogliptin (inhibitor of DPP-IV) and N-acetylated alogliptin	About 70%–80% of the administered drug is bound to plasma proteins Primary excretion: bile and gut Secondary excretion: kidneys
Year of approval	2006	2007	2009	2013	2011
Brand name	Januvia	Galvus	Onglyza	Nesina	Tradjenta

mechanisms such as protein modifications, including oxidant-induced modifications in phosphorylation state, alterations in gene regulation including transcriptomic and transcriptional modifications with the affected proteins being either direct insulin signaling molecules or oxidant-sensitive signaling pathways that interfere with the insulin signaling cascade, have been postulated to contribute to reactive oxygen species (ROS)-induced insulin resistance (69). Excessive levels of ROS inhibit insulin gene expression and insulin production as well as damage islet tissue. Anti-diabetes effects can be enhanced using plant-based DPP-IV inhibitors, such as alkaloids, phenolic acid, flavonoids, quercetin, and coumarin, possessing antioxidant characteristics (70, 71).

Antioxidants derived from plants, such as kinsenosides and flavonoids, exhibit anti-diabetic and antioxidant properties and assist in maintaining the function of pancreatic β-cells *in vivo* (71, 72). The majority of studies examining the role of plant-derived antioxidants in protecting β-cells focus on flavonoids. The administration of flavonoids to diabetic animals boosts the antioxidant potential of β-cells by increasing both enzymatic and non-enzymatic antioxidants, consequently limiting ROS generation and lipid peroxidation in β-cells and protecting them against autophagy, apoptosis, or necroptosis (73, 74). Diabetes is associated with an increase in the expression of pro-apoptotic genes (*e.g.*, caspases) and a decrease in the expression of anti-apoptotic genes (*e.g.*, Bcl-2 proteins) in β-cells. Flavonoids have been proven to protect β-cell viability by limiting these gene expression changes (Figure 4) (71, 75).

Flavonoids, which include flavanols, flavonols, flavones, flavanones, isoflavones, and anthocyanins, also possess DPP-4 inhibitory activity—for instance, the flavonoid-rich fraction of *Pilea microphylla* displayed antidiabetic efficacy in rats with diabetes generated by a high-fat diet and streptozotocin by lowering DPP-IV. This was accomplished while simultaneously increasing the endogenous antioxidant status in the liver of mice (38). Similarly, supplements derived from citrus bioflavonoids suppress the DPP-4 enzyme and also have higher free radical scavenging capabilities (27, 76). It has also been reported that an ethanolic extract of the leaves of *Psidium guajava* contains seven major flavonol-glycosides, all of which inhibit DPP-IV in a dose-dependent manner (29). Additionally, it was demonstrated through an *in vitro* bioassay that three

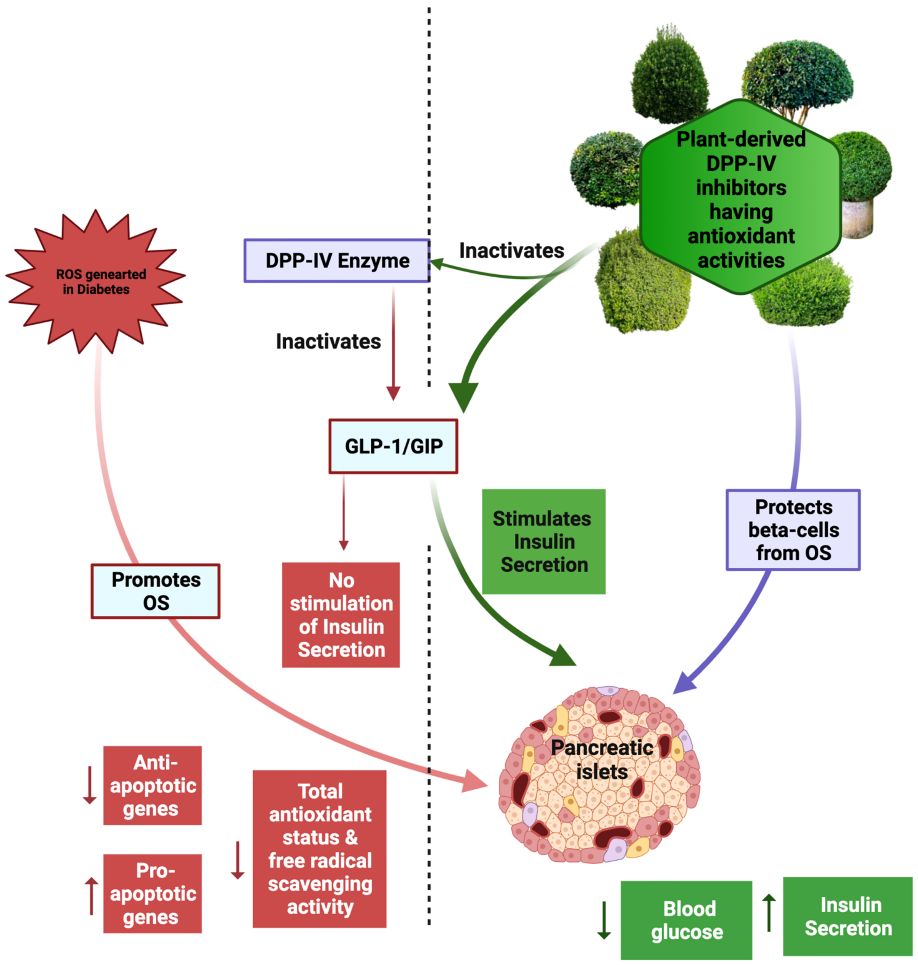


FIGURE 4
Role of dipeptidyl peptidase inhibitors with antioxidant properties in protecting pancreatic β-cells and treating diabetes. GLP-1, glucagon-like peptide-1; GIP, glucose-dependent intestinal polypeptide; OS, oxidative stress.

flavonol glycosides extracted from the seeds of *Lens culinaris* possessed DPP-IV inhibitory activity in a concentration-dependent manner (47). Antioxidants derived from plants with antidiabetic qualities, such as acting as DPP-4 inhibitors, are regarded to be the most effective strategy for keeping a normal β-cell physiology and treating diabetes (77). The following sections address plants that have DPP-4 inhibitor actions, either with or without known antioxidant properties.

In vitro and *in vivo* studies of DPP-IV inhibitors derived from plants

The effectiveness of the DPP-IV inhibition offered by a variety of tropical plants is being evaluated. In addition, in prior studies, a wide variety of organic solvents, plant parts, and extraction procedures were employed to isolate different bioactive components. The percentage yield of the extracts and the number of bioactive compounds are profoundly influenced by the polarity of the organic solvents (39, 78).

Urena lobata: Pharmacokinetic difference between *in vitro* and *in vivo* inhibition efficacy against DPP-IV

Recent studies on the root and the aqueous leaf extract of *U. lobata* reported its anti-diabetic competency in streptozotocin-induced diabetic rats, in compliance with the fact that the polarity of the organic solvent affects the solubility of bioactive compounds and its antioxidant property, which is supported by *in vitro* and *in vivo* generated results (79, 80). As a result of the research performed *in vitro*, the findings of the alcoholic extract portrayed its inhibition efficacy by expressing an IC_{50} value of

1,654.64 μg/ml. This value demonstrated that the alcoholic extract was four times more effective than the aqueous extract, which had an IC_{50} value of 6,489.88 μg/ml. However, the *in vitro* inhibitory activity of standard vildagliptin was superior to both extracts (IC_{50} = 57.44 μg/ml) (39, 78). *In vivo*, however, pharmacokinetic indices indicated that the aqueous extract was more effective than the alcoholic extract because water positively influences the absorption of active compounds in the serum by synergistically forming a complex and enhancing the inhibition efficacy against DPP-IV as well as the bioavailability of GLP-1 in the serum (81). Because the extracts contain sterols, which are known to alter the conformational structure of active compounds and subsequently reduce the bioactivity within the gastrointestinal (GI) tract, the alcoholic extract displays a lower level of *in vivo* inhibitory activity (39, 82, 83).

Pueraria tuberose: In vitro and in vivo inhibition efficacy against DPP-IV

From the *in vivo* studies undertaken previously using *P. tuberose* methanolic root extract (PTME) as a potential herbal treatment on the liver homogenates of alloxan-induced diabetic rats, it was reported that PTME possessed an abundance of flavonoids and significantly reduced DPP-IV activity in a time-dependent manner from 16 DPP-IV activity in a time-dependent manner fromto 4 μM/unit as compared with alloxan control (17.5 μM/unit) within a period of 40 days, which proved that PTME was a promising candidate to cure DPP-IV-induced liver disorders (84). From another investigation done, the aqueous extract was found to be potent enough to upregulate antioxidant SOD levels and downregulate DPP-IV mRNA expression, which apparently led to a concomitant reduction in stress, changes in the structural complexity of villi, increase of the intestinal patch, subsequent increase of the rate of nutrient absorption, and augmentation of GLP-1 and GIP secretion by causing an enhanced intestinotrophic effect (85). Besides this, *in vitro* results also supported the data as a potential DPP-IV inhibitor by exhibiting a considerable IC_{50} value of 17.4 mg/ml. In addition, when *in vivo* studies were undertaken on glucose-fed rats administered with the aqueous extract, the plasma glucose concentration during 60 min was reduced by 27.68%, and plasma DPP-IV activity was reduced by approximately onefold as compared with untreated rats. Additionally, a sharp rise of 1.2-fold was also observed in the plasma GLP-1 concentration compared with the untreated control (84).

Withania coagulans: In vitro inhibition efficacy against DPP-IV

As one of the prospective candidates, *W. coagulans* was chosen, and its effectiveness was checked using root, leaf, and fruits with 100 and 80% methanol as an organic solvent. Among all the plant parts, a root extract of the mature plant with 100% methanol exerted the maximum 50% inhibition efficacy against DPP-IV at a concentration of 8.76 μg/ml, whereas 80% methanol expressed its 50% inhibition at a concentration of 21.03 μg/ml. However, both extracts showed lesser efficacy than diprotin, the standard drug expressing its 50% inhibition efficacy at a concentration of 4.13 μg/ml (86).

Commiphora mukul: Choice of extraction method highly influences the extract yield

Recent findings have unveiled that the alcoholic extract of *C. mukul* gum resin with an abundance of antioxidants significantly proclaimed its antihyperglycemic activity by modulating the key glucose-metabolizing enzymes of the liver and kidney and further promoting normal blood glucose homeostasis (87–89).

With an ideology to get the maximum yield, optimization of procedure was an essential need which was undertaken by microwave-assisted extraction (MAE) method and conventional Soxhlet extraction (CSE) method resulting from giving a yield of 2.5%–3% and 2%, respectively, by using ethyl acetate as the solvent of interest (90). MAE proved to show a better yield than the Soxhlet extraction method because it was found to be less laborious, required less solvent, with improved quality of extract, and more economical (91). Moreover, by taking Hydro alcohol (HA) extract into practice for obtaining *in vitro* results, the maximum inhibitory activity imparted by C. *mukul* was 92%, with an IC_{50} value of 17 μM.

Ferrula asafetida: *In vitro* inhibition efficacy against DPP-IV

When experiments were undertaken by choosing *F. asafoetida* to assess DPP-IV inhibition activity using methanol, ethanol, water, and methanol–ethanol as organic solvents, it was observed that, among all the fractions, ethanol and ethanol–methanol fractions showed a maximum inhibitory effect with 24% and 22%, respectively (45).

Desmodium gangeticum: *In vitro* inhibition efficacy against DPP-IV

By choosing *Desmodium gangeticum* as one of the potential DPP-IV inhibitors, it was noted that the aqueous extract exhibited 73.21 % inhibition at 1,000 µg/ml concentration with an IC_{50} value of 255.5 µg/ml, which was inferior concerning its efficacy when compared with the standard drug diprotin expressing IC_{50} =10 µg/ml by 78.3% (92).

Moringa oleifera: *In vitro* DPP-IV inhibitory activity

According to the data, the leaf extract of *Moringa oleifera* was considered one of the potential candidates with significant antihyperglycemic activity, particularly in the ethanol and ethyl acetate extracts (93). Recent findings suggested that the extracts successfully reduced the blood glucose levels, increased the insulin levels, decreased the inflammatory cytokines and HOMA-R values, and improved the PPAR gamma levels. Nevertheless, the extract failed to prove its inhibition efficacy against DPP-IV when a set of *in vivo* experiments was performed (94). However, by undergoing ADMET analysis to evaluate the pharmacokinetic indices, myriads of different compounds from the extract were screened. Seven out of all could make it through *in silico* analysis by undergoing molecular docking, and just one compound with a specific conformation that exhibited the highest inhibitory activity with maximum binding energy was selected. Its *in vitro* evaluation was also undertaken, where it responded with an IC_{50} value of 798 nM (95).

Morus alba: Sample pre-treatment influences the *in vitro* DPP-IV inhibitory activity

Recent data has also reported *Morus alba* (also called white mulberry) as a potential DPP-IV inhibitor. MAE was used to obtain the extract from dried stem bark using ethanol with and without hydrolyzed acid. In addition, it was observed that the proportion of bioactive compounds in the *Morus alba* ethanolic extract without acid hydrolysis was only 0.04% but that the percentage of these compounds increased to 0.16% when the extraction was carried out with acid hydrolysis (96). Furthermore, previous research findings suggested that the compounds from *M. alba* root bark and fruits have also been reported to have anti-diabetic effects on STZ-induced mice by stimulating insulin secretion but lack the bioactive compounds which could exert DPP-IV Inhibition (97–100). The experiments were conducted to check the efficacy of stem bark as a DPP-IV inhibitor, wherein it showed a considerable inhibitory activity of 23%, which was 0.33 times the inhibitory activity of the standard drug sitagliptin (96).

Pterocarpus marsupium: *In vitro* and *in vivo* inhibition against DPP-IV

Pterocarpus marsupium was selected to treat hypoglycemia by utilizing various parts of the plant, and the DPP-IV inhibitor activity of the plant was investigated in both *in vitro* and *in vivo* conditions. Based on the findings, it was possible to determine an IC_{50} value of 273 µg/ml. In addition, the experimental conditions impacted the inhibitory half-life of the enzyme, which was 462.3 min, whereas the *in vivo* studies also reported that the extract could successfully increase the GLP-1 levels compared with the control group; the highest peak for GLP-1 was detected within 2 h (32).

Curcligo latifolia: *In vitro* inhibition against DPP-IV

As one of the potential options for combating hyperglycemia and hyperinsulinemia, the Hypoxidaceae family member *C. latifolia* has been selected. The root and the fruit extracts were prepared using the subcritical water extraction method. Both extracts expressed inhibitory potential against DPP-IV, and from the results, it was portrayed that the root extract (66.15%) exhibited better inhibition against DPP-IV than the fruit extract (42.79%).

Melicope latifolia: *In vitro* inhibition against DPP-IV

In the latest studies, the anti-diabetic and antioxidant potential of *M. latifolia* has been demonstrated, and the bark extract obtained from the plant has been investigated using various solvents. Methanol showed the highest yield out of all the evaluated extracts. Moreover, the *in vitro* results depicted that chloroform extract depicted the maximum inhibitory strength against DPP-IV (IC_{50} value = 221.58 µg/ml), whereas the hexane extract expressed the minimum inhibitory potential.

Phytochemical screening and *in silico* analysis

Poor pharmacokinetic characteristics are a common reason many drugs never make it to the market (101) (Table 2). Therefore, it is crucial to develop lead compounds that are easily absorbed orally, efficiently delivered to the site of action,

TABLE 2 Inhibitory role of different tropical plant extracts against dipeptidyl peptidase-IV enzyme.

Plant	Family	Part used in the investigation	Organic solvent	IC_{50} value/percentage inhibition	Extraction method	References
Urena lobata	*Malvaceae*	Root	Water Ethanol	6,489.88 µg/ml 1,654.64 µg/ml	Decoction	(78)
Pueraria tuberose	*Fabaceae*	Root	Water Methanol	17.4 mg/ml Activity is reduced in a time-dependent manner	Decoction Continuous soxhlet	(84, 102)
Withania coagulans	*Solanacea*	Root	Methanol (80%, 100%) Ethanol	8.76 µg/ml 21.03 µg/ml	Hot percolation	(86, 103)
Ferrula asafetida	*Umbelliferae*	Seed	Methanol Ethanol Water Methanol–ethanol	24%	Percolation	(45)
Desmodium gangeticum	*Leguminosae*	Aerial parts	Water	255.5 µg/ml	Maceration	(92)
Moringa oleifera	*Moringaceae*	Leaf	Ethanol Ethyl acetate Hexane Aqueous	798 nm	Percolation	(93, 94)
Morus alba	*Moraceae*	Stem bark	Methanol	23%	MAE	(96)
Pterocarpus marsupium	*Leguminosae*	Root Stem Leaf	Petroleum ether Methanol Ethyl acetate Chloroform	273.73 µg/ml	Soxhlet and maceration	(32)
Curcligo latifolia	*Hypoxidaceae*	Root Fruit	Methanol Ethanol Acetonitrile	7.02% to 66.15% 2.69% to 42.79% (increased with respect to concentration dependent)	Subcritical water extraction	(104)
Melicope latifolia	*Rutaceae*	Bark	Methanol Chloroform Hexane	990.21 µg/ml 221.58 µg/ml 5,872.03 µg/ml	Maceration	(105)

not readily converted into harmful metabolic products *en route* to the site of action, and rapidly excreted from the body. The acronym ADMET is commonly used to describe the aforementioned characteristics (absorption, distribution, metabolism, excretion, and toxicity). Although Lipinski's "rule of five" (106) is an early version of an integrated approach, its parameters should be used for guidance rather than as strict cutoffs. This mnemonic predicts that poor absorption or permeation is more likely when there are more than five hydrogen-bond donors and more than 10 hydrogen-bond acceptors, the molecular weight is greater than 500, and the calculated partition coefficient (logP) is greater than five. The same holds for the methods used to deal with problems caused by specific ADMET attributes (107).

A broad variety of bioactive compounds have been identified through the use of various plants and various ways of extraction. In addition, over the course of time, certain novel bioactive compounds have been explored for their potential as DPP-IV inhibitors through the use of *in silico* analysis (Figure 5). With the aid of advanced computational biotechnological studies, scientists can foretell the potential drug candidates in the pharmaceutical industry. New ligands are needed for the targets of known structure, wherein the potential compounds are screened for their adequate efficacy against a target of interest, and also the therapeutic dosage approval is possible by analyzing the ADMET properties to be able to abide by the Lipinski rule and investigate the drug likeliness of the compound (108, 109). The score of the free binding energy is directly proportional to the binding affinity. The binding energy with a lower value reinforced the synthesis of a strong binding molecule by exhibiting potential biological activity and binding orientation field (109, 110).

By generating an ensemble of ligand conformers that are subsequently firmly docked into the binding site, fast rigid exhaustive docking (FRED; part of the OpenEye Scientific Software) enables ligand conformational flexibility. Fast computations are possible with FRED, which is useful when screening libraries containing hundreds of thousands of molecules (111). A FRED docking study indicated that inhibition of DPP-IV is one of the mechanisms by which berberine exerts its hypoglycemic impact (13). Amini et al. (2016) performed molecular docking experiments and modeled the DPP-IV inhibitory activities of a variety of new aminomethyl-piperidones using a quantitative structure–activity

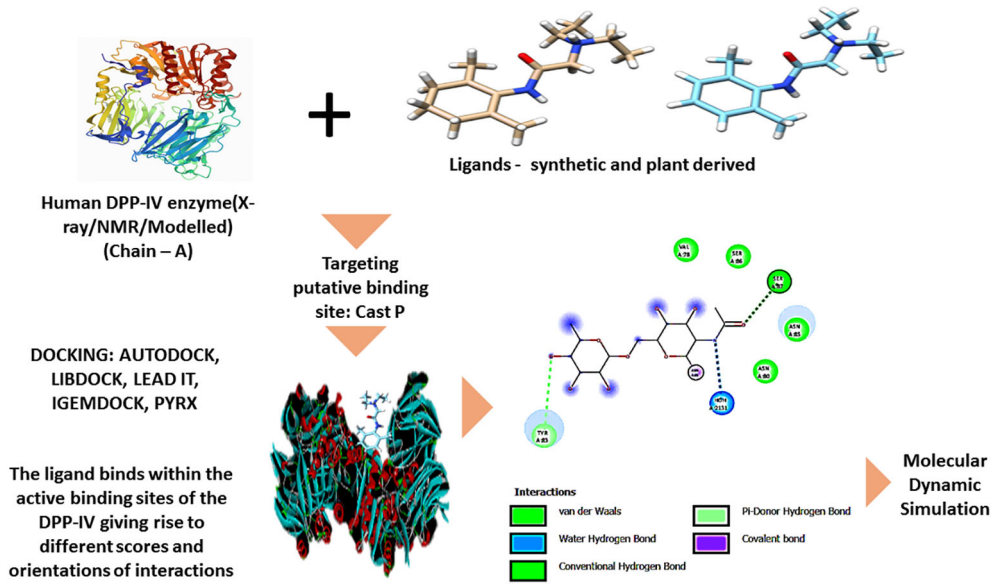


FIGURE 5
General mechanism of molecular docking. With a view to check the inhibition efficacy of different phytocompounds against the dipeptidyl peptidase (DPP)-IV putative binding site, *in silico* analysis can be performed by exploring various software, and in accordance with the same, different binding scores and orientations can be known, which are further projected towards molecular dynamics simulation. The ribbon structure of the human DPP-IV and the molecular structure of the ligand were downloaded from the protein data bank of PubMed and PubChem, respectively. The molecular interaction figure is recited after Kim et al. (2018) (47) under creative common license attribution.

relationship (QSAR) technique (112). This paper demonstrates the utility of a hybrid docking-QSAR approach to the study of ligand–protein interactions. In another study, three of the sugars found in fructooligosaccharides (*i.e.*, 1-kestose, nystose, and 1-β-fructofuranosyl nystose) are powerful inhibitors of dipeptidyl peptidase-IV as determined by molecular docking with Gold and Glide software (113). Furthermore, with the results of the isobologram and combination index analysis, the synergistic effects were also evaluated showing nystose and panose combinations having the greatest synergistic effects (114). In a recent report, the interaction of flavonoids with DPP-IV has been studied elaborately (115). Among a panel of 70 structurally different flavonoids that were tested for their inhibitory properties, myricetin, hyperoside, narcissoside, cyanidin 3-O-glucoside, and isoliquiritigenin exhibited concentration-dependent stronger inhibitory effects against DPP-4. Furthermore, fluorescence quenching studies revealed that these five flavonoid molecules could efficiently suppress the intrinsic fluorescence of DPP-4 by forming an unstable complex through spontaneous binding. While myricetin's complex with DPP-4 was mostly stabilized by hydrogen bonds and van der Waals forces, electrostatic forces may play a significant role in maintaining the complexes of the other four flavonoids with DPP-4. AutoDock 4.2 software was used to perform the molecular docking simulation, which further confirmed the binding interactions between DPP-4 and the five flavonoids chosen. The binding sites of hyperoside, narcissoside, cyaniding 3-O-glucoside, and isoliquiritigenin were found within the active site cavity of DPP-4, while myricetin's binding site was found in a minor cavity close to the active pockets (115).

Most of the flavonoids and terpenoids in the herbs have potency as antioxidants (116), antiseptics, and anti-inflammatory, and sterols are known for their anti-inflammatory property, but the pharmacological influence of alkaloids is difficult to predict because of the presence of many biological activities. Anti-diabetic herbs have many active compounds which could either work synergistically or antagonistically (117).

To reduce the pathogenesis of diabetes and its complications, flavonoids and polyphenolics have played an extremely significant role by potentiating GLUT-4 (Figure 1) isomer expression and uptake (118). Flavonoids portray their characteristic antidiabetic potency by promoting carbohydrate digestion, insulin signaling, secretion, glucose uptake, and adipose deposition (119). From the previous findings, a strong notion was developed that the phytocompounds have a potency to regulate GLUT-4 translocation through insulin signaling pathways, namely, the PI3K-Akt pathway and AMPK-dependent pathway (120). Moreover, even the phenols trigger the secretion of GLP-1 from the L cells of the large intestine, causing the elevation of the half-life of GLP-1 and the inhibition

of DPP-IV enzymatic activity (121). Synergistically, many phytocompounds also showcased their antioxidant and anti-inflammatory effect by suppressing oxidation and inflammatory signaling pathways and further preventing the risk of developing diabetes-related complications.

The flavonoids obtained from the water and ethanolic leaf extract of *U. lobata* were chrysoeriol and gossypetin. Chrysoeriol is a flavone exhibiting anti-inflammatory properties, found to be soluble in water and alkali solution, and after undergoing liquid chromatography (LC)–mass spectrometry (MS), this bioactive compound was found to be present in both the ethanolic and aqueous leaf extracts, which were further explored by undergoing molecular docking to check its inhibition activity as a ligand against the enzyme DPP-IV, and its efficacy was proven by portraying the binding energy of -4.6 kcal/mol (78). Besides this, gossypetin was the other prominent flavone identified in the aqueous and ethanolic leaf extracts of *U. lobata* by undergoing LC–MS screening, and the inhibition activity of this bioactive compound was investigated by calculating the free binding energy, which was -5.20 kcal/mol (78).

Two of the most potent flavonoids, puererone and robinin, were obtained by undergoing HPLC–MS analysis from the aqueous root extract of *P. tuberose*, which was exploited through computational studies to investigate their potency as DPP-IV inhibitors. Both flavones identified were competitive inhibitors of DPP-IV as they docked directly into all the three putative active sites of DPP-IV by exhibiting direct interaction, hydrogen bonding, and pi–pi interaction to substantiate the strong binding affinity and inhibition activity towards DPP-IV by possessing free binding energies of 7.543 and 7.376 kcal/mol, respectively, which further validated the efficacy of PTWE (84).

To select a potent phytochemical for the development of a plant-based DPP-IV inhibitor, the fruit extract of *W. coagulans* was screened and subjected to computational analysis, and from the analysis, hydrogen bonding was analyzed for various bioactive compounds against the target enzyme. In accordance with the same, even the binding energies of the competent compounds were detected to range from -7.2 to -9.8 kcal/mol, further inhibiting the target enzyme irreversibly. Among all the phytocompounds screened, sitoindoside IX showed the maximum inhibitory activity (-9.8 kcal/mol), which was even higher than the standard drug sitagliptin (103).

To analyze the DPP-IV inhibitory activity of *Ferrula asafoetida* methanolic seed extract, 12 different bioactive compounds were isolated and characterized by undergoing gas chromatography (GC)–MS analysis; they were further screened to examine their inhibitory role by going through docking studies using AUTODOCK software. Among the 12 different bioactive compounds, four bioactive compounds—namely, hexadecanoic acid, methyl tetradecanoate, ethoxy-disilane, and 9,12-octadecadienoic acid—were found to be potent enough and can be used for *in vivo* studies (122).

Among different plants, even the seeds of *Moringa oleifera* could show its efficacy as a DPP-IV inhibitor when being addressed to different docking programs. This resulted in generating 23 different competent compounds as ligands to develop compatible configurations. This investigation revealed that seven out of 23 candidate compounds had higher LibDock scores than the standard vildagliptin. These seven were further subjected to CDOCKER program, and three of them showed the best potency as DPP-IV inhibitors by possessing binding energy better than vildagliptin. Among the three, compound 1, a unique urethane O-ethyl-4-[L-rhamnosyloxy) benzyl] carbamate, possessed the highest binding energy (-84.99 kcal/mol) (95).

Five different bioactive compounds were detected from the methanolic leaf extract of *Gynura bicolor*, which were characterized using HPLC and LC–MS analysis and subjected to docking to identify the compounds with strong binding efficacy. Among the evaluated bioactive compounds, 3-caffeoyl quinic acid showed the highest binding energy (-29.07 kJ/mol), which was higher than sitagliptin (-25.04 kJ/mol), linagliptin (-27.57 kJ/mol), and saxagliptin (-23.45 kJ/mol) and subsequently proved to be a promising inhibitor of the enzyme to regulate incretin activity (123).

By evaluating the *in vitro* activity of *Curcligo latifolia*, fractionation was performed, and five different bioactive compounds were obtained, which were then analyzed for their drug-like properties; phlorizin exhibited the highest binding energy (-10.9 kcal/mol), which was higher than that of the reference drug sitagliptin (-8.6 kcal/mol) (104), whereas the bioactive compounds of *Melicope latifolia* were isolated from chloroform extract by undergoing LC–MS. It was observed that, among the four different compounds, p-coumarate featured the maximum binding efficacy towards the target molecule (-5.7 kcal/mol) (105).

Consortium of plant extracts—Polyherbal formulation

It has been discovered that several mechanisms in the treatment of diabetes effectively control the progress as well as alter the deteriorating condition of patients. Conventional medicines have been discouraged due to their adverse effects and withdrawal symptoms. In lieu of getting diabetes control, several herbal mixtures have been estimated and analyzed for treating diabetic patients. A traditional therapeutic herbal strategy has been practiced for the past many years. Furthermore, this strategy also confers its maximum effectiveness due to the synergistic action of several herbs to achieve better potency as compared with that of an individual herb, and this gave rise to the mechanistic concept of polyherbalism or polypharmacy, which could be looked at as one of the comprehensive approaches to address diabetes and its emerging complications (85, 124–126).

Moreover, nanoformulations could be used to address the limitations of herbal medicinal products, such as low stability and restricted absorption, which hinder their development as medicinal agents (127). Extracts from plants and isolated phytochemicals have been nanoformulated in a variety of ways to improve their therapeutic efficacy as nanoformulations and are proven to possess superior characteristics compared with the respective plant extracts or isolated phytochemicals. Prior research on peptides obtained from oat globulin revealed potent DPP4-inhibiting activity (128). Solid lipid nanoparticle-embedded oat globulin peptides were reported by Su et al. (2020) as stable and being able to maintain their capacity to inhibit DPP4, while non-embedded peptides suffered secondary hydrolysis by proteases in gastrointestinal fluids and lost their inhibitory effects (129). In a glucose-induced diabetic zebrafish model, *Eysenhardtia polystachya* (EP)-loaded silver nanoparticles (AgNPs) were likewise reported to boost pancreatic β-cell survival and insulin secretion-enhanced hyperglycemia. In addition, EP-AgNPs restored insulin production in insulinoma cell line (INS-1; an established model for studying the function of pancreatic islet β-cells) cells induced by H_2O_2, indicating that this may be due to cytoprotection against oxidative damage (130). Bark methanol/water extract from EP contains bioactive components including chalcones, flavonoids, and dihydrochalcones, which play a determining role in the phytofabrication of AgNPs (130).

Compared to the control Chang liver cells, glucose absorption was enhanced by the *Leonotis leonurus* extract-nanostructured lipid carriers (NLCs) (131). Under hyperglycemic conditions, INS-1 cells exposed to the NLCs released more insulin than the control untreated cells and the unformulated-extract-treated cells (131). A rise in the ATP/ADP ratio results from glucose metabolism in β-cells, which triggers insulin release. Increased intracellular Ca^{2+} stimulates insulin secretion by closing ATP-sensitive K^+-channels, depolarizing the cell membrane, and activating voltage-gated Ca^{2+}-channels (132). It has been demonstrated that a component derived from the *Leonotis sibiricus* plant can boost insulin production in INS-1E cells while simultaneously promoting cell proliferation (133). It is possible that the rise in insulin release caused by the extract NLC is attributable to the formulation's small particle size and high bioavailability (134), both of which lead to efficient cellular absorption, which is necessary for the effect to be achieved.

Paul et al. (2014) described an α-eleostearic acid (ESA)-rich nanoemulsion (NE) formulation and its anti-diabetic and antioxidative effects (135). Bitter gourd seed oil (BGO) contains 30%–50% ESA (136), whose antioxidative activity in scavenging ROS has been described (137). In plasma and liver homogenate fractions, the antioxidative defense system of rats with alloxan-induced T2DM was considerably impaired compared with the normal control group. Evidently, the administration of synthesized BGO-NE alleviated the oxidative stress state by activating the antioxidative defense mechanism (as demonstrated by an improvement in GPx, SOD, and CAT activities) (135).

Clinical status of phytochemicals

For decades now, it has been established that endothelial dysfunction and, ultimately, atherosclerosis are directly linked to the increase in oxidative stress that occurs during the progression of diabetic hyperglycemia. According to prior research, plasma antioxidants, including tocopherol, carotene, lutein, lycopene, retinol, and ascorbic acid, decline significantly in diabetes (138). Many phytochemicals are utilized to treat various diseases, and they play a crucial role in the fight against free radicals by targeting myriads of enzymes in several metabolic pathways.

Recent studies show that quercetin and resveratrol are used to increase insulin sensitivity, whereas beta-glucans and basic acids have been clinically used to stimulate glycogen synthesis and gluconeogenesis. Scirupsin B and myricetin are clinically proven for efficacy by targeting amylase from the salivary gland. Curcumin and turmerin have been recorded to inhibit glucosidase in the small intestine. Additionally, chicoric acid and lupanine acids are administered for insulin secretion from β-cells. Even berberine and pectin imparted their efficacy for reducing fasting blood glucose, postprandial blood glucose, and hemoglobin A1c (HbA1c) (139). The previous studies also showed that the seed extract of *Castanospermum australe* possesses very strong DPP-IV inhibitory alkaloids, including castanospermine, 7-deoxy-6-epi-castanospermine, and australine, and these are depicted as their efficacy in the management of hyperglycemia in rats (42). Recent studies have also been undertaken with garlic bulb, which exhibited a substantial DPP-IV inhibition efficacy (IC50 = 70.88 μg/ml), and this was because of the presence of caffeic acid 3-glucoside, malonylgenistin, and calenduloside E which have very high binding energy Table 3 (49). The recent *in silico* studies performed on the plant *Amberboa ramose* determined that the compound 5-hydroxy-7,8 dimethoxyflavone can be considered a promising candidate for serving well for all the purposes of drug likeliness by following the Lipinski's rule (140). The phytochemicals extracted from *Commiphora mukul* are guggulsterone E, guggulsterone Z, and glucogallin, and these compounds have potent DPP-IV inhibitor activity compared with vildagliptin (standard plant drug). Additionally, phytochemicals like pzrogallol, beta-glucogallin, and gallic acid purified from the plant *Phyllanthus emblica* possess considerable inhibitory activity against the DPP-IV target compound (141).

Among many other medicinal plants, *Terminalia arjuna* is a natural DPP-IV inhibitor with significant cardio-protective effects owing to the presence of some active ingredients,

TABLE 3 Different bioactive compounds and their respective binding energy value.

Plant	Phytochemicals	Bioactive compounds	Binding energy (kcal/mol)	References
Urena lobata	Flavonoids and flavones Sterols Glucoside	Gossypetin	-5.20	(78)
		Chrysoeriol	-4.66	
		Stigmasterol	-7.42	
		β-Sitosterol	-6.59	
		Mangiferin	-7.66	
Pueraria tuberose	Flavonoids	Robinin	7.543	(84)
		Puererone	7.376	
		Anhydrotuberosin	7.149	
		Daidzin	7.042	
		Tuberosin	6.965	
Withania coagulans	Sterols	Withanolide-D	-9.2	(103)
		Withanone	-7.9	
		Sitoindoside	-9.8	
		Withaferin	-8.1	
		Withacoagulin H	-8.9	
		Withanolide E	-7.6	
		Withangulatin A	-8.8	
Ferrula asafetida	Sterols	Hexadecanoic acid	-3.0	(122)
		Methyl tetra decanoate	-2.8	
		Ethoxydi (tert-butyl) silane	-2.4	
		9,12-Octadecadienoic acid	-2.7	
Moringa oleifera	Sterols and peptides	Urethane	-84.99	(93, 95)
		Isothiocyanate	-81.10	
		Dipeptide	-47.36	
		2-Butyloxycarbonyloxy-1(ethanol extract)	-5.583	
		Eicosanoic acid	-3.852	
		Cis-11-eicosenoic acid	-3.00	
Gynura bicolor	Sterols	3-Caffeoylquinic acid	-29.07	(123)
		5-O-Caffeoylquinic acid	-27.32	
		3,4-Dicaffeoylquinic acid	-27.17	
		Trans-5-p-coumarylquinic acid	27.11	
Curcligo latifolia	Polyphenol Flavonoid Sterol	Phlorizin	-10.9	(104)
		Scandenin	-9.3	
		Mundulone	-9.3	
		Berberine	-8.9	
		Dimethyl caffeic acid	-7.1	
Melicope latifolia	Sterols Flavonoids	β-Sitosterol Halfordin Methyl-p-coumarate Protocatechuic acid	Maximum binding energy was of compound 3 (-5.7)	(105)

including arjungenin, ellagic acid, and arjunic acid. These active compounds show superior DPP-IV inhibitory activity compared with synthetic inhibitors (64). Nowadays, combinations of ethnomedicine are used, and their efficacy is enhanced by adding bioactivity-determining related ingredients—for example, the efficacy of some of the tea extracts is enhanced by adding bioactive compounds like epigallocatechin-3-O-gallate, kaempferol rutinoside, myricetin-3-0-glucoside, and theogallin. The ethanolic extract of *Eucalyptus citriodora* has active compounds such as rhodomyrtosone B, rhodomyrtosone E, and quercitroside. These compounds have remarkable *in vivo* and *in vitro* effects on managing hyperglycemia. These biochemical compounds enhance insulin functionality in 3T3-L1 cells by improving plasma insulin, glucose tolerance in the high-fat-diet-fed obese rats, and attenuating plasma DPP-IV with a concomitant rise in GLP-1 levels (142). Moreover, a hot water extract from *Heritiera fomes* possesses some bioactive compounds similar to quercetin. These compounds exhibit

antidiabetic action, proved using BRIN-BD11 cells and high-fat-fed rats. A significant improvement in glucose tolerance and plasma DPP-IV was observed in rats fed with the abovementioned compounds. It also led to a decrease in intestinal disaccharidase activity while increasing the GI tract motility and transit time (143). In one of the experiments done on the quinine tree (*Rauvolfia caffra sond*), the efficacy of crude extract was found to be exterminated because of the dwindling alliances between alkaloids and saponins. Assessing fractions containing saponins and alkaloids revealed decreased antioxidant and DPP-IV activity. Based on these results, alkaloids and saponins should be kept separate during drug formulation (144).

Using bioactive compounds alone or in combination for their synergistic effects is thoroughly studied for their effectiveness against free radicals. The synergism and antagonism of two potential compounds of the crude extract are well known to be strongly influenced by the structure and spatial conformation of the antioxidants involved. It has been stated recently that several compounds can have synergistic effects in particular ratios, as at a particular concentration one bioactive compound can enhance the bioavailability of the other bioactive compound. With an increase of weaker antioxidants like hydroquinone and resveratrol, synergism decreases. Maximum synergism was observed when rutin hydrate was paired with resveratrol in 3:1 proportion, whereas maximum antagonism was observed when rutin hydrate was paired with hydroquinone in 1:3 proportion (145, 146). Moreover, studies have demonstrated that the efficacy of different combinations is also influenced by molar ratio, and the externalization of synergy strongly depends on the inflow of free radicals (147). In one of the studies, the combination of *Terminalia arjuna* and *Commiphora mukul* exhibited synergism and augmented antioxidant activity as measured by the normalization of superoxide radicals and nitric oxide levels (148).

Moreover, several polyherbal formulations like BGR-34 (blood glucose regulator) were marketed by Aimil Pharmaceuticals, which was approved by the AYUSH ministry, Government of India. It was named after 34 different active phytoconstituents formulated with a consortium of six different plant extracts, including the stem extract of *Berberis aristata*, *Pterocarpus marsupium*, and *Tinospora cardiofolia*, the leaf extract of *Gymnema sylvestre*, and the seed extract of *Trigonella foenum graecum.* It has exhibited DPP-IV action along with its cardioprotective effects, reduced glycated Hb level, enhanced antioxidant action, and regulated glucose homeostasis (124, 149, 150). Similarly, Insulin Management Expert-9, which was developed by the Central Council of Ayurvedic Sciences (CCRAS), is a consortium of five different plant extracts, including the seed extract of *Mangifera indica* and *Syzigium cumini*, the fruit extract of *Momordica charantia*, the leaf extract of *Gymnae sylvestre*, and the exudates of *Asphaltum punjabinum.* Its effects led to the regeneration of pancreatic β-cells, stimulation of insulin production, decrease in insulin resistance, delayed insulin resistance, delayed intestinal absorption, and reduced sugar cravings (124, 149, 150).

Flavonoids (Figure 6) are the most abundant plant polyphenolics that are consumed on a regular basis by humans, which can modulate DPP-4 activity, thus exerting their anti-diabetic effects (115). Whether key flavonoid subclasses (anthocyanins, flavones, flavonols, flavanones, and flavan-3-ols) in the diet are connected with the incidence of T2DM has been the subject of a number of research. Several randomized clinical trials (RCTs) have demonstrated the importance of flavonoids in T2DM, especially in long-term trials, but short-term therapies had no discernible impact on blood glucose levels or insulin resistance. An 8-week RCT by Hall et al. (2006) (151) and a 6-month RCT using soy protein and isoflavones did not find a beneficial effect on the plasma concentrations of insulin and glucose in postmenopausal women (152). In another study, green tea extracts containing 456 mg of catechins (a type of flavonoid) daily did not have noticeable effects on blood glucose levels or insulin resistance in an RCT lasting for 2 months (153). However, the long-term randomized, double-blind, placebo-controlled clinical investigation indicated the benefit of green tea extract on patients with T2DM and lipid abnormalities. For the duration of the 16-week research, the therapeutic group took 500 mg of green tea extract three times daily, whereas the control group received cellulose at the same dose and frequency. An increase in GLP-1, likely by inhibiting DPP-4, and a decrease in triglycerides and insulin resistance were noticed in the therapeutic group who received green tea extract (154). Additionally, Curtis et al. (2012) reported that postmenopausal women with T2DM who took flavan-3-ols and isoflavones for a year exhibited significant reductions in estimated peripheral insulin resistance and persistent improvements in lipid profile and insulin sensitivity (155). Similarly, a meta-analysis of 24 RCTs by Shrime et al. (2011) concluded that consuming flavonoid-rich cocoa significantly improved insulin resistance (156), and a meta-analysis of the effects of cocoa, chocolate, and flavan-3-ols from 42 RCTs was summarized by Hooper et al. (2012), who noticed significant reductions in insulin resistance and fasting serum insulin after cocoa or chocolate interventions (157). Notably, flavan-3-ols are among the most abundant bioactive components and account for 82.5% of the total flavonoid consumption among adults in the United States (158).

While the consumption of anthocyanin-rich foods, notably blueberries and apples/pears, was related to a decreased risk of T2DM in US men and women, no significant relationships were reported between total flavonoid intake and other flavonoid subclasses and T2DM risk (159). Dietary intakes of the flavonoids quercetin (found in fruits and vegetables) and myricetin (found in tea, berries, fruits, vegetables, and medicinal herbs) were found to be marginally associated with a reduced risk of T2DM in a study involving 10,054 Finnish men

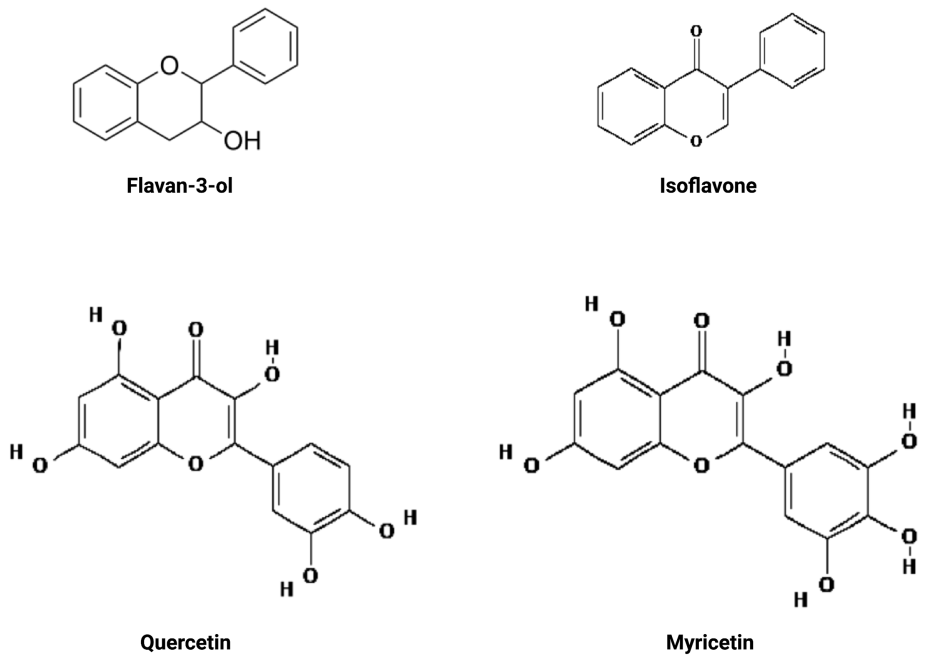


FIGURE 6
Flavonoids exhibiting an association with a reduced risk of type 2 diabetes mellitus in clinical studies.

and women (160). Because of the contradictory results, well-designed, long-term studies are required to determine whether flavonoids offer protection against T2DM.

Discussion

Since antiquity, natural product leads have been enriched with bioactive compounds encompassing a huge arena compared with small synthetic molecules (161). Myriads of bioactive compounds have made an incredible contribution to pharmacotherapy. They are far superior to structural leads based on diversified chemical properties. The presence of structural complexity and scaffold diversity is the most distinguishing and prominent feature which has characterized natural drugs to be better and more advantageous than synthetic drugs (162, 163). The existence of higher molecular mass, a larger number of sp3 carbon atoms, more oxygen atoms, and lesser nitrogen and halogen atoms serve to be hydrogen donors and acceptors with greater molecular rigidity over synthetic compounds. Earlier synthetic drugs were preferred over herbal ones because of some shortcomings. To isolate and identify bioactive compounds from plant origin is a challenging task as plants can have a varied chemical composition influenced by various parameters like environmental conditions and choice of extraction methods (164) which can create obstructions during isolation. Henceforth, new advanced technologies have been introduced to reduce the risk of degradation of plant extracts. Microwave, ultrasonic-assisted, and accelerated solvent extraction have outcompeted and flourished over conventional techniques. Currently, the application of ionic liquids, also known as designer solvents, have been used for MAE as well as UAE, have been strongly preferred over other classical solvents for their novel properties, and have replaced several volatile organic compounds in solvent extraction (165). Recent findings have also suggested that adapting the supercritical fluid extraction methodology for extracting various natural products results in better yield (164, 166). As a deduction, herbal leads are perpetuated with a humongous repository of compounds to delineate various pharmacognostic products. With evolving times, people are getting more inclined towards adopting a healthy lifestyle and are required to get nutritionally enriched products with more antioxidants and bioactive compounds; sometimes, these are lost while undergoing processing. Hence, improvement of physicochemical properties is needed along with retention of phenolic compounds by maintaining all the organoleptic characteristics in the final product. Therefore, many emerging treatments are needed to preserve the characteristics of the food product, and by following the block freeze crystallization (BFC) technique, the entire solution is subjected to a freezing process, which is further thawed by microwave heating with separation and extraction of cryoconcentrated solution by centrifugation and vacuum from the frozen matrix. Maintaining concentration is the major trump

card of this technique. An advancement of adding a filter to centrifugal BFC is to increase solute efficiency and solute yield. While undergoing experiments with green tea extract, the number of phenolics and antioxidant concentrations was increased by following the BFC technique (167, 168). Moreover, it is well established that obtaining a powder form of pomegranate fruit by performing BFC and spray drying resulted in greater retention of antioxidants as well as significant increases in physical parameters such as solubility index, bulk density, and hygroscopicity (169). The previous studies conducted on fruits of *Citrus sinensis* demonstrated that freeze drying technique expressed a higher content of phenol and also antioxidants. Flavonoids (hesperidin) were found to be decreased during heat drying (170). Due to the heat-sensitive nature of phenol compounds and the activation of polyphenol oxidase and peroxidase during the thermal drying process, phenolic compounds are destroyed. In contrast, polyphenol oxidase enzyme activity was reduced in freeze drying, which was conducted at a lower temperature. So, instead of using a heat drying process, freeze drying is the best alternative for preserving and quality extraction of bioactive compounds (170). A study on the preservation of antioxidant capacity and health-promoting components in frozen baby mustard also found that blanching before freezing diminishes antioxidant capacity levels as well as the contents of high-content glucosinolates and ascorbic acid (171).

With the recent progression in advanced drug discovery, several *in silico* approaches have been re-strategized for testing and synthesizing new phytochemicals as anti-diabetic compounds (172). Moreover, in terms of pharmacological and pharmacokinetic synergism, polyherbal formulation offers a better therapeutic effect compared with a single multi-component drug; hence, this strategy produced maximum inhibition with minimal adverse effects (124). Although phytochemicals have gained much interest as therapeutic negotiators, a gap exists between the *in vitro* examination and *in vivo* outcomes, eventually impeding clinical performance. To compensate for its efficacy in this regard, the application of nanotechnology has proven to be the best approach by turning all the odds associated with their pharmacokinetic profile. In

TABLE 4 Different marketed polyherbal drugs with their composition and mechanism of action as dipeptidyl peptidase-IV inhibitors.

Plant	Failed antidiabetic action of plant extracts	Nanophytomedicine: mode of action	Reference
Leonotis leonurus (, ajor phytoconstituent: marrubin)	*In vitro* experiments were performed on Chang liver cells and INS-1 cells exposed to hyperglycemic conditions. The extract was able to induce insulin secretion and upregulate the GLUT-2 gene The extract could not portray its effectiveness in the gastrointestinal tract because of its big size and poor solubility and bioavailability	Leaf extracts fabricated with homogenized nanolipid carriers demonstrated its efficacy on INS-1 pancreatic cells and changed the liver cells exposed to hyperglycemic conditions by improving the size of the formulation to increase the solubility and bioavailability	(131)
Ficus religiosa	Insulin-sensitizing action and hypoglycemic action were reduced	Solid lipid nanoparticles fabricated with extract induced hypoglycemic effect insulin secretion	(174)
Momordica Charantia (major phytoconstituent: alpha eleostearic acid)	The bitter gourd oil enriched with conjugated linolenic acid is known to increase the antioxidant activity of enzymes within *in vivo* systems Deprivation of bioavailability in the gastrointestinal tract was a constraint which inhibited its efficacy against reactive oxygen species	Since bioavailability was a constraint, hence, to upskill extract formulation, henceforth oil-based nanoemulsions were formulated with low dosage, maximum bioavailability, and efficacy against reactive oxygen species	(135)
Argyeria nervosa	The aqueous leaf extract has proven to be a potent antidiabetic formulation for the presence of polyphenols The extracts expressed limited efficacy against the target enzymes because of poor solubility	With an intention to obtain maximum antidiabetic efficacy and effectiveness against free radicals, functional groups of different phytochemicals were coupled to a silver nanoparticle surface in order to reduce the surface area and to obtain maximum antidiabetic activity	(175)
Eysenhardtia polystachya	The methanolic and aqueous extract prepared is enriched with an abundance of flavonoids to combat diabetic complications The plant extract showed its limited efficacy because of poor bioavailability within the gastrointestinal tract	With a rationale to improve and showcase antidiabetic effects, extracts are fabricated with nanoparticles to ameliorate insulin resistance and hyperglycemia in INS-1 cells and zebra fish, respectively, by increasing the bioavailability with minimal dosage	(130)
Marsilea quadrifolia	The plant extract was found to be enriched with phenolics and flavonoids which could impart its antidiabetic property The extract with poor bioavailability impeded glucose availability to 3T3L adipose cells	Flavonoids and polyphenols of the extract attached to the surface of AuNPs improved the bioavailability and induced transmitted GLUT -4 vesicle to the cell membrane and its uptake in adipocytes Functional groups of different phytochemicals coupled to gold nanoparticles resulted to the formation of biogenic gold nanoparticles, which further reduced the surface area by improving the cellular viability and glucose availability in 3T3 adipocytes	(176)
Oat derived peptides	Peptides is potent enough to inhibit DPP-IV inhibitory activity, but these peptides are degraded by gastrointestinal tract hydrolysis	Nanocrystallization of solid nanoparticles was undertaken in order to protect the oat-based bioactive peptides in a simulated gastric fluid environment with a view to inhibit dipeptidyl peptidase-IV	(129)

accordance with the same from recent findings, nanocarrier-assembled nanoparticles fabricated with bioactive compounds act as hypoglycemia agents, which, in turn, strongly influence the effectiveness of the anti-diabetic agents to improve drug penetration within the GI tract against the specific target, perpetuating the hypoglycemic effect with minimal side effects (173). In line with the discussion above about DPP-IV inhibition by the methanolic and aqueous extract of *U. lobata*, it was understood that methanolic extract expressed better *in vitro* results than aqueous extract. However, on the contrary, aqueous extract expressed better *in vivo* results, but not methanolic extract because of lack of solubility and bioavailability within the GI tract, and to improvise the efficacy, the methanolic extract could be fabricated with the nanoformulations for enhancing the bioavailability within the GI tract.

In summary, the DPP-IV inhibitory properties of phytochemicals have sparked an interest in their potential role in glycemia management. Results from *in vitro* and animal research highlight the potential of these natural inhibitors as plant-based constituents to complement pharmacotherapy in the regulation of blood glucose levels; however, human studies on these compounds are limited. The next stage in researching plant-derived DPP-IV inhibitors is to gather crucial data on the bioaccessibility and bioavailability of these bioactive compounds as well as clinical proof of their usefulness in humans and probable Table 4 interactions with presently prescribed medicines. DPP-IV inhibitors derived from plants may be developed as dietary supplements to minimize the risk of developing hyperglycemia or they may be used with anti-diabetic medications currently approved for glycemic management in T2DM; however, additional research is required to determine the strategies that will result in economically producible levels of these inhibitors.

Concluding remarks

At the molecular level, DPP-IV inhibitors have proved their functional role by holding an important position in drug discovery by hampering the deterioration of incretin hormones and further preserving their endogenous and blood glucose levels. Additionally, it also aids in the regeneration of pancreatic β-cell mass and enhances skeletal muscle cell proliferation. Nowadays, pharmacognosy has flourished enough to design herbal drugs far more reliable and superior to synthetic drugs with the least adverse effects. This review article aims to shed some light on the potency of different isolated bioactive compounds, polyherbal formulations, nanophytomedicines, and their functional role in efficacious treatment for T2DM. With anticipation of promoting pharmacognosy and phytochemistry to young researchers, this review is entailed with all the information which will be a steppingstone to designing and developing an herbal drug against the target enzyme to maintain endogenous incretin levels.

Author contributions

Original concept, writing the original draft, editing, and methodology: SC. Writing the original draft, editing, and methodology: SM. Writing the original draft, editing, and methodology: SV. Writing the original draft, editing, methodology, and proofreading: DS. Writing the original draft, methodology, and proofreading: PM. Writing the original draft, editing, methodology, and proofreading: BP. All authors contributed to the article and approved the submitted version.

Funding

Schemes [number ECR/2016/001984 by SERB, DST, Government of India, and 1188/ST, Bhubaneswar, dated 01.03.17, ST-(Bio)-02/2017, DST, Government of Odisha, India] to BP are acknowledged.

Acknowledgments

The support from Iowa State University, President Science College, and St. Xavier's College, Gujurat, India, and from Odisha University of Agriculture and Technology, respectively, are highly acknowledged.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Roden M, Shulman GI. The integrative biology of type 2 diabetes. *Nature* (2019) 576:51–60. doi: 10.1038/S41586-019-1797-8

2. Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: a review of current trends. *Oman Med J* (2012) 27:269–73. doi: 10.5001/OMJ.2012.68

3. Murea M, Ma L, Freedman BI. Genetic and environmental factors associated with type 2 diabetes and diabetic vascular complications. *Rev Diabetes Stud* (2012) 9:6. doi: 10.1900/RDS.2012.9.6

4. Huang X, Liu G, Guo J, Su ZQ. The PI3K/AKT pathway in obesity and type 2 diabetes. *Int J Biol Sci* (2018) 14:1483. doi: 10.7150/IJBS.27173

5. Henquin JC. Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* (2000) 49:1751–60. doi: 10.2337/DIABETES.49.11.1751

6. Ramachandran A, Snehalatha C, Shetty AS, Nanditha A. Trends in prevalence of diabetes in Asian countries. *World J Diabetes* (2012) 3:110. doi: 10.4239/WJD.V3.I6.110

7. Gamage K, Wasana P, Attanayake AP, Perera A, Jayatilaka W, Weerarathna TP. Natural drug leads as novel DPP-IV inhibitors targeting the management of type 2 diabetes mellitus. *J Of Complementary Med Res* (2020) 11:43-53. doi: 10.5455/jcmr.2020.11.01.06

8. Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. *Cell Metab* (2013) 17:819–37. doi: 10.1016/J.CMET.2013.04.008

9. Hussain MA, Laimon-Thomson E, Mustafa SM, Deck A, Song B. Detour ahead: Incretin hormone signaling alters its intracellular path as β-cell failure progresses during diabetes. *Front Endocrinol (Lausanne)* (2021) 12:665345/BIBTEX. doi: 10.3389/FENDO.2021.665345/BIBTEX

10. Müller TD, Finan B, Bloom SR, D'Alessio D, Drucker DJ, Flatt PR, et al. Glucagon-like peptide 1 (GLP-1). *Mol Metab* (2019) 30:72–130. doi: 10.1016/J.MOLMET.2019.09.010

11. Pratley RE, Salsali A. Inhibition of DPP-4: a new therapeutic approach for the treatment of type 2 diabetes. *Curr Med Res Opin* (2007) 23:919–31. doi: 10.1185/030079906X162746

12. Röhrborn D, Wronkowitz N, Eckel J. DPP4 in diabetes. *Front Immunol* (2015) 6:386/BIBTEX. doi: 10.3389/FIMMU.2015.00386/BIBTEX

13. Al-Masri IM, Mohammad MK, Tahaa MO. Inhibition of dipeptidyl peptidase IV (DPP IV) is one of the mechanisms explaining the hypoglycemic effect of berberine. *J Enzy Inhibit Med Chem* (2009) 24:1061–6. doi: 10.1080/14756360802610761

14. Bower AM, Real Hernandez LM, Berhow MA, de Mejia EG. Bioactive compounds from culinary herbs inhibit a molecular target for type 2 diabetes management, dipeptidyl peptidase IV. *J Agric Food Chem* (2014) 62:6147–58. doi: 10.1021/JF500639F

15. Harnedy PA, O'Keeffe MB, Fitzgerald RJ. Purification and identification of dipeptidyl peptidase (DPP) IV inhibitory peptides from the macroalga palmaria palmata. *Food Chem* (2015) 172:400–6. doi: 10.1016/J.FOODCHEM.2014.09.083

16. Kalhotra P, Chittepu VCSR, Osorio-Revilla G, Gallardo-Velázquez T. Structure¯Activity relationship and molecular docking of natural product library reveal chrysin as a novel dipeptidyl peptidase-4 (DPP-4) inhibitor: An integrated in silico and *In vitro* study. *Molecules* (2018) 23. doi: 10.3390/MOLECULES23061368

17. Lammi C, Zanoni C, Arnoldi A, Vistoli G. Peptides derived from soy and lupin protein as dipeptidyl-peptidase IV inhibitors: *In vitro* biochemical screening and in silico molecular modeling study. *J Agric Food Chem* (2016) 64:9601–6. doi: 10.1021/ACS.JAFC.6B04041

18. Mojica L, Luna-Vital DA, González de Mejía E. Characterization of peptides from common bean protein isolates and their potential to inhibit markers of type-2 diabetes, hypertension and oxidative stress. *J Sci Food Agric* (2017) 97:2401–10. doi: 10.1002/JSFA.8053

19. Saleem S, Jafri L, Haq IU, Chang LC, Calderwood D, Green BD, et al. Plants fagonia cretica l. and hedera nepalensis k. Koch contain natural compounds with potent dipeptidyl peptidase-4 (DPP-4) inhibitory activity. *J Ethnopharmacol* (2014) 156:26–32. doi: 10.1016/J.JEP.2014.08.017

20. Ayachi H, Merad M, Ghalem S. Study of interaction between dipeptidyl peptidase-4 and products extracted from the stevia plant by molecular modeling. *Int J Pharm Sci Rev Res* (2013) 23(1):87–90.

21. Singh AK, Jatwa R, Joshi J. Cytoprotective and dipeptidyl peptidase- IV (DPP-IV/CD26) inhibitory roles of ocimum sanctum and momordica charantia extract. *Asian J Pharm Clin Res* (2014) 7:115–20.

22. Ekayanti M, Sauriasari R, Elya B. Dipeptidyl peptidase IV inhibitory activity of fraction from white tea ethanolic extract (Camellia sinensis (L.) kuntze) ex vivo. *Pharmacogn J* (2018) 10:190–3. doi: 10.5530/pj.2018.1.32

23. Riyanti S, Suganda AG, Sukandar EY. Dipeptidyl peptidase –IV inhibitory activity of some Indonesian medicinal plants. *Asian J Pharm Clin Res* (2016) 9:375–7.

24. Bisht R, Bhattacharya S, Jaliwala YA. Evaluating the use of desmodium gangeticum as alpha glucosidase and DPPIV inhibitor for type-II diabetes. *Am J Phytomed Clin Ther* (2014) 2(4):530–9.

25. Borde MK, Mohanty I, Suman R, Deshmukh Y. Dipeptidyl peptidase-IV inhibitory activities of medicinal plants: Terminalia arjuna, commiphora mukul, gymnema sylvestre, morinda citrifolia, emblica officinalis. *Asian J Pharm Clin Res* (2016) 9:180–2. Available at: https://innovareacademics.in/journals/index.php/ajpcr/article/view/10894

26. Cásedas G, Les F, González-Burgos E, Gómez-Serranillos MP, Smith C, López V. Cyanidin-3-O-glucoside inhibits different enzymes involved in central nervous system pathologies and type-2 diabetes. *South Afr J Bot* (2019) 120:241–6. doi: 10.1016/J.SAJB.2018.07.001

27. Gupta A, Jacobson GA, Burgess JR, Jelinek HF, Nichols DS, Narkowicz CK, et al. Citrus bioflavonoids dipeptidyl peptidase-4 inhibition compared with gliptin antidiabetic medications. *Biochem Biophys Res Commun* (2018) 503:21–5. doi: 10.1016/J.BBRC.2018.04.156

28. Lin YS, Chen CR, Wu WH, Wen CL, Chang CI, Hou WC. Anti-α-glucosidase and anti-dipeptidyl peptidase-IV activities of extracts and purified compounds from vitis thunbergii var. taiwaniana. *J Agric Food Chem* (2015) 63:6393–401. doi: 10.1021/ACS.JAFC.5B02069/SUPPL_FILE/JF5B02069_SI_001.PDF

29. Eidenberger T, Selg M, Krennhuber K. Inhibition of dipeptidyl peptidase activity by flavonol glycosides of guava (Psidium guajava l.): A key to the beneficial effects of guava in type II diabetes mellitus. *Fitoterapia* (2013) 89:74–9. doi: 10.1016/J.FITOTE.2013.05.015

30. Dey B, Mitra A, Katakam P, Singla RK. Exploration of natural enzyme inhibitors with hypoglycemic potentials amongst eucalyptus spp. by *in vitro* assays. *World J Diabetes* (2014) 5:209. doi: 10.4239/WJD.V5.I2.209

31. Martinez G. *Comprehensive medicinal chemistry. volume 1, general perspective - the future of drug discovery*. Martinez A, Gil C, editors. Madrid, Spain: Centro de Investigaciones Biologicas-CSIC (2017).

32. Kosaraju J, Dubala A, Chinni S, Khatwal RB, Satish Kumar MN, Basavan D. A molecular connection of pterocarpus marsupium, Eugenia jambolana and gymnema sylvestre with dipeptidyl peptidase-4 in the treatment of diabetes. *Pharm Biol* (2014) 52:268–71. doi: 10.3109/13880209.2013.823550

33. Yogisha S, Raveesha KA. Dipeptidyl peptidase IV inhibitory activity of mangifera indica. *J Nat Prod* (2010) 3:76–9.

34. Ansari P, Hannon-Fletcher MP, Flatt PR, Abdel-Wahab YHA. Effects of 22 traditional anti-diabetic medicinal plants on DPP-IV enzyme activity and glucose homeostasis in high-fat fed obese diabetic rats. *Biosci Rep* (2021) 41. doi: 10.1042/BSR20203824

35. Quek A, Kassim NK, Ismail A, Latif MAM, Shaari K, Tan DC, et al. Identification of dipeptidyl peptidase-4 and α-amylase inhibitors from melicope glabra (Blume) t. g. Hartley (Rutaceae) using liquid chromatography tandem mass spectrometry, *In vitro* and in silico methods. *Molecules* (2020) 26:1-35. doi: 10.3390/MOLECULES26010001

36. Gao Y, Zhang Y, Zhu J, Li B, Li Z, Zhu W, et al. Recent progress in natural products as DPP-4 inhibitors. *Future Med Chem* (2015) 7:1079–89. doi: 10.4155/FMC.15.49

37. Wang HJ, Chiang BH. Anti-diabetic effect of a traditional Chinese medicine formula. *Food Funct* (2012) 3:1161–9. doi: 10.1039/C2FO30139C

38. Bansal P, Paul P, Mudgal J, G. Nayak P, Thomas Pannakal S, Priyadarsini KI, et al. Antidiabetic, antihyperlipidemic and antioxidant effects of the flavonoid rich fraction of pilea microphylla (L.) in high fat diet/streptozotocin-induced diabetes in mice. *Exp Toxicol Pathol* (2012) 64:651–8. doi: 10.1016/J.ETP.2010.12.009

39. Purnomo Y, Soeatmadji DW, Sumitro SB, Widodo MA. Inhibitory activity of urena lobata leaf extract on dipeptidyl peptidase-4 (DPP-4): Is it different *in vitro* and *in vivo*? *Medi Plants* (2018) 10:99–105. doi: 10.5958/0975-6892.2018.00016.3

40. Wang Z, Yang L, Fan H, Wu P, Zhang F, Zhang C, et al. Screening of a natural compound library identifies emodin, a natural compound from rheum palmatum linn that inhibits DPP4. *PeerJ* (2017) 1-14. doi: 10.7717/PEERJ.3283/SUPP-5

41. Fan J, Johnson MH, Lila MA, Yousef G, de Mejia EG. Berry and citrus phenolic compounds inhibit dipeptidyl peptidase IV: Implications in diabetes management. *Evid Based Complement Alternat Med* (2013) 2013:1-13. doi: 10.1155/2013/479505

42. Bharti SK, Krishnan S, Kumar A, Rajak KK, Murari K, Bharti BK, et al. Antihyperglycemic activity with DPP-IV inhibition of alkaloids from seed extract of castanospermum australe: Investigation by experimental validation and molecular docking. *Phytomedicine* (2012) 20:24–31. doi: 10.1016/J.PHYMED.2012.09.009

43. Wang F, Yu G, Zhang Y, Zhang B, Fan J. Dipeptidyl peptidase IV inhibitory peptides derived from oat (Avena sativa l.), buckwheat (Fagopyrum esculentum), and highland barley (Hordeum vulgare trifurcatum (L.) trofim) proteins. *J Agric Food Chem* (2015) 63:9543–9. doi: 10.1021/ACS.JAFC.5B04016

44. Velarde-Salcedo AJ, Barrera-Pacheco A, Lara-González S, Montero-Morán GM, Díaz-Gois A, González De Mejia E, et al. *In vitro* inhibition of dipeptidyl peptidase IV by peptides derived from the hydrolysis of amaranth (Amaranthus hypochondriacus l.) proteins. *Food Chem* (2013) 136:758–64. doi: 10.1016/J.FOODCHEM.2012.08.032

45. Yarizade A, Kumleh HH, Niazi A. *In vitro* antidiabetic effects of ferula assa-foetida extracts through dipeptidyl peptidase IV and α-glucosidase inhibitory activity. *Asian J Pharm Clin Res* (2017) 10:357–60. doi: 10.22159/AJPCR.2017.V10I5.16648

46. Kumar V, Sachan R, Rahman M, Sharma K, Al-Abbasi FA, Anwar F. Prunus amygdalus extract exert antidiabetic effect *via* inhibition of DPP-IV: in-silico and *in-vivo* approaches. *J Biomol Struct Dyn* (2021) 39:4160–74. doi: 10.1080/07391102.2020.1775124

47. Kim BR, Kim HY, Choi I, Kim JB, Jin CH, Han AR. DPP-IV inhibitory potentials of flavonol glycosides isolated from the seeds of lens culinaris: *In vitro* and molecular docking analyses. *Molecules* (2018) 23:1-10. doi: 10.3390/MOLECULES23081998

48. Chakrabarti R, Bhavtaran S, Narendra P, Varghese N, Vanchhawng L, Sham Shihabudeen MH, et al. Dipeptidyl peptidase-IV inhibitory activity of berberis aristata. *J Nat Prod* (2011) 4:158–63.

49. Kalhotra P, Chittepu VCSR, Osorio-Revilla G, Gallardo-Velazquez T. Phytochemicals in garlic extract inhibit therapeutic enzyme DPP-4 and induce skeletal muscle cell proliferation: A possible mechanism of action to benefit the treatment of diabetes mellitus. *Biomolecules* (2020) 10:1-16. doi: 10.3390/BIOM10020305

50. Zhang L, Zhang ST, Yin YC, Xing S, Li WN, Fu XQ. Hypoglycemic effect and mechanism of isoquercitrin as an inhibitor of dipeptidyl peptidase-4 in type 2 diabetic mice. *RSC Adv* (2018) 8:14967–74. doi: 10.1039/C8RA00675J

51. Gallwitz B. Clinical use of DPP-4 inhibitors. *Front Endocrinol (Lausanne)* (2019) 10:389/BIBTEX. doi: 10.3389/FENDO.2019.00389/BIBTEX

52. Biftu T, Sinha-Roy R, Chen P, Qian X, Feng D, Kuethe JT, et al. Omarigliptin (MK-3102): a novel long-acting DPP-4 inhibitor for once-weekly treatment of type 2 diabetes. *J Med Chem* (2014) 57:3205–12. doi: 10.1021/JM401992E

53. Barnett A. DPP-4 inhibitors and their potential role in the management of type 2 diabetes. *Int J Clin Pract* (2006) 60:1454–70. doi: 10.1111/J.1742-1241.2006.01178.X

54. Deacon CF. Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: a comparative review. *Diabetes Obes Metab* (2011) 13:7–18. doi: 10.1111/J.1463-1326.2010.01306.X

55. del Prato S, Rosenstock J, Garcia-Sanchez R, Iqbal N, Hansen L, Johnsson E, et al. Safety and tolerability of dapagliflozin, saxagliptin and metformin in combination: *Post-hoc* analysis of concomitant add-on versus sequential add-on to metformin and of triple versus dual therapy with metformin. *Diabetes Obes Metab* (2018) 20:1542–6. doi: 10.1111/DOM.13258

56. Scheen AJ. The safety of gliptins : updated data in 2018. *Expert Opin Drug Saf* (2018) 17:387–405. doi: 10.1080/14740338.2018.1444027

57. Goldenberg RM. Choosing dipeptidyl peptidase-4 inhibitors, sodium-glucose cotransporter-2 inhibitors, or both, as add-ons to metformin: Patient baseline characteristics are crucial. *Clin Ther* (2017) 39:2438–47. doi: 10.1016/J.CLINTHERA.2017.10.016

58. Kumar S, Mittal A, Mittal A. A review upon medicinal perspective and designing rationale of DPP-4 inhibitors. *Bioorg Med Chem* (2021) 46. doi: 10.1016/J.BMC.2021.116354

59. Scheen AJ. A review of gliptins in 2011. *Expert Opin Pharmacother* (2012) 13:81–99. doi: 10.1517/14656566.2012.642866

60. FDA Drug Safety Communication. *FDA Warns that DPP-4 inhibitors for type 2 diabetes may cause severe joint pain* (2016). Available at: https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-fda-warns-dpp-4-inhibitors-type-2-diabetes-may-cause-severe-joint-pain (Accessed October 4, 2022).

61. Gupta V, Kalra S. Choosing a gliptin. *Indian J Endocrinol Metab* (2011) 15:298. doi: 10.4103/2230-8210.85583

62. Pathak R, Bridgeman MB. Dipeptidyl peptidase-4 (DPP-4) inhibitors in the management of diabetes. *Pharm Ther* (2010) 35:509.

63. Chahal H, Chowdhury TA. Gliptins: a new class of oral hypoglycaemic agent. *QJM: Int J Med* (2007) 100:671–7. doi: 10.1093/QJMED/HCM081

64. Mohanty IR, Borde M, Kumar CS, Maheshwari U. Dipeptidyl peptidase IV inhibitory activity of terminalia arjuna attributes to its cardioprotective effects in experimental diabetes: In silico, *in vitro* and *in vivo* analyses. *Phytomedicine* (2019) 57:158–65. doi: 10.1016/J.PHYMED.2018.09.195

65. Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. *Cardiovasc Diabetol* (2005) 4:5. doi: 10.1186/1475-2840-4-5

66. Chan SMH, Ye JM. Strategies for the discovery and development of anti-diabetic drugs from the natural products of traditional medicines. *J Pharm Sci* (2013) 16:207–16. doi: 10.18433/J3T60G

67. Esmail Al-Snafi A, Majid WJ, Ali Talab T, Al-Battat HA, Author C. Medicinal plants with antidiabetic effects-an overview (Part 1). *IOSR J Pharm* (2019) 9:9–46.

68. Chainy GBN, Sahoo DK. Hormones and oxidative stress: an overview. *Free Radic Res* (2020) 54:1–21. doi: 10.1080/10715762.2019.1702656

69. Bashan N, Kovsan J, Kachko I, Ovadia H, Rudich A. Positive and negative regulation of insulin signaling by reactive oxygen and nitrogen species. *Physiol Rev* (2009) 89:27–71. doi: 10.1152/PHYSREV.00014.2008/ASSET/IMAGES/LARGE/Z9J0010924990009.JPEG

70. Sharifi-Rad J, Cruz-Martins N, López-Jornet P, Lopez EPF, Harun N, Yeskaliyeva B, et al. Natural coumarins: Exploring the pharmacological complexity and underlying molecular mechanisms. *Oxid Med Cell Longev* (2021) 2021. doi: 10.1155/2021/6492346

71. Ghorbani A, Rashidi R, Shafiee-Nick R. Flavonoids for preserving pancreatic beta cell survival and function: A mechanistic review. *Biomed Pharmacother* (2019) 111:947–57. doi: 10.1016/J.BIOPHA.2018.12.127

72. Oh YS. Plant-derived compounds targeting pancreatic beta cells for the treatment of diabetes. *Evid Based Complement Alternat Med* (2015) 2015. doi: 10.1155/2015/629863

73. Annadurai T, Muralidharan AR, Joseph T, Hsu MJ, Thomas PA, Geraldine P. Antihyperglycemic and antioxidant effects of a flavanone, naringenin, in streptozotocin–nicotinamide-induced experimental diabetic rats. *J Physiol Biochem* (2012) 68:307–18. doi: 10.1007/S13105-011-0142-Y

74. Coskun O, Kanter M, Korkmaz A, Oter S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat pancreas. *Pharmacol Res* (2005) 51:117–23. doi: 10.1016/J.PHRS.2004.06.002

75. Lee D, Kim KH, Lee J, Hwang GS, Lee HL, Hahm DH, et al. Protective effect of cirsimaritin against streptozotocin-induced apoptosis in pancreatic beta cells. *J Pharm Pharmacol* (2017) 69:875–83. doi: 10.1111/JPHP.12719

76. He JZ, Shao P, Liu JH, Ru QM. Supercritical carbon dioxide extraction of flavonoids from pomelo (Citrus grandis (L.) osbeck) peel and their antioxidant activity. *Int J Mol Sci* (2012) 13:13065–78. doi: 10.3390/IJMS131013065

77. Singh AK, Yadav D, Sharma N, Jin JO. Dipeptidyl peptidase (DPP)-IV inhibitors with antioxidant potential isolated from natural sources: A novel approach for the management of diabetes. *Pharmaceuticals* (2021) 14. doi: 10.3390/PH14060586

78. Purnomo Y, Soeatmadji DW, Sumitro SB, Widodo MA. Original article. *Asian Pac J Trop BioMed* (2015) 8:645–9. doi: 10.1016/J.APJTB.2015.05.014

79. Omonkhua AA, Onoagbe IO. Evaluation of the long-term effects of urena lobata root extracts on blood glucose and hepatic function of normal rabbits. *J Toxicol Environ Health Sci* (2011) 3:204–13.

80. Sekendar Ali M, Omar Faruq K, Aziz Abdur Rahman M, Aslam Hossain M. Antioxidant and cytotoxic activities of methanol extract of urena lobata (L) leaves. *Pharma Innovation-Journal* (2013) 2.

81. Goodman LS, Louis S, Gilman A, Hardman JG, Gilman AG, Limbird LE. *Goodman & gilman's the pharmacological basis of therapeutics*. (New York: McGraw-Hill, Medical Publishing Division). (1996). 1905.

82. Stevens MM, Honerkamp-Smith AR, Keller SL. Solubility limits of cholesterol, lanosterol, ergosterol, stigmasterol, and β-sitosterol in electroformed lipid vesicles. *Soft Matter* (2010) 6:5882–90. doi: 10.1039/C0SM00373E

83. Morris GM, Lim-Wilby M. Molecular docking. *Methods Mol Biol* (2008) 443:365–82. doi: 10.1007/978-1-59745-177-2_19/TABLES/1

84. Srivastava S, Yadav D, Bhusan Tripathi Y. DPP-IV inhibitory potential of methanolic extract of pueraria tuberosa in liver of alloxan induced diabetic model. *Biosci Biotechnol Res Asia* (2018) 15:01–4. doi: 10.13005/BBRA/2602

85. Choudhury H, Pandey M, Hua CK, Mun CS, Jing JK, Kong L, et al. An update on natural compounds in the remedy of diabetes mellitus: A systematic review. *J Tradit Complement Med* (2018) 8:361. doi: 10.1016/J.JTCME.2017.08.012

86. Kempegowda PK, Zameer F, Narasimashetty CK, Kollur SP, Murari SK. Inhibitory potency of *Withania somnifera* extracts against DPP-4: an *in vitro*

evaluation. *Afr J Traditional Complementary Altern Medicines* (2018) 15:11–25. doi: 10.4314/ajtcam.v15i1

87. Bellamkonda R, Rasineni K, Singareddy SR, Kasetti RB, Pasurla R, Chippada AR, et al. Antihyperglycemic and antioxidant activities of alcoholic extract of commiphora mukul gum resin in streptozotocin induced diabetic rats. *Pathophysiology* (2011) 18:255–61. doi: 10.1016/J.PATHOPHYS.2010.10.002

88. Ramesh B, Karuna R, Sreenivasa Reddy S, Sudhakara G, Saralakumari D. Ethanolic extract of commiphora mukul gum resin attenuates streptozotocin-induced alterations in carbohydrate and lipid metabolism in rats. *EXCLI J* (2013) 12:556.

89. Ramesh B, Saralakumari D. Antihyperglycemic, hypolipidemic and antioxidant activities of ethanolic extract of commiphora mukul gum resin in fructose-fed male wistar rats. *J Physiol Biochem* (2012) 68:573–82. doi: 10.1007/S13105-012-0175-X

90. Asghari J, Ondruschka B, Mazaheritehrani M. Extraction of bioactive chemical compounds from the medicinal Asian plants by microwave irradiation. *J Medi Plants Res* (2011) 5:495–506.

91. Mubarik S, Aftab M, Tariq A. Issue 2 article 4 part of the biodiversity commons, and the biology commons recommended citation recommended citation javad. *J Bioresource Manage* (2016) 3. doi: 10.35691/JBM.6102.0051

92. Bisht R, Bhattacharya S. Effect of various extracts of desmodium gangeticum on streptozotocin-nicotinamide induced type-2 diabetes. *Asian J Plant Sci Res* (2013) 3:28–34.

93. Nwakulite A, Obeagu EI, Nwanjo HU, Nwosu DC, Nnatuanya IN, Eze R, et al. Studies on molecular docking of moringa oleifera leaf phytochemical constituents on alpha glucosidase, alpha amylase and dipeptidyl peptidase. *J Pharm Res Int* (2021) 33:239–45. doi: 10.9734/JPRI/2021/V33I28A31527

94. Anwer T, Safhi MM, Makeen HA, Alshahrani S, Siddiqui R, Sivakumar SM, et al. Antidiabetic potential of moringa oleifera lam. leaf extract in type 2 diabetic rats, and its mechanism of action. *Trop J Pharm Res* (2021) 20:97–104. doi: 10.4314/tjpr.v20i1.15

95. Yang Y, Shi CY, Xie J, Dai JH, He SL, Tian Y. Identification of potential dipeptidyl peptidase (DPP)-IV inhibitors among moringa oleifera phytochemicals by virtual screening, molecular docking analysis, ADME/T-based prediction, and *In vitro* analyses. *Molecules* (2020) 25:1-12. doi: 10.3390/MOLECULES25010189

96. Agusfina M, Saputri FC, Sakti AS, Mun'im A. Difference of acidic adding effect in ethanol extraction of white mulberry stem bark (Morus alba) and DPP-4 inhibiting activity screening for identifying its antidiabetic potential. *Pharmacogn J* (2019) 11:790–5. doi: 10.5530/pj.2019.11.126

97. Guo C, Li R, Zheng N, Xu L, Liang T, He Q. Anti-diabetic effect of ramulus mori polysaccharides, isolated from morus alba l., on STZ-diabetic mice through blocking inflammatory response and attenuating oxidative stress. *Int Immunopharmacol* (2013) 16:93–9. doi: 10.1016/J.INTIMP.2013.03.029

98. Jiao Y, Wang X, Jiang X, Kong F, Wang S, Yan C. Antidiabetic effects of morus alba fruit polysaccharides on high-fat diet- and streptozotocin-induced type 2 diabetes in rats. *J Ethnopharmacol* (2017) 199:119–27. doi: 10.1016/J.JEP.2017.02.003

99. Kim SB, Chang BY, Hwang BY, Kim SY, Lee MK. Pyrrole alkaloids from the fruits of morus alba. *Bioorg Med Chem Lett* (2014) 24:5656–9. doi: 10.1016/J.BMCL.2014.10.073

100. Zhang Y, Ren C, Lu G, Cui W, Mu Z, Gao H, et al. Purification, characterization and anti-diabetic activity of a polysaccharide from mulberry leaf. *Regul Toxicol Pharmacol* (2014) 70:687–95. doi: 10.1016/J.YRTPH.2014.10.006

101. Darvas F, Keseru G, Papp A, Dorman G, Urge L, Krajcsi P. In silico and ex silico ADME approaches for drug discovery. *Curr Top Med Chem* (2002) 2:1287–304. doi: 10.2174/1568026023392841

102. Bharti R, Chopra BS, Raut S, Khatri N. Pueraria tuberosa: A review on traditional uses, pharmacology, and phytochemistry. *Front Pharmacol* (2020) 11:582506. doi: 10.3389/FPHAR.2020.582506

103. Ram H, Kumar P, Purohit A, Kashyap P, Kumar S, Kumar S, et al. Improvements in HOMA indices and pancreatic endocrinal tissues in type 2-diabetic rats by DPP-4 inhibition and antioxidant potential of an ethanol fruit extract of withania coagulans. *Nutr Metab (Lond)* (2021) 18:1–17. doi: 10.1186/S12986-021-00547-2/TABLES/4

104. Zabidi NA, Ishak NA, Hamid M, Ashari SE, Mohammad Latif MA. Inhibitory evaluation of curculigo latifolia on α-glucosidase, DPP (IV) and *in vitro* studies in antidiabetic with molecular docking relevance to type 2 diabetes mellitus. *J Enzyme Inhib Med Chem* (2021) 36:109–21. doi: 10.1080/14756366.2020.1844680

105. Quek A, Kassim NK, Lim PC, Tan DC, Mohammad Latif MA, Ismail A, et al. α-amylase and dipeptidyl peptidase-4 (DPP-4) inhibitory effects of melicope latifolia bark extracts and identification of bioactive constituents using *in vitro* and in silico approaches. *Pharm Biol* (2021) 59:964–73. doi: 10.1080/13880209.2021.1948065

106. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Delivery Rev* (1997) 23:3–25. doi: 10.1016/S0169-409X(96)00423-1

107. Schnider P. (2021), 1–15. doi: 10.1039/9781788016414-00001

108. Meng X-Y, Zhang H-X, Mezei M, Cui M. Molecular docking: a powerful approach for structure-based drug discovery. *Curr Comput Aided Drug Des* (2011) 7:146–57. doi: 10.2174/157340911795677602

109. Ferreira LG, dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. *Molecules* (2015) 20:13384–421. doi: 10.3390/MOLECULES200713384

110. Bikadi Z, Hazai E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. *J Cheminform* (2009) 1:1–16. doi: 10.1186/1758-2946-1-15/FIGURES/3

111. Lape M, Elam C, Paula S. Comparison of current docking tools for the simulation of inhibitor binding by the transmembrane domain of the sarco/endoplasmic reticulum calcium ATPase. *Biophys Chem* (2010) 150:88. doi: 10.1016/J.BPC.2010.01.011

112. Amini Z, Fatemi MH, Gharaghani S. Hybrid docking-QSAR studies of DPP-IV inhibition activities of a series of aminomethyl-piperidones. *Comput Biol Chem* (2016) 64:335–45. doi: 10.1016/J.COMPBIOLCHEM.2016.08.003

113. Bharti SK, Krishnan S, Kumar A, Rajak KK, Murari K, Bharti BK, et al. Antidiabetic activity and molecular docking of fructooligosaccharides produced by aureobasidium pullulans in poloxamer-407-induced T2DM rats. *Food Chem* (2013) 136:813–21. doi: 10.1016/J.FOODCHEM.2012.08.083

114. Bharti SK, Krishnan S, Kumar A, Gupta AK, Ghosh AK, Kumar A. Mechanism-based antidiabetic activity of fructo- and isomalto-oligosaccharides: Validation by *in vivo*, in silico and *in vitro* interaction potential. *Process Biochem* (2015) 50:317–27. doi: 10.1016/J.PROCBIO.2014.10.014

115. Pan J, Zhang Q, Zhang C, Yang W, Liu H, Lv Z, et al. Inhibition of dipeptidyl peptidase-4 by flavonoids: Structure–activity relationship, kinetics and interaction mechanism. *Front Nutr* (2022) 9:892426. doi: 10.3389/FNUT.2022.892426

116. Ilango S, Sahoo DK, Paital B, Kathirvel K, Gabriel JI, Subramaniam K, et al. A review on annona muricata and its anticancer activity. *Cancers* (2022) 14:4539. doi: 10.3390/CANCERS14184539

117. Evans WC. *Trease and evans' pharmacognosy, Sixteenth Edition* (USA: Elsevier) (2009). 1–603.

118. Gannon NP, Conn CA, Vaughan RA. Dietary stimulators of GLUT4 expression and translocation in skeletal muscle: a mini-review. *Mol Nutr Food Res* (2015) 59:48–64. doi: 10.1002/MNFR.201400414

119. Vinayagam R, Xu B. Antidiabetic properties of dietary flavonoids: a cellular mechanism review. *Nutr Metab* (2015) 12:1–20. doi: 10.1186/S12986-015-0057-7

120. Md Sayem AS, Arya A, Karimian H, Krishnasamy N, Hasamnis AA, Hossain CF. Action of phytochemicals on insulin signaling pathways accelerating glucose transporter (GLUT4) protein translocation. *Molecules* (2018) 23. doi: 10.3390/MOLECULES23020258

121. Domínguez Avila JA, Rodrigo García J, González Aguilar GA, de la Rosa LA. The antidiabetic mechanisms of polyphenols related to increased glucagon-like peptide-1 (GLP1) and insulin signaling. *Molecules* (2017) 22. doi: 10.3390/MOLECULES22060903

122. Vijaya Nagini D, Krishna MSR, Karthikeyan S. Identification of novel dipeptidyl peptidase -IV inhibitors from ferula asafoetida through GC-MS and molecular docking studies. *Res J Pharm Technol* (2020) 13:5072–6. doi: 10.5958/0974-360X.2020.00888.4

123. Rozano L, Abdullah Zawawi MR, Ahmad MA, Jaganath IB. Computational analysis of gynura bicolor bioactive compounds as dipeptidyl peptidase-IV inhibitor. *Adv Bioinf* (2017) 2017:1-17. doi: 10.1155/2017/5124165

124. Karole S, Shrivastava S, Thomas S, Soni B, Khan S, Dubey J, et al. Polyherbal formulation concept for synergic action: A review. *J Drug Delivery Ther* (2019) 9:453–66. doi: 10.22270/JDDT.V9I1-S.2339

125. Gordon A, Buch Z, Baute V, Coeytaux R. Use of ayurveda in the treatment of type 2 diabetes mellitus. *Glob Adv Health Med* (2019) 8. doi: 10.1177/2164956119861094

126. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of ayurveda. *Pharmacogn Rev* (2014) 8:73–80. doi: 10.4103/0973-7847.134229

127. Wickramasinghe ASD, Kalansuriya P, Attanayake AP. Nanoformulation of plant-based natural products for type 2 diabetes mellitus: From formulation design to therapeutic applications. *Curr Ther Res Clin Exp* (2022) 96:100672. doi: 10.1016/J.CURTHERES.2022.100672

128. Wang F, Zhang Y, Yu T, He J, Cui J, Wang J, et al. Oat globulin peptides regulate antidiabetic drug targets and glucose transporters in caco-2 cells. *J Funct Foods* (2018) 42:12–20. doi: 10.1016/J.JFF.2017.12.061

129. Su L, Zhou F, Yu M, Ge R, He J, Zhang B, et al. Solid lipid nanoparticles enhance the resistance of oat-derived peptides that inhibit dipeptidyl peptidase IV in simulated gastrointestinal fluids. *J Funct Foods* (2020) 65:103773. doi: 10.1016/J.JFF.2019.103773

130. Garcia Campoy AH, Pérez Gutiérrez RM, Manriquez-Alvirde G, Muñiz Ramirez A. Protection of silver nanoparticles using eysenhardtia polystachya in peroxide-induced pancreatic β-cell damage and their antidiabetic properties in zebrafish. *Int J Nanomed* (2018) 13:2601. doi: 10.2147/IJN.S163714

131. Odei-Addo F, Shegokar R, Müller RH, Levendal R-A, Frost C. Nanoformulation of leonotis leonurus to improve its bioavailability as a potential antidiabetic drug. *3 Biotech* (2017) 7:344. doi: 10.1007/S13205-017-0986-0

132. Ashcroft FM, Proks P, Smith PA, Ämmälä C, Bokvist K, Rorsman P. Stimulus-secretion coupling in pancreatic beta cells. *J Cell Biochem* (1994) 55 Suppl:54–65. doi: 10.1002/JCB.240550007

133. Schmidt S, Jakab M, Jav S, Streif D, Pitschmann A, Zehl M, et al. Extracts from leonurus sibiricus l. increase insulin secretion and proliferation of rat INS-1E insulinoma cells. *J Ethnopharmacol* (2013) 150:85–94. doi: 10.1016/J.JEP.2013.08.013

134. Jin X, Zhang ZH, Li SL, Sun E, Tan XB, Song J, et al. A nanostructured liquid crystalline formulation of 20(S)-protopanaxadiol with improved oral absorption. *Fitoterapia* (2013) 84:64–71. doi: 10.1016/J.FITOTE.2012.09.013

135. Paul D, Dey TK, Mukherjee S, Ghosh M, Dhar P. Comparative prophylactic effects of α-eleostearic acid rich nano and conventional emulsions in induced diabetic rats. *J Food Sci Technol* (2014) 51:1724. doi: 10.1007/S13197-014-1257-2

136. Saha SS, Ghosh M. Ameliorative role of conjugated linolenic acid isomers against oxidative DNA damage induced by sodium arsenite in rat model. *Food Chem Toxicol* (2010) 48:3398–405. doi: 10.1016/J.FCT.2010.09.011

137. Dhar P, Ghosh S, Bhattacharyya DK. Dietary effects of conjugated octadecatrienoic fatty acid (9 cis, 11 trans, 13 trans) levels on blood lipids and nonenzymatic *in vitro* lipid peroxidation in rats. *Lipids* (1999) 34:109–14. doi: 10.1007/S11745-999-0343-2

138. Bharti SK, Krishnan S, Kumar A, Kumar A. Antidiabetic phytoconstituents and their mode of action on metabolic pathways. *Ther Adv Endocrinol Metab* (2018) 9:81–100. doi: 10.1177/2042018818755019

139. Alam S, Sarker MMR, Sultana TN, Chowdhury MNR, Rashid MA, Chaity NI, et al. Antidiabetic phytochemicals from medicinal plants: Prospective candidates for new drug discovery and development. *Front Endocrinol (Lausanne)* (2022) 13:800714. doi: 10.3389/FENDO.2022.800714

140. Paul RK, Ahmad I, Patel H, Kumar V, Raza K. Phytochemicals from amberboa ramosa as potential DPP-IV inhibitors for the management of type-II diabetes mellitus: Inferences from in-silico investigations. *J Mol Struct* (2023) 1271:134045. doi: 10.1016/J.MOLSTRUC.2022.134045

141. Mohanty I, Kumar C, Borde M. Antidiabetic activity of commiphora mukul and phyllanthus emblica and computational analysis for the identification of active principles with dipeptidyl peptidase IV inhibitory activity. *Indian J Pharmacol* (2021) 53:384–7. doi: 10.4103/IJP.IJP_69_19

142. Ansari P, Choudhury ST, Abdel-Wahab YHA. Insulin secretory actions of ethanol extract of eucalyptus citriodora leaf, including plasma DPP-IV and GLP-1 levels in high-Fat-Fed rats, as well as characterization of biologically effective phytoconstituents. *Metabolites* (2022) 12:757. doi: 10.3390/METABO12080757

143. Ansari P, Hannan JA, Abdel-Wahab YHA, Flatt PR. Antidiabetic and insulinotropic properties of bark of heritiera fomes: inhibits starch digestion, protein glycation, DPP-IV activity, and glucose absorption in gut. *Planta Med* (2021) 87:YRW9. doi: 10.1055/S-0041-1736789

144. Milugo TK, Omosa LK, Ochanda JO, Owuor BO, Wamunyokoli FA, Oyugi JO, et al. Antagonistic effect of alkaloids and saponins on bioactivity in the quinine tree (Rauvolfia caffra sond.): further evidence to support biotechnology in traditional medicinal plants. *BMC Complement Altern Med* (2013) 13. doi: 10.1186/1472-6882-13-285

145. Joshi T, Deepa PR, Sharma PK. Effect of different proportions of phenolics on antioxidant potential: Pointers for bioactive Synergy/Antagonism in foods and nutraceuticals. *Proc Natl Acad Sci India Sect B Biol Sci* (2022). doi: 10.1007/S40011-022-01396-6

146. Roell KR, Reif DM, Motsinger-Reif AA. An introduction to terminology and methodology of chemical synergy-perspectives from across disciplines. *Front Pharmacol* (2017) 8:158/BIBTEX. doi: 10.3389/FPHAR.2017.00158/BIBTEX

147. Ilyasov I, Beloborodov V, Antonov D, Dubrovskaya A, Terekhov R, Zhevlakova A, et al. Flavonoids with glutathione antioxidant synergy: Influence of free radicals inflow. *Antioxidants* (2020) 9:1–20. doi: 10.3390/ANTIOX9080695

148. Rizvanov AA, Kumar B, Padma S, Editors G. *Lecture notes in bioengineering. advances in biomedical engineering and technology select proceedings of ICBEST* (2018). Available at: http://www.springer.com/series/11564 (Accessed September 30, 2022).

149. Gajarmal DA, Kumar Rath D. Review on promotions of ayurveda product, bgr-34 through multimedia. *J Drug Res* (2016) 5:16–29.

150. Gupta BP, Sharma I, Kohli N, Sharma S, Rathi A, Sharma AK. Preliminary clinical assessment and non- toxicity evaluation of an ayurvedic formulation BGR-34 in NIDDM. *J Tradit Complement Med* (2018) 8:506. doi: 10.1016/J.JTCME.2017.11.004

151. Hall WL, Vafeiadou K, Hallund J, Bugel S, Reimann M, Koebnick C, et al. Soy-isoflavone-enriched foods and markers of lipid and glucose metabolism in postmenopausal women: interactions with genotype and equol production. *Am J Clin Nutr* (2006) 83:592–600. doi: 10.1093/AJCN.83.3.592

152. Liu YJ, Zhan J, Liu XL, Wang Y, Ji J, He QQ. Dietary flavonoids intake and risk of type 2 diabetes: A meta-analysis of prospective cohort studies. *Clin Nutr* (2014) 33:59–63. doi: 10.1016/J.CLNU.2013.03.011

153. Fukino Y, Shimbo M, Aoki N, Okubo T, Iso H. Randomized controlled trial for an effect of green tea consumption on insulin resistance and inflammation markers. *J Nutr Sci Vitaminol (Tokyo)* (2005) 51:335–42. doi: 10.3177/JNSV.51.335

154. Liu CY, Huang CJ, Huang LH, Chen IJ, Chiu JP, Hsu CH. Effects of green tea extract on insulin resistance and glucagon-like peptide 1 in patients with type 2 diabetes and lipid abnormalities: A randomized, double-blinded, and placebo-controlled trial. *PloS One* (2014) 9:e91163. doi: 10.1371/JOURNAL.PONE.0091163

155. Curtis PJ, Dhatariya K, Sampson M, Kroon PA, Potter J, Cassidy A. Chronic ingestion of flavan-3-ols and isoflavones improves insulin sensitivity and lipoprotein status and attenuates estimated 10-year CVD risk in medicated postmenopausal women with type 2 DiabetesA 1-year, double-blind, randomized, controlled trial. *Diabetes Care* (2012) 35:226–32. doi: 10.2337/DC11-1443

156. Shrime MG, Bauer SR, McDonald AC, Chowdhury NH, Coltart CEM, Ding EL. Flavonoid-rich cocoa consumption affects multiple cardiovascular risk factors in a meta-analysis of short-term studies. *J Nutr* (2011) 141:1982–8. doi: 10.3945/JN.111.145482

157. Hooper L, Kay C, Abdelhamid A, Kroon PA, Cohn JS, Rimm EB, et al. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. *Am J Clin Nutr* (2012) 95:740–51. doi: 10.3945/AJCN.111.023457

158. Ock KC, Sang JC, Song WO. Estimated dietary flavonoid intake and major food sources of U.S. adults. *J Nutr* (2007) 137:1244–52. doi: 10.1093/JN/137.5.1244

159. Wedick NM, Pan A, Cassidy A, Rimm EB, Sampson L, Rosner B, et al. Dietary flavonoid intakes and risk of type 2 diabetes in US men and women. *Am J Clin Nutr* (2012) 95:925–33. doi: 10.3945/AJCN.111.028894

160. Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, et al. Flavonoid intake and risk of chronic diseases. *Am J Clin Nutr* (2002) 76:560–8. doi: 10.1093/AJCN/76.3.560

161. Lachance H, Wetzel S, Kumar K, Waldmann H. Charting, navigating, and populating natural product chemical space for drug discovery. *J Med Chem* (2012) 55:5989–6001. doi: 10.1021/JM300288G

162. Atanasov AG, Zotchev SB, Dirsch VM, Orhan IE, Banach M, Rollinger JM, et al. Natural products in drug discovery: advances and opportunities. *Nat Rev Drug Discovery* (2021) 20:200–16. doi: 10.1038/s41573-020-00114-z

163. Feher M, Schmidt JM. Property distributions: differences between drugs, natural products, and molecules from combinatorial chemistry. *J Chem Inf Comput Sci* (2003) 43:218–27. doi: 10.1021/CI0200467

164. Bucar F, Wube A, Schmid M. Natural product isolation–how to get from biological material to pure compounds. *Nat Prod Rep* (2013) 30:525–45. doi: 10.1039/C3NP20106F

165. Delazar A, Nahar L, Hamedeyazdan S, Sarker SD. Microwave-assisted extraction in natural products isolation. *Methods Mol Biol* (2012) 864:89–115. doi: 10.1007/978-1-61779-624-1_5

166. Sarker SD, Nahar L. An introduction to natural products isolation. *Methods Mol Biol* (2012) 864:1–25. doi: 10.1007/978-1-61779-624-1_1

167. da Silva Haas IC, de Espindola JS, de Liz GR, Luna AS, Bordignon-Luiz MT, Prudêncio ES, et al. Gravitational assisted three-stage block freeze concentration process for producing enriched concentrated orange juice (Citrus sinensis l.): Multi-elemental profiling and polyphenolic bioactives. *J Food Eng* (2022) 315:110802. doi: 10.1016/J.JFOODENG.2021.110802

168. Meneses DL, Ruiz Y, Hernandez E, Moreno FL. Multi-stage block freeze-concentration of green tea (Camellia sinensis) extract. *J Food Eng* (2021) 293:110381. doi: 10.1016/J.JFOODENG.2020.110381

169. Orellana-Palma P, Guerra-Valle M, Gianelli MP, Petzold G. Evaluation of freeze crystallization on pomegranate juice quality in comparison with

conventional thermal processing. *Food Biosci* (2021) 41. doi: 10.1016/J.FBIO.2021.101106

170. Kumar D, Ladaniya MS, Gurjar M, Kumar S. Impact of drying methods on natural antioxidants, phenols and flavanones of immature dropped citrus sinensis l. osbeck fruits. *Sci Rep* (2022) 12:1–12. doi: 10.1038/s41598-022-10661-7

171. Zhang F, Zhang J, Di H, Xia P, Zhang C, Wang Z, et al. Effect of long-term frozen storage on health-promoting compounds and antioxidant capacity in baby mustard. *Front Nutr* (2021) 8:665482/BIBTEX. doi: 10.3389/FNUT.2021.665482/BIBTEX

172. Pinzi L, Rastelli G. Molecular docking: Shifting paradigms in drug discovery. *Int J Mol Sci* (2019) 20. doi: 10.3390/IJMS20184331

173. Dewanjee S, Chakraborty P, Mukherjee B, de Feo V. Plant-based antidiabetic nanoformulations: The emerging paradigm for effective therapy. *Int J Mol Sci* (2020) 21. doi: 10.3390/IJMS21062217

174. Ganesan P, Ramalingam P, Karthivashan G, Ko YT, Choi DK. Recent developments in solid lipid nanoparticle and surface-modified solid lipid nanoparticle delivery systems for oral delivery of phyto-bioactive compounds in various chronic diseases. *Int J Nanomed* (2018) 13:1569–83. doi: 10.2147/IJN.S155593

175. Saratale GD, Saratale RG, Benelli G, Kumar G, Pugazhendhi A, Kim DS, et al. Anti-diabetic potential of silver nanoparticles synthesized with argyreia nervosa leaf extract high synergistic antibacterial activity with standard antibiotics against foodborne bacteria. *J Clust Sci* (2017) 28:1709–27. doi: 10.1007/S10876-017-1179-Z

176. Chowdhury A, Kunjiappan S, Bhattacharjee C, Somasundaram B, Panneerselvam T. Biogenic synthesis of marsilea quadrifolia gold nanoparticles: a study of improved glucose utilization efficiency on 3T3-L1 adipocytes. *In Vitro Cell Dev Biol Anim* (2017) 53:483–93. doi: 10.1007/S11626-017-0136-3

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 04 January 2023
DOI 10.3389/fendo.2022.1092837

OPEN ACCESS

EDITED BY
Luna Samanta,
Ravenshaw University, India

REVIEWED BY
Jelena Djordjevic,
Faculty of Biology, University of Belgrade, Serbia
Aleksandra Klisic,
Primary Health Care Center Podgorica, Montenegro
Mohamed A. Haidara,
Cairo University, Egypt

*CORRESPONDENCE
Mirjana T. Macvanin
mirjana.macvanin@vin.bg.ac.rs
Esma R. Isenovic
isenovic@yahoo.com

SPECIALTY SECTION
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 08 November 2022
ACCEPTED 12 December 2022
PUBLISHED 04 January 2023

CITATION
Macvanin MT, Gluvic Z, Zafirovic S, Gao X, Essack M and Isenovic ER (2023) The protective role of nutritional antioxidants against oxidative stress in thyroid disorders. *Front. Endocrinol.* 13:1092837. doi: 10.3389/fendo.2022.1092837

The protective role of nutritional antioxidants against oxidative stress in thyroid disorders

Mirjana T. Macvanin[1]*, Zoran Gluvic[2], Sonja Zafirovic[1], Xin Gao[3,4], Magbubah Essack[3,4] and Esma R. Isenovic[1]*

[1]Department of Radiobiology and Molecular Genetics, VINČA Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia, [2]Clinic for Internal Medicine, Department of Endocrinology and Diabetes, Zemun Clinical Hospital, School of Medicine, University of Belgrade, Belgrade, Serbia, [3]Computational Bioscience Research Center (CBRC), King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia, [4]Computer Science Program, Computer, Electrical and Mathematical Sciences and Engineering Division (CEMSE), King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia

An imbalance between pro-oxidative and antioxidative cellular mechanisms is oxidative stress (OxS) which may be systemic or organ-specific. Although OxS is a consequence of normal body and organ physiology, severely impaired oxidative homeostasis results in DNA hydroxylation, protein denaturation, lipid peroxidation, and apoptosis, ultimately compromising cells' function and viability. The thyroid gland is an organ that exhibits both oxidative and antioxidative processes. In terms of OxS severity, the thyroid gland's response could be physiological (i.e. hormone production and secretion) or pathological (i.e. development of diseases, such as goitre, thyroid cancer, or thyroiditis). Protective nutritional antioxidants may benefit defensive antioxidative systems in resolving pro-oxidative dominance and redox imbalance, preventing or delaying chronic thyroid diseases. This review provides information on nutritional antioxidants and their protective roles against impaired redox homeostasis in various thyroid pathologies. We also review novel findings related to the connection between the thyroid gland and gut microbiome and analyze the effects of probiotics with antioxidant properties on thyroid diseases.

KEYWORDS

oxidative stress, reactive oxygen species, thyroid disease, nutritional antioxidants, thyroid-gut axis, gut microbiome, antioxidant probiotic

1 Introduction

Cellular redox homeostasis depends on a dynamic equilibrium between prooxidant production and its elimination. Reactive oxygen species (ROS), together with reactive nitrogen species (RNS), represent the most important prooxidants whose excessive accumulation leads to oxidative stress (OxS) and molecular damage (1, 2). ROS are molecules with an oxygen atom, and unpaired electrons are primarily generated as by-products of ATP synthesis in mitochondrial respiratory chains (1) or during inflammation (3, 4). The concentration of ROS determines their physiological role (5). When present at low concentrations, ROS are involved in signaling processes essential for normal cellular functions (6, 7), whereas high ROS concentration leads to DNA, lipid, and protein damage and apoptosis (8).

ROS are crucial in thyroid function because they are essential in the initial stages of thyroid hormone synthesis during iodide oxidation (9). Also, the process whereby thyroid peroxidase (TPO) catalyzes thyroxine (T4) and triiodothyronine (T3) during its synthesis in thyroid follicles involves ROS (10). In addition, thyroid hormones affect the mitochondrial activity and modulate ROS production (10). The dependence of normal thyroid function on ROS implies that the thyroid is continuously exposed to ROS and, thus, particularly sensitive to oxidative damage (10). Therefore, to protect the integrity of the thyroid, it is mandatory that the thyroid antioxidant defence system effectively regulates and balances ROS production and elimination (11, 12).

Aerobic organisms have evolved multiple antioxidant and repair systems for protection against OxS. Enzymes that decompose ROS, such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) provide the primary antioxidant defence (5, 7, 8, 13). In contrast, ROS-induced damage repair systems eliminate damaged cells through autophagy and apoptosis processes (14, 15). However, the capacity of intrinsic antioxidant systems is not always sufficient to prevent damage caused by excessive accumulation of ROS. Thus, non-enzymatic mechanisms based on the action of molecules with antioxidant properties such as glutathione (GSH), thioredoxin, coenzyme Q10, and exogenous antioxidants, including various polyphenolic compounds, ascorbic acid, tocopherol retinol, and β-carotene, that may also support antioxidant systems are essential. The use of nutritional antioxidants as supplementary substances that delay and/or prevent the oxidation of cellular components has shown the potential to protect human organs, including the thyroid gland, against oxidative damage by reinforcing the body's antioxidant defence and increasing total antioxidant capacity (5, 16, 17).

Recently, a search for natural nutritional antioxidants from biological resources has gained substantial attention. Of particular interest are probiotics which represent live non-pathogenic microorganisms that can restore microbial balance in the gastrointestinal tract upon appropriate administration (18). Evidence demonstrates that probiotic bacteria exert significant antioxidant effects *in vitro* and *in vivo* (19–22), and the connection between the thyroid gland and gut microbiome is well-established. Furthermore, it has been documented that dysbiosis, an imbalance in gut microbiota, is associated with impaired thyroid function and pathogenesis of thyroid disorders such as Hashimoto's and Graves' disease (23). In this review, we discuss the protective role of exogenous nutritional antioxidants in the context of various thyroid disorders. We also review novel findings related to the connection between the thyroid gland and gut microbiome and analyze the effects of probiotics with antioxidant properties on thyroid diseases.

2 Search strategy

We searched MEDLINE and PubMed for all English and non-English articles with English abstracts published between 1977 and 2022. The leading search terms were: oxidative stress, reactive oxygen species, thyroid disease, nutritional antioxidants, thyroid-gut axis, gut microbiome, and antioxidant probiotics. The search retrieved original peer-reviewed research articles, which were further analyzed, focusing on the role of nutritional antioxidants in thyroid diseases. We specifically focused on including the most recent findings published in the past five years.

3 Oxidative stress

Oxidative stress is a disbalance caused by excessive production of prooxidant substances such as ROS and RNS and/or the antioxidant systems working inefficiently (14, 24, 25). ROS include superoxide anion, hydroxyl radical, and hydrogen peroxide, which are produced *in vivo* primarily by the mitochondrial respiratory chain during aerobic metabolism (26). RNS family includes peroxynitrite, generated *via* a reaction between nitric oxide (NO) and superoxide, and nitrosoperoxycarbonate, generated *via* a reaction between peroxynitrite and carbon dioxide. Under physiological conditions, ROS plays a vital role in maintaining cellular homeostasis by regulating the endogenous antioxidant pool (27–30) and participating in host defence and hormone synthesis (31, 32). In thyrocytes, ROS production is essential for their functional role (33) since TPO-mediated hormone synthesis depends on the action of dual oxidases (DUOX), enzymes responsible for H_2O_2 production (34). However, when ROS and RNS are present in excessive amounts and/or in the form of highly reactive free radicals such as superoxide anion and hydroxyl radical, they oxidize susceptible

biomolecules such as membrane lipids, cellular proteins, and nucleic acids, leading to disruption of normal cellular functions (35). Lipid peroxidation is a process in which oxidants such as free radicals or non-radical species attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids (PUFAs) (36). The main lipid peroxidation products are hydroperoxides, such as propanal, hexanal, 4-hydroxynonenal, and malondialdehyde (MDA) (37). Phospholipids, cholesterol, and glycolipids are also targets of potentially lethal peroxidative modifications (38). ROS can also cause damage to DNA by oxidizing nucleoside bases (39). For example, guanine oxidation produces 8-oxo guanine (8-oxoG), which may lead to G-T or G-A transversions if unrepaired. The guanine and deoxyguanosine oxidation products 8-oxoG and its nucleotide 8-oxo-2′-deoxyguanosine (8-oxodG) are ROS-mediated DNA lesions considered the most significant biomarkers for oxidative DNA damage (40). Oxidized bases are usually recognized and repaired by the base excision pathway (BER). Still, when they co-occur on opposing strands, BER can lead to the generation of double-stranded DNA breaks (41). ROS accumulation also induces mitochondrial DNA lesions, strand breaks, and DNA degradation (42). In addition, increased ROS levels are responsible for protein oxidation that can rapidly contribute to the augmentation of OxS by directly affecting cell structure, cell signaling, and essential enzymatic metabolic processes. Several modes of ROS-mediated protein oxidation are reported, including metal-catalyzed oxidation, oxidation-induced cleavage, amino acid oxidation, and the conjugation of lipid peroxidation products (43).

Excessive ROS accumulation is an important factor in the pathogenesis of different diseases. For instance, an elevated ROS production by the respiratory chain is observed in obesity as a response to metabolic overload caused by excess macronutrients and increased substrate availability (44). Mitochondrial dysfunction and endothelial reticulum stress contribute to metabolic perturbances in the adipose tissue of obese patients (45). Consequent ROS accumulation leads to cell damage and pathogenesis of inflammatory and cardiovascular diseases (46). Furthermore, mitochondrial ROS acts as signaling molecules mediating pro-inflammatory cytokines' production, further reinforcing the connection between OxS and inflammation (47).

Several enzymatic and non-enzymatic defence mechanisms that guard cells against free radical damage have been identified in different cellular localizations, including mitochondria, plasma membrane, endoplasmic reticulum, peroxisomes, and cytosol. For example, enzymes SOD, Cat, and GPx, and transition-metal binding proteins, such as transferrin, ferritin, and ceruloplasmin, inactivate free radicals (48). Three forms of SOD are known in mammals: cytoplasmic SOD (SOD1), mitochondrial SOD (SOD2), and extracellular SOD (SOD3) (49). SOD belongs to a group of metalloenzymes that catalyzes the dismutation of superoxide anion to hydrogen peroxide and molecular oxygen, while Cat decomposes hydrogen peroxide to water and molecular oxygen (50). In high H_2O_2 levels, GPx also participates in detoxification by converting lipid peroxides to the corresponding alcohols. Hydrosoluble molecules with free radical scavenging properties such as ascorbic acid, albumin, bilirubin, urates and thiols, liposoluble coenzyme Q10, and vitamin E interfere with the lipid peroxidation by neutralizing the free radicals. In particular, liposoluble scavengers in cellular membranes have high diffusion rates, enabling them to abolish the radical chain reactions by immediately converting them into more stable and less reactive molecules (46). Additional defence mechanisms that reconstruct damaged molecules involve using specific phospholipase that removes peroxidized fatty acids, allowing the reacylation of damaged molecules (51, 52).

4 General overview of thyroid diseases

Thyroid hormones have a considerable impact on the cellular oxidative stress processes which is ascribed to their role in cellular metabolism and oxygen consumption (53). Thyroid hormones are produced by thyroid gland, released into circulation, and transported to all organs and cells where they exert their effect. An important role in production of thyroid hormones has hypothalamic-pituitary-thyroid axis. Hypothalamus production of thyrotropin-releasing hormone (TRH) stimulates anterior pituitary gland to secrete thyroid-stimulating hormone (TSH), which affects thyroid gland and leads to production of thyroid hormones. Thyroid gland mainly produces T4, a prohormone which needs to convert to T3 to become biologically active. T4 comprises about 80% of secreted thyroid hormones, while the other 20% is T3. Increased plasma values of thyroid hormones in circulation activate negative feedback loop and inhibit release of TSH (54, 55).

Thyroid hormones exhibit profound metabolic effects characterized by an increased rate of both catabolic and anabolic reactions, resulting in an overall acceleration of the basal metabolism which is associated with increased oxygen consumption, respiratory rate, energy expenditure, and heat production (56). In addition, altered thyroid hormones levels may cause changes in the number and activity of mitochondrial respiratory chain components which represent the principal cellular site of ROS production, ultimately leading to changes in the cellular redox environment and increased ROS generation (57, 58). For instance, it has been reported that hypothyroidism-induced dysfunction of the mitochondrial respiratory chain is associated with increased production of free radicals (59) Thus, excess TSH in hypothyroidism may modulate oxidative stress processes (60) by augmenting the accumulation of ROS that result from both increased generation of free radicals and diminished capacity of the antioxidative defense systems.

Hypothyroidism is associated with an increased risk of atherosclerosis due to its metabolic effects (61, 62). It is commonly accompanied by hyperlipidemia which results from a disbalance between the rates of fatty acids' synthesis and degradation and is characterized by elevated total cholesterol and low-density lipoprotein-cholesterol (LDL-C), thus providing the substrate for ROS-mediated lipid peroxidation (63–66). Interestingly, products of lipid peroxidation may further increase overall cellular oxidative stress by facilitating the generation of free radicals through the formation of adducts with proteins, which increases direct free radical-induced protein oxidation (67).

In thyroid diseases, metabolic disorders associated with low-grade inflammation can also lead to an increased oxidative stress (68). For instance, chronic low-grade inflammation observed in Hashimoto's thyroiditis causes endothelial dysfunction which represents an early step in the development of atherosclerosis. Endothelial dysfunction is characterized by the reduction of bioavailability of NO, resulting in impaired endothelium-dependent vasodilation (69) and increased oxidative stress (70). However, it should be mentioned that there is still no consensus in the literature regarding the connection between hypothyroidism and oxidative stress. Some studies report increased oxidative stress in hypothyroidism while other suggest that hypometabolic state that is prevalent in hypothyroidism may protects tissues from oxidative damage.

Thyroid diseases are considered the most commonly reported endocrine diseases in clinical practice, followed by lipid and carbohydrate disorders (71–73). The primary thyroid condition that affects thyroid functionality presents as hyperthyroidism or hypothyroidism (Figure 1.). Thyroid dysfunction could manifest fully (clinically) or latently (subclinically). Based on duration, thyroid dysfunction could be persistent or transitional (73–75). The natural history of thyroid disorders can negatively affect the morphology and function of target tissues if left untreated or improperly treated (55, 73, 76). Thus, the leading causes of death in patients with thyroid dysfunctions are the consequences of atherosclerosis acceleration and the worsening of pre-existing cardiovascular and central nervous system diseases (77, 78). Such endpoints depend on pronounced OxS and diminished antioxidant defense systems at the molecular level (79–81).

5 Thyroid diseases and OxS

Different thyroidopathies have been shown to cause increased ROS production and evident OxS-induced damage to thyroid cells. This relationship is reciprocal since thyroid conditions can worsen OxS and increase ROS production, exacerbating oxidative damage. Thyroid hormones increase ROS release in the mitochondrial respiratory chain (25, 82, 83). Hypothyroidism contributes to OxS through an inefficient antioxidant defence system, opposite to hyperthyroidism, where increased ROS production promotes OxS and oxidative damage of thyroid cells (Figure 1) (46). According to published research,

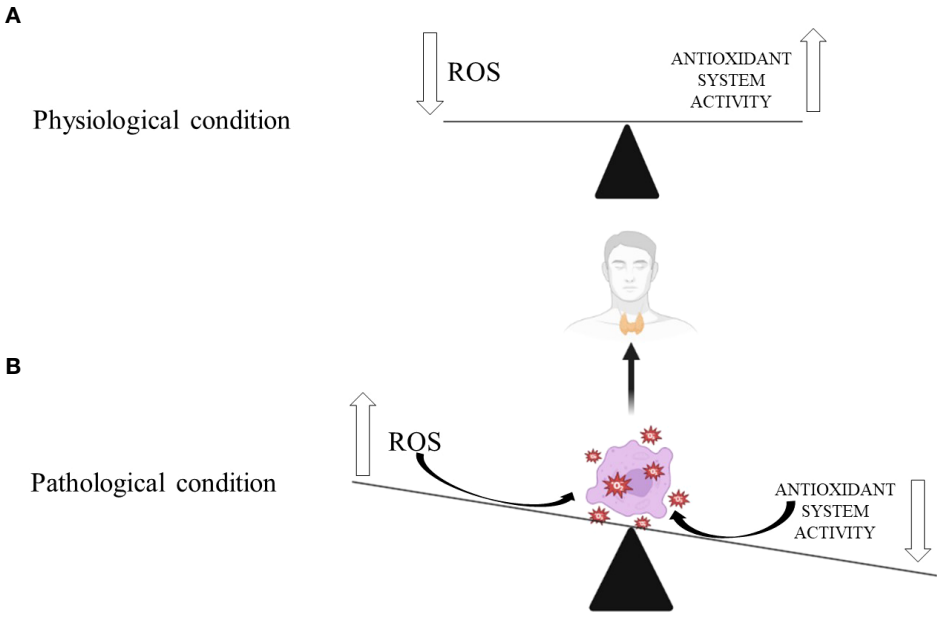


FIGURE 1
Thyroid gland's response to oxidative stress in: **(A)** physiological and **(B)** pathological conditions. Biorender.com was used to generate part of the Figure.

preventive dietary antioxidant therapy may partially correct the redox imbalance, making it a viable method for preventing the onset of many chronic thyroidopathies (25).

5.1 Thyroid dysfunctions and OxS

5.1.1 Hypothyroidism

Even in its subclinical form, hypothyroidism reduces antioxidant system activity, which promotes OxS, causing oxidative damage and altered lipid metabolism in thyroid cells (25, 46, 84). MDA, a by-product of ROS-induced lipid peroxidation, has also been found in higher serum concentrations in hypothyroid patients (79). Even though levothyroxine considerably reduces lipid peroxidation, the serum MDA levels never reach the levels seen in healthy individuals (85). In addition, accumulated oxygen free radicals in thyroid cells may inhibit TPO function and interfere with the synthesis and secretion of thyroid hormones, causing hypothyroidism (34, 86).

5.1.2 Hyperthyroidism

ROS generation is increased by hyperthyroidism (87). The increased intracellular ATP consumption, increased tissue oxygen consumption and oxidative phosphorylation, overexpression of adrenergic receptors, and a decrease in antioxidant defensive mechanisms are the mechanisms of free radicals overproduction that favour OxS in hyperthyroid patients (46). In addition, patients with hyperthyroidism have increased rates of lipid peroxidation compared to euthyroid people, which is a sign of oxidative damage to membrane lipids (82). The link between hyperthyroidism and deteriorating OxS is suggested by the positive association between thyroid hormones and MDA, TSH, and GSH (83).

5.2 Thyroid disorders and OxS

5.2.1 Nodular goitre

OxS promotes thyroid cell proliferation (88, 89). Elevated MDA levels were observed in tissues collected from patients with toxic and non-toxic multinodular goitre, accompanied by reduced activity of SOD, GPx, and selenium content compared to adjacent, healthy thyroid tissue. Tissues of benign thyroid nodules show significantly reduced total antioxidant status (TAS) and reduced oxidative stress index (OSI) (90). The presence of elevated OxS parameters in toxic multinodular goitre and decreased plasma GPx and GR activities were also demonstrated (91). These findings suggest an impaired redox balance and antioxidant defence in patients with toxic thyroid nodules and nodular goitre.

Additionally, rare loss-of-function germline mutations of Kelch-like ECH-associated protein 1 (KEAP1) could be detected in nodular goitre leading to Nrf2 pathway activation that favours transcription of cytoprotective and antioxidant enzymes (92). The thyroid nodule size may change in both directions over time. The decrease in the size of thyroid nodules may result from supplementation with extracts of plants with antioxidant and anti-inflammatory properties (93).

5.2.2 Autoimmune thyroid diseases

5.2.2.1 Hashimoto thyroiditis

By interacting with TPO and thyroglobulin (TG) and promoting immunogenicity by altering their morphology and function, NADPH-oxidases (NOXs) involvement in the production of hydrogen peroxide (H_2O_2) regarding thyroid hormone synthesis may be related to the pathophysiology of AITD (86, 94, 95). More specifically, it has been demonstrated that an increase in ROS encourages the cleavage of TG into smaller fragments, which exposes the immune system to novel epitopes and intensifies the autoimmune response (96). OxS indicators are significantly higher when Hashimoto thyroiditis is associated with thyroid dysfunction. According to certain studies, the markers of worsened OxS in patients with Hashimoto thyroiditis were closely related to the levels of TG or TPO antibodies (25, 97–99).

Because it increases ROS production and lowers antioxidant levels, excessive iodine consumption is considered an additional risk factor for developing AITD. In people with Hashimoto thyroiditis, anti-TPO antibodies depend on GSH levels and exhibit an inverse correlation (89, 100). Additionally, there is a favourable association between total oxidative status (TOS) and OSI and both antibodies (anti-TG and anti-TPO). Reduced GSH levels seem to be a decisive factor in OxS activation and the development of Hashimoto thyroiditis (101, 102). Additionally, it has been demonstrated that elevated TOS and OSI parameters may precede the development of hypothyroidism in autoimmune thyroiditis and may serve as indicators of thyroid cell injury (101–103). Areas with lower-selenium soil have been linked to increased Hashimoto thyroiditis in humans (104). Also, genetic interactions between minor alleles in the selenoprotein S gene (*SELENOS*) and the nuclear factor erythroid 2-related factor 2 gene (*NFE2L2*) increase chronic thyroiditis incidences (105).

5.2.2.2 Graves' disease

The most typical cause of hyperthyroidism is Graves' disease (GD). Its natural history appears to be heavily influenced by oxidative DNA damage. Untreated GD sufferers were shown to have much more DNA damage than patients with toxic nodular goitre and healthy people (106). The highest level of OxS markers was recorded in hyperthyroid GD patients, especially ones with relapsing disease (107, 108). Although both thiamazole and propylthiouracil effectively restore ROS and the antioxidative defence systems, some authors evidenced

propylthiouracil as more efficacious (109). The unique mechanism of how OxS leads to GD is disrupting self-tolerance. The thyroid-stimulating antibodies (TSAb) present in GD are engaged in oxidation processes. As the markers of OxS show a positive correlation with TSAb, it may indicate that these variables may be involved in the breakdown of redox balance (110). In patients with GD, activating the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway may help restore thyroid function (105).

5.2.3 Thyroid cancer

Increased production of ROS has been shown to favour cancer development (111). However, ROS can also trigger cell senescence and death, acting as an anti-tumorigenic agent (112). Disturbed genomic integrity induces oxidative genetic damage, DNA oxidation, the activation of proto-oncogenes, and the inactivation tumour suppressor genes leading to proliferative effects and mutagenesis (12, 113, 114). According to Krohn et al., DNA damage, a precursor to tumorigenesis, is thought to be caused by OxS (115). Resultant oxidative DNA base lesions have the potential to mutate some genetic material, which would impair the integrity of the genome by preventing transcription and replication and by generating mutagenesis (116). The oxidized form of guanine, 8-oxo-2′-deoxyguanosine (8-oxo-dG), is a valuable marker of oxidative DNA damage during carcinogenesis (116, 117). When compared to matched normal thyroid tissue, both benign (human follicular adenomas, or FTAs) and malignant (follicular (FTC) and papillary thyroid carcinoma (PTC)) lesions were found to have elevated nuclear levels of 8-oxo-dG (118) which most likely reflects the detrimental effects of prolonged exposure to chronic OxS seen during thyroid cancer (113, 118).

According to an analysis of the redox balance, sera antioxidant levels were lower in thyroid cancer patients than in healthy controls, and OxS marker sera levels in thyroid cancer patients were significantly higher than in the control samples (119). In addition, high concentrations of MDA in blood were detected in thyroid cancer patients, which unequivocally indicated reduced blood antioxidative capacity (120, 121).

A disturbed balance between serum OxS and antioxidant defence system markers is typically encountered in thyroid cancer patients compared to healthy individuals (119, 120, 122, 123). The ineffective defence mechanism cannot neutralize ROS overproduction in thyroid cancer cells, leading to OxS (122). A significant difference in GPx activity and MDA levels was seen between the thyroid cancer patients before and after thyroidectomy in a study examining the change in OxS markers. Although thyroidectomy dramatically improved the oxidative status in favour of antioxidants, lipid peroxidation levels remained much more significant than in healthy thyroid people (25, 121, 124). Also, PTC patients exhibit a worse oxidative profile than patients with autoimmune thyroid disease and higher oxidative process rates than healthy individuals (125). Moreover, thyroid cancer risk was observed to be higher in obese people, and female patients with type 2 diabetes mellitus (T2DM) are more likely to have the extra-glandular invasion of PTC than male T2DM patients (126, 127).

In PTC and anaplastic thyroid carcinoma, somatic *KEAP1* and *NFE2L2* mutations activating the Nrf2 pathway were discovered (128, 129). Although the significance of such pathway activation in thyroid tumours is still unclear, it may help cancer cells survive (105, 130). In addition, tumour tissue exhibit a higher quantity of ROS, which was linked to the decreased expression of selenium antioxidant proteins in cancer cells compared to healthy cells (122). Furthermore, compared to normal thyroid tissue, antioxidant catalase expression was significantly reduced in human thyroid tumours (131). These results show oxidant/antioxidant system in thyroid cancer tissue is imbalanced (12, 132).

6 Nutrition and OxS

Proper nutritional intake is mandatory for overall well-being and better human health. However, dietary habits have an impact on human health and can lead to the development of a variety of disorders and diseases. The most prevalent diet in the world is the Western-style diet, characterized by an increased intake of refined food with a high caloric index and an increased amount of sugars and salt, while the intake of vegetables, fruits, and fish is reduced (133). Negative consequences of nutritional habits associated with a Western-style diet may lead to inflammation and production of free radicals (134) through the secretion of numerous pro-inflammatory molecules such as interleukin (IL)- 6, IL-1b, IL-8, and C-reactive protein (CRP), leading to the development of autoimmune disorders either directly, due to inflammation or disturbed immune balance, or indirectly due to increased fat depositions and the development of obesity. Obesity has the most severe consequences since it is associated with systemic inflammation, hypertension, and hypercholesterolemia, which represent conditions that increase the risk of developing cardiovascular disease and T2DM.

It is believed that the state after taking a meal (postprandial state) is pro-inflammatory and pro-oxidative, and the type of food mainly consumed affects the occurrence of OxS. As mentioned earlier, the increased intake of proteins (processed, red meats), sugars, salt, saturated and trans fat, and refined carbohydrates, which are characteristic of the Western-style diet, leads to the development of many diseases, which have their basis in the occurrence of OxS (135). Furthermore, a diet based on an increased intake of carbohydrates and fats leads to increased production of free radicals, directly affecting mitochondrial metabolism. Also, animals fed a high-fat diet have been shown to have increased OxS and dysfunctional mitochondria (136).

To reduce the development of obesity, cancer, diabetes, and cardiovascular diseases, WHO recommended a diet that includes an increased intake of fruits, vegetables, nuts, fish, and unsaturated fatty acids. This type of diet is represented in certain coastal regions of the world and has received the popular name Mediterranean diet. The natural antioxidants, as a result of proper nutritional habits, provide indirect protection by decreasing the production of cytokines and reducing OxS (134).

Numerous exogenous antioxidant molecules (nutritional antioxidants) have been shown to play an important role in excessive ROS accumulation in organisms. Here we will discuss several nutritional antioxidants with a confirmed protective antioxidant role. For instance, monounsaturated fatty acids (MUFA) such as oleic acid that are present in high amounts in olive oil and certain nuts decrease ROS production and exert protection against OxS (137). In addition, oleic acid showed anti-inflammatory effects by decreasing obesity and cytokine production and reducing cardiovascular mortality (134). The anti-inflammatory actions of MUFAs are based on their ability to counteract the effects of long-chain saturated fatty acids on hepatocytes, which include reducing endoplasmic reticulum stress, restricting lipotoxicity induced by accumulation of saturated fatty acids, decreasing ROS production, and inhibiting nuclear factor-κB (NF-kB) transcription factors by binding peroxisome proliferator-activated receptor γ (PPARγ) and G-protein coupled surface receptor 120 (GPR120) (138). *In vitro*, MUFA has shown the ability to induce the expression of the adiponectin gene *via* PPARγ activation, which would result in decreased production of pro-inflammatory molecules such as IL-6 and tumour necrosis factor (TNF)-alpha (139)

Polyunsaturated fatty acids, such as omega-3 fatty acids (n-3 PUFA), are mostly found in eggs, nuts, and fish, whereas omega-6 fatty acid (n-6 PUFA) is predominantly present in sunflower and other vegetable oils. The ratio of n-3 PUFA/n-6 PUFA is of the utmost importance since its disbalance may activate pro-inflammatory pathways (140) n-3 and n-6 PUFA exert opposite effects on the immune system, whereas n-3 PUFA have an anti-inflammatory effect while n-6 PUFA induces a pro-inflammatory action (141). The anti-inflammatory effects of n-3 PUFA are based on their ability to decrease endogenous concentrations of ROS and expression of NF-kB and promote activation of genes involved in antioxidant protection.

Resveratrol (3,4′,5-trihydroxy-trans-stilbene) is a natural polyphenol nonflavonoid compound primarily found in grapes, red wine, berries, and peanuts. It has been shown that long-term treatment with reservatrol prolongs lifespan and reduces OxS (142). In addition, resveratrol was shown to possess cardiovascular protective capacity (143–145) and exhibit antidiabetic, anti-inflammatory, and antioxidant effects (146–150), as well as the ability to suppress the proliferation of a variety of tumour cells (151, 152). Also, resveratrol positively affects obesity, reducing triglycerides and glucose levels. The anti-inflammatory effects resulting from using resveratrol can be seen in the reduction of increased levels of interleukin and TNF in obese mice (15). The antioxidant effects of resveratrol were confirmed in many studies. For example, resveratrol significantly inhibited ROS production by polymorphonuclear leukocytes treated with formyl methionyl leucyl phenylalanine (153) and reduced OxS markers like glycated albumin levels in serum and 8-hydroxyguanosine in urine in stroke-prone spontaneously hypersensitive rats (154). Due to its lipophilic nature, resveratrol can bind to lipoprotein particles, which seems crucial for its antioxidant effects (155). Resveratrol consumption increases plasma antioxidant levels and decreases lipid peroxidation (156). It also reduces intracellular ROS and prevents LDL oxidation in endothelial cells (157) by inhibiting lipoxygenases (158). The mechanism by which resveratrol prevents LDL oxidation is based on its ability to chelate copper and scavenge ROS (159).

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a natural polyphenol derived from the rhizomes of the herbs from genus *Curcuma*, particularly from *Curcuma longa* (turmeric), *Curcuma amada, Curcuma zedoaria, Curcuma aromatic* and *Curcuma raktakanta*. (160–162). It has multiple positive effects on the organism, acting as an antioxidant and decreasing inflammation. Using macrophages, Lin et al. showed that curcumin treatment increased levels of SOD and Cat while decreasing levels of ROS (163). The anti-inflammatory effect of curcumin is most likely associated with its ability to inhibit cyclooxygenase-2 (COX-2), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS) (164). In addition, curcumin positively affects body weight and glucose, increases GPx activity (165), and decreases FFA, triglycerides, and cholesterol concentrations in diabetic rats (15).

Berberine (5,6-dihydro-9,10-dimethoxybenzo [g]-1,3-benzodioxolo [5,6-a] quinolizinium) is a plant alkaloid found and derived from numerous families of plants, such as *Annonaceae, Menispermaceae, Papaveraceae, Ranunculaceae*, etc. (166). Barberine has been shown to have numerous positive effects such as decreasing cholesterol levels and reducing weight and adipose tissue in obese mice. In addition, berberine decreases obesity- and diabetes-related inflammation (167). In the atherosclerotic mouse model, berberine activating the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway decreases OxS (15, 168). Ma et al. (169) showed diabetic animal models administered berberine activates the Nrf2 pathway and decreases OxS.

It is important to mention micronutrients with antioxidant properties, such as vitamins. Since the body cannot synthesize sufficient amounts of vitamins, it is necessary to take them through food or supplements. Vitamin E acts as a regulator of cellular metabolism, and its deficiency leads to anaemia, dysregulation of energy metabolism, irregular mitochondrial function, and tissue damage resulting from increased lipid peroxidation (16). Vitamin D deficiency affects muscle function and leads to irregular cardiovascular function. Also, a lack of Vitamin D is connected with disrupted mitochondrial

function and thus increased inflammation and OxS (16). However, it should be emphasized that an increased intake of vitamins has the opposite effect, increasing OxS. For example, it is well known that vitamin C has antioxidant properties; it reacts with ROS creating a product with poor reactivity that does not have detrimental effects. On the other hand, vitamin C may undergo the Fenton reaction, in which a highly reactive free radical is formed (170).

7 Nutritional antioxidants and thyroid disease

7.1 Trace elements

7.1.1 Iodine

An average adult body contains around 15 to 20 mg of iodine located predominantly in the thyroid gland, which performs an essential role in synthesizing thyroid hormones. In addition to being a component of the thyroid hormone, iodine can act as an antioxidant and antiproliferative agent (171). Its uptake is mediated by the sodium/iodide symporter (NIS), expressed in thyroid cells and extrathyroidal tissues, including the stomach and salivary glands. The iodine content in food is determined by its amount in the soil. Since seafood and seaweed are rich sources of iodine, a diet based on high seafood consumption is sufficient. Likewise, fortifying salt and milk products with iodine ensures an adequate amount of dietary iodine (172, 173). Although iodine deficiency was associated with goitre and thyroid nodules, PTC appears to be more common in areas with high iodine intake, which points to the complex relationship between iodine intake and thyroid disease (174). For instance, excessive iodine intake is associated with a transient reduction of thyroid hormone synthesis for approximately 24 hours after ingestion, known as the Wolff-Chaikoff effect (175). In patients with autoimmune thyroid disease or on anti-thyroid drug therapy, increased iodine intake can induce hypothyroidism, whereas, in patients with diffuse nodular goitre or latent Grave's disease, it can cause hyperthyroidism (176).

7.1.2 Zinc

Zinc is regarded as an antioxidative trace element because it is a co-factor of the enzyme SOD, which scavenges free radicals. Zinc is essential for normal thyroid function since it is required for the activity of enzyme 1,5′-deiodinase which catalyzes the conversion of T4 to T3. In addition, zinc plays a vital role in the thyroid hormones' metabolism by regulating thyrotropin-releasing hormone (TRH) and TSH synthesis and modulating the structures of essential transcription factors involved in synthesizing thyroid hormones (177, 178). Zinc deficiency affects the thyroid gland by impairing TRH, TSH, T3, and T4 synthesis. In animal studies, free levels of T3 and T4 were reduced by approximately 30% (179), and a similar trend was observed in studies of human subjects. Hypothyroid patients often present with reduced levels of zinc. In a study designed to evaluate zinc metabolism in patients with thyroid disease, plasma and erythrocyte zinc concentration and urinary zinc excretion were investigated in hypo- and hyperthyroid patients (180). The mean concentration of plasma zinc in hypothyroid patients was lower than that of healthy control subjects, whereas no statistically significant differences were observed in plasma zinc values between hyperthyroid patients and control subjects. However, erythrocyte zinc concentration was significantly decreased in hyperthyroid patients compared to hypothyroid patients and accompanied by an increased urinary zinc excretion resulting from increased muscle tissue catabolism in hyperthyroid patients. The findings of this study suggest that abnormal zinc metabolism commonly occurs in thyroid dysfunctions (180) (Table 1).

7.1.3 Selenium

Selenium is an essential trace mineral whose functions in the organism are mainly connected to its antioxidant properties (190). Selenium is essential to antioxidant enzymes such as GPx (183) and is involved in thyroid and immune system functions. The thyroid gland has the highest concentration of selenium in the body, which is predominantly stored in the thyrocytes in the form of selenoproteins, such as deiodinases, GPx, and thioredoxin reductases (181, 182). Adequate selenium intake is mandatory for the normal function of thyrocytes, and selenium deficiency is associated with the decreased synthesis of thyroid hormones (191), increased thyroid volume, and increased number of thyroid nodules (182, 192). Selenium has been shown to affect T-cell differentiation and modulate the T-helper (Th) cells' responses. Th cells are cytokine-producing cells that are divided into subgroups 1 and 2 depending on their mechanisms of action; Th1 cells are involved in cell-mediated immunity, whereas Th2 cells participate in antibody-mediated immunity. Th1 cytokine production generally tends to exert pro-inflammatory effects and may lead to autoimmune conditions such as Hashimoto's thyroiditis. Th2-induced hyperproduction of the thyroid autoantibodies observed in In Graves' disease results in hyperthyroidism. Selenium deficiency has been associated with Th2 cell response, whereas higher selenium levels favour Th1 response (193). These findings may explain the beneficial effects of selenium supplementation in autoimmune thyroid diseases (181, 194), such as reduced levels of anti-thyroid antibodies, improved thyroid structure and metabolism, and ameliorated clinical symptoms (181, 195). Dietary forms of selenium include selenomethionine present in plant products and inorganic selenium forms used for supplementation (196). No indication of an increased risk of thyroid cancer in either selenium deficiency or exogenous supplementation has been reported (182) (Table 1).

TABLE 1 The role of nutritional antioxidants in thyroid function.

Nutritional antioxidant	Role in thyroid function	Reference
Iodine	Essential for the synthesis of thyroid hormones Antioxidant Antiproliferative agent	171)
Zinc	Required for the activity of enzyme 1,5′-deiodinase which catalyzes the conversion of T4 to T3 Regulator of TRH and TSH synthesis	(177, 178)
Selenium	Constituent of selenoproteins Cofactor of Gpx, deiodinases and thioredoxin reductases	(181–183)
Resveratrol	Mediates the levels of TSH and iodide uptake in thyrocytes by decreasing sodium/iodide symporter expression	(184)
Berberine	Decreases the abundance of pathogenic bacteria in the gut Increases the content of beneficial bacteria in the gut	(185)
Inositol	Regulates thyroid hormone synthesis by forming H_2O_2 in thyrocytes Involved in TSH signaling pathway	(187)
L-carnitine	inhibit thyroid hormone entry into the nucleus of hepatocytes, neurons, and fibroblasts	(186, 187)
Probiotics	*Lactobacilli* and *Bifidobacteriaceae* supplementation increase levothyroxine availability Reduce thyroid hormone serum fluctuation Increase the availability of bacterial enzymes sulfatases and ß-glucuronidases that regulate iodothyronines deconjugation	(188, 189)

TRH, thyrotropin-releasing hormone; TSH, thyroid stimulating hormone; GPx, glutathione peroxidase; H_2O_2, hydrogen peroxide.

7.2 Natural polyphenols and alkaloids

As an antioxidant polyphenolic compound and a free radical scavenger, resveratrol has attracted interest for the potential treatment of thyroid diseases accompanied by increased ROS production, such as autoimmune thyroiditis and hyperthyroidism (197). In addition, resveratrol may help treat thyroid cancer since it can induce apoptosis of thyroid cancer cells by increasing the abundance and phosphorylation of p53 tumour suppressor protein (p53) (198, 199). *In vitro* and *in vivo* studies have also demonstrated that resveratrol mediates the levels of TSH and iodide uptake in thyrocytes by decreasing NIS expression (184). However, the observed effects also resulted in significant proliferative action of thyrocytes; thus, resveratrol may be a thyroid-disrupting compound and a goitrogen (184). Currently, data from clinical studies on resveratrol's effect on the thyroid in humans are absent, and all literature evidence is based on studies performed in cell cultures and animal models. Therefore, proper randomized clinical trials are mandatory to reach the final verdict on the potential use of resveratrol in treating thyroid diseases.

Alkaloid antioxidant berberine was recently reported to exert positive effects in treating GD. When supplemented in combination with methimazole, berberine significantly altered the microbiota composition of patients, decreasing the abundance of the pathogenic bacteria *Chryseobacterium indologenes* and *Enterobacter hormaechei* while simultaneously increasing the content of the beneficial bacteria *Lactococcus lactis* (185). In addition, berberine supplementation resulted in significantly elevated enterobactin production, improving iron functioning and restoring thyroid function in patients with Graves' disease (185) (see Table 1).

7.3 Inositol

Inositol (also known as vitamin B8) is a carbohydrate compound that is an essential component of the plasma membrane phospholipids and has an important role in synthesizing secondary messengers in the cells (200). Inositol is involved in signaling hormones such as TSH, insulin, and gonadotropins. Myoinositol (Myo), a cyclic polyol with six hydroxyl groups, is the most abundant isoform of inositol, mainly derived from the dietary intake of fruits, beans, and nuts. In contrast, its endogenous production is generated either from glucose by enzymatic reactions or by *de novo* catabolism of phosphatidylinositol (PI), phosphoinositides (PIP), and inositol phosphates (IP). Myo has a crucial role in thyroid function and autoimmune diseases due to its regulation of thyroid hormone synthesis by forming H_2O_2 in thyrocytes. Myo is involved in the TSH signaling pathway; thus, depleted levels of Myo may cause the pathogenesis of thyroid diseases such as hypothyroidism (187). It has been observed that TSH levels significantly decreased in patients with subclinical hypothyroidism, with or without autoimmune thyroiditis, after treatment with Myo in combination with selenium (201, 202). Studies of patients with Hashimoto's thyroiditis and subclinical hypothyroidism showed that supplementation of Myo and selenomethionine significantly decreased TSH, TPOAb, and TGAb concentrations, while simultaneously increasing thyroid hormones levels and

restoring euthyroid state in patients with autoimmune thyroiditis (203, 204). In addition, the combined treatment with Myo and selenomethionine was found to have an ameliorating effect on nodular thyroid disease by promoting a significant reduction of thyroid nodules size and number and regression of their stiffness (205). Additional *in vitro* and *in vivo* studies are required to investigate the mechanism of this effect and the potential use of Myo, alone or in combination with selenomethionine, as a novel clinical treatment for the general management of autoimmune thyroiditis and thyroid nodules (Table 1).

7.4 L-carnitine

L-Carnitine (3-Hydroxy-4-(trimethylazaniumyl) butanoate) is a biological compound that is ubiquitous in mammalian tissues and fluids where it is required for β-oxidation of fatty acids by facilitating their transport in the form of acyl-carnitine esters across the mitochondrial inner membrane (206). In addition, L-carnitine possesses significant antioxidant properties reflected in its ability to scavenge superoxide anion radical and hydrogen peroxide and chelate metal ions such as ferrous ions (207). L-carnitine was shown to positively impact cardiac function through reduced oxidative stress, inflammation, and necrosis of cardiac myocytes (208). As much as 75% of L-carnitine comes from the dietary intake of red meat and dairy products (Table 2), whereas only 25% is generated by endogenous biosynthesis. Muscles are the main reservoir of carnitine, storing 95% of the total amount of 120 mmol present in the adult human body (209)

The anti-thyroid effect of L-carnitine is based on its ability to inhibit thyroid hormone entry into the nucleus of hepatocytes, neurons, and fibroblasts (186, 210). As a result, rather than being

TABLE 2 Classification of nutritional oxidants with protective roles against oxidative stress in thyroid diseases and their naturally occurring sources.

Nutritional antioxidant	Natural source
Vitamins	
Vitamin E	Plant oils (wheat germ, sunflower, safflower, and soybean oil), nuts (almonds, peanuts), sunflower seeds, fruits, and vegetables
Vitamin D	Cod liver oil, salmon, swordfish, tuna fish, sardines, egg yolk, beef liver, dairy and plant milk fortified with vitamin D
Vitamin C	Citrus fruits (oranges, lemon, grapefruit), kiwi, strawberries, vegetables (bell peppers, tomatoes, broccoli, cabbage, cauliflower, white potatoes)
Inositol (vitamin B8)	Fruits (cantaloupe, citrus fruits), fibre-rich foods (beans, brown rice, sesame seeds, corn, wheat bran), nuts (almonds, peanuts)
Trace elements	
Iodine	Seafood, seaweed, iodized table salt, dairy, eggs, chicken, beef liver
Zinc	Seafood, meat
Selenium	Brazil nuts, seafood, meat
Monounsaturated fatty acids (MUFA)	
Oleic acid	Olive and almond oil, nuts (hazelnuts, pecans, almonds)
Polyunsaturated fatty acids (PUFA)	
Omega-3 fatty acids	Seafood, nuts and seeds (flaxseed, chia seeds, and walnuts), plant oils (flaxseed oil, soybean oil, and canola oil)
Omega-6 fatty acids	Vegetable oils (sunflower, corn, and grapeseed oil), nuts (walnuts, pine nuts)
Polyphenolic compounds	
Resveratrol	Grapes, red wine, berries, peanuts
Curcumin	Rhizomes of the herbs from genus *Curcuma* (*Curcuma longa* (turmeric), *Curcuma amada, Curcuma zedoaria, Curcuma aromatic* and *Curcuma raktakanta*)
Alkaloids	
Berberine	Plants (*Annonaceae, Menispermaceae, Papaveraceae, Ranunculaceae*)
Biological compounds	
Carnitine	Red meat, dairy products

a direct inhibitor of thyroid gland function, it acts as a peripheral antagonist of thyroid hormone action (186). The first controlled clinical trial demonstrating the beneficial effects of L-carnitine in reducing elevated thyroid hormone circulating levels was conducted in 50 women receiving TSH-suppressive (L-T4) therapy for cytologically benign thyroid nodules (210). L-carnitine supplementation was shown to be effective in reversing and preventing symptoms of hyperthyroidism (210). Consequent studies showed that severe forms of GD-related hyperthyroidism, including thyroid storms, may be effectively treated with L-carnitine (211–213), which may be partly explained by increased levels of thyroid hormones deplete the tissue deposits of L-carnitine (214). Interestingly, decreased concentration of L-carnitine was also found in the skeletal muscles of hypothyroid patients (215), suggesting that L-carnitine depletion in skeletal muscles may contribute to myopathy associated with either hypothyroidism or hyperthyroidism. A recent study demonstrated that L-carnitine supplementation might alleviate fatigue symptoms in hypothyroid patients (216). Further clinical studies are required to establish the usefulness of L-carnitine supplementation in hypothyroidism (see Table 1).

7.5 Probiotics

Probiotics are live non-pathogenic microorganisms with beneficial health effects for their hosts (217). Probiotics regulate the composition of the intestinal microbiota, stimulate humoral and cellular immunity; decrease the frequency and duration of diarrhoea; and eliminate harmful metabolites in the colon, such as ammonium and procancerogenic enzymes. In addition, certain probiotic strains possess antioxidant activity and may reduce damage caused by OxS (218). Probiotics improve metabolic diseases such as obesity and diabetes by modulating intestinal microbiota composition (219–221). Furthermore, the oxidative stress in patients with T2DM can be ameliorated by multispecies probiotics (222).

Probiotic bacteria possess their antioxidant defence systems, such as enzymes SOD and Cat, and can chelate metal ions, such as ferrous and cupric ions, preventing them from catalyzing oxidation (19, 22, 223). In addition, probiotics can stimulate the host's antioxidant defence systems and increase the activity of antioxidant enzymes (224). For instance, intact cells and cell-free extracts of *Bifidobacterium animalis* 01 can scavenge hydroxyl radicals and superoxide anion *in vitro*, whereas *in vivo*, they increase the antioxidative enzyme activity (20). Lactic acid bacteria strains can defend against peroxide radicals, superoxide anions, and hydroxyl radicals (225, 226). Human studies have shown elevated SOD and GPx activities, and improved total antioxidant status in T2DM patients supplemented with *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 (227). *Lactobacillus rhamnosus* supplementation was shown to exert significant antioxidant protection in conditions of increased physical stress (228). In addition, probiotics produce various antioxidant metabolites, such as GSH and folate. *Lactobacillus fermentum* strains, E-3 and E-18, contain very high levels of GSH (226), which can, together with selenium-dependent GPx, eliminate hydroxyl radicals and peroxynitrite (229).

The enormous complexity of human microbiota is reflected in the finding that adult human organisms typically contain 10^{14} bacteria in the gut, which is approximately ten times more bacterial cells than the number of human cells (230), with at least 400 different bacterial species (231). Most bacterial species in a healthy human microbiota belong to the genera *Bacteroidetes* and *Firmicutes* (232), whereas *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Cyanobacteria*, are less abundant (233). Furthermore, microbiota composition varies depending on its localization in the gastrointestinal system; therefore *Bacilli* class of the *Firmicutes* and *Actinobacteria* is enriched in the small intestine, whereas the *Bacteroidetes* family of the *Firmicutes* is predominantly present in the colon (234).

Gut microbiota in patients with thyroid diseases has a different composition compared to the healthy controls and typically contains a decreased content of *Lactobacillaceae* and *Bifidobacteriaceae*. (23). The family *Lactobacillacae* has important antioxidant properties and may exert protective effects on the thyroid. (235), and its decreased content may cause higher oxidative stress in the thyroid (23). In addition, opportunistic pathogens in gut microbiota were shown in patients suffering from thyroid disease (Zhang, 235). Gut microbiota dysbiosis negatively affects the regulation of anti-inflammatory and immune system responses and appears to be associated with autoimmune diseases, inflammation, and some types of cancer (189, 236, 237). For instance, thyroid cancer is associated with the increased presence of *Clostridiaceae*, *Neisseria*, and *Streptococcus*, whereas in patients with thyroid nodules, a relative increase of *Streptococcus* and *Neisseria* compared to healthy controls was observed (235). Increased abundance of *Neisseria* has been linked to inflammatory disorders (238). In contrast, *Clostridiaceae* and *Streptococcus* were associated with carcinogenic effects and a higher risk of carcinomas (239, 240), and those three seem to have a role in thyroid carcinogenesis (23).

Probiotic supplementation has substantial beneficial effects on thyroid hormones and thyroid function. It was demonstrated that *Lactobacillus reuteri* supplementation improves thyroid function in mice by increasing free T4 and thyroid mass (241). Microbiota modulation by probiotic supplementation of *Lactobacilli* and *Bifidobacteriaceae* increased levothyroxine availability in humans and stabilized thyroid function. Probiotics were shown to be beneficial in lowering serum hormone fluctuations (188), partly because iodothyronines deconjugation is regulated by bacterial enzymes sulfatases and ß-glucuronidases whose availability could be increased by

probiotic supplementation (189). Finally, probiotics influence the uptake of minerals relevant to thyroid function, including selenium, iodine, iron, and zinc, and a synergistic effect of probiotics and trace elements on the total antioxidant capacity was observed *in vivo*. Although probiotic supplementation shows promising potential in improving thyroid function in thyroid diseases, further human studies on the effects of probiotics as adjuvant therapy for thyroid diseases are required (Table 1).

8 Conclusions

Besides the impairment of cellular redox homeostasis in thyroid gland cells, thyroid diseases significantly contribute to systemic redox imbalance. In that way, thyroid diseases are organ-confined and promote histological changes in distant organs by disturbing the vital cellular pathways. Therefore, the simultaneous treatment of thyroid diseases and substituting of nutraceutical antioxidants could beneficially affect different molecular mechanisms enabling the recovery of disturbed redox balance. Se levels are lower in people with thyroid dysfunctions, such as subclinical or overt hypothyroidism (242). In order to determine whether Se supplementation may impact the progression of autoimmune thyroid disease, some trials carried out in regions where the population has a diffusely low or borderline Se status inconsistently suggest that Se supplementation may cause a decrease in thyroid autoantibodies (243, 244). The population heterogeneity, various Se formulations and the length of Se supplementation, as well as different thyroid function test and Se measurement strategies, are among the reasons for the study conclusions inconsistency (244). The benefit of Se supplementation could be expected in patients living in regions with low Se availability or who have low- or sub-optimal Se levels. The supplementation must be attentive as the reference ranges of Se blood levels are narrow, and the risk of insufficient or toxic supplementation is possible (245, 246).

Accumulating evidence support the existence of a thyroid-gut axis and displays important correlations between the composition of the gut bacteria and thyroid function. Dysbiosis, a common finding in thyroid disorders, not only promotes local inflammation of the intestinal membrane but also directly affects thyroid hormone levels *via* its own deiodinase activity and TSH inhibition. In addition, gut microbiota can modulate the absorption of trace minerals, such as iodine, selenium, and zinc, that are essential for thyroid function, including iron. For instance, iodine deficiency may lead to goiter, whereas high iodine intake may induce thyroid dysfunction in susceptible patients. Supplementation with antioxidative probiotics has shown beneficial effects in thyroid diseases thus representing a potential adjuvant therapy for thyroid disorders. The advances in the field of microbiome research envision the future possibility of personalized treatment with probiotics that are specifically adjusted to individual patients. Nevertheless, more data from adequately powered human studies are required for further evaluation of the impact of gut microbiota on thyroid diseases and the potential for possible therapeutic interventions.

Author contributions

MM wrote the article. ZG wrote the article. SZ wrote the article. ME wrote the article, XG wrote and critically reviewed the article and EI wrote and critically reviewed the article. All authors contributed to the article and approved the submitted version.

Acknowledgments

This work is part of the collaboration between the Department of Radiobiology and Molecular Genetics, "VINČA" Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia, Clinic for Internal Medicine, Department of Endocrinology and Diabetes, Zemun Clinical Hospital, School of Medicine, University of Belgrade, Belgrade, Serbia, and KAUST. The research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No#451-03-9/2021-14/200017) and King Abdullah University of Science and Technology (KAUST) through grant awards Nos. BAS/1/1624-01-01, FCC/1/1976-20-01, FCC/1/1976-26-01, and Contract No#OSR 4129.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer JD declared a shared affiliation with the authors MM, ZG, SZ, EI to the handling editor at the time of review.

Publisher's note

References

1. Jakubczyk K, Dec K, Kałduńska J, Kawczuga D, Kochman J, Janda K. Reactive oxygen species - sources, functions, oxidative damage. *Pol Merkur Lekarski* (2020) 48:124–7.

2. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat Rev Mol Cell Biol* (2020) 21:363–83. doi: 10.1038/s41580-020-0230-3

3. Shekhova E. Mitochondrial reactive oxygen species as major effectors of antimicrobial immunity. *PloS Pathog* (2020) 16:e1008470. doi: 10.1371/journal.ppat.1008470

4. Yang S, Lian G. ROS and diseases: role in metabolism and energy supply. *Mol Cell Biochem* (2020) 467:1–12. doi: 10.1007/s11010-019-03667-9

5. Di Marzo N, Chisci E, Giovannoni R. The role of hydrogen peroxide in redox-dependent signaling: Homeostatic and pathological responses in mammalian cells. *Cells* (2018) 7:156. doi: 10.3390/cells7100156

6. Sies H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biol* (2017) 11:613–9. doi: 10.1016/j.redox.2016.12.035

7. Sies H, Berndt C, Jones DP. Oxidative stress. *Annu Rev Biochem* (2017) 86:715–48. doi: 10.1146/annurev-biochem-061516-045037

8. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. *Curr Biol* (2014) 24:R453–462. doi: 10.1016/j.cub.2014.03.034

9. Massart C, Hoste C, Virion A, Ruf J, Dumont JE, Van Sande J. Cell biology of H2O2 generation in the thyroid: investigation of the control of dual oxidases (DUOX) activity in intact ex vivo thyroid tissue and cell lines. *Mol Cell Endocrinol* (2011) 343:32–44. doi: 10.1016/j.mce.2011.05.047

10. Thanas C, Ziros PG, Chartoumpekis DV. The Keap1/Nrf2 signaling pathway in the thyroid-2020 update. *Antioxidants (Basel)* (2020) 9:1082. doi: 10.3390/antiox9111082

11. Poncin S, Gérard AC, Boucquey M, Senou M, Calderon PB, Knoops B, et al. Oxidative stress in the thyroid gland: from harmlessness to hazard depending on the iodine content. *Endocrinology* (2008) 149:424–33. doi: 10.1210/en.2007-0951

12. Ameziane El Hassani R, Buffet C, Leboulleux S, Dupuy C. Oxidative stress in thyroid carcinomas: biological and clinical significance. *Endocr Relat Cancer* (2019) 26:R131–r143. doi: 10.1530/erc-18-0476

13. Sies H. Oxidative stress: a concept in redox biology and medicine. *Redox Biol* (2015) 4:180–3. doi: 10.1016/j.redox.2015.01.002

14. Filomeni G, De Zio D, Cecconi F. Oxidative stress and autophagy: the clash between damage and metabolic needs. *Cell Death Differ* (2015) 22:377–88. doi: 10.1038/cdd.2014.150

15. Gu Y, Han J, Jiang C, Zhang Y. Biomarkers, oxidative stress and autophagy in skin aging. *Ageing Res Rev* (2020) 59:101036. doi: 10.1016/j.arr.2020.101036

16. Margaritelis NV, Paschalis V, Theodorou AA, Kyparos A, Nikolaidis MG. Antioxidants in personalized nutrition and exercise. *Adv Nutr* (2018) 9:813–23. doi: 10.1093/advances/nmy052

17. Guo Q, Li F, Duan Y, Wen C, Wang W, Zhang L, et al. Oxidative stress, nutritional antioxidants and beyond. *Sci China Life Sci* (2020) 63:866–74. doi: 10.1007/s11427-019-9591-5

18. Williams NT. Probiotics. *Am J Health Syst Pharm* (2010) 67:449–58. doi: 10.2146/ajhp090168

19. Lin MY, Yen CL. Antioxidative ability of lactic acid bacteria. *J Agric Food Chem* (1999) 47:1460–6. doi: 10.1021/jf981149l

20. Shen Q, Shang N, Li P. *In vitro* and *in vivo* antioxidant activity of bifidobacterium animalis 01 isolated from centenarians. *Curr Microbiol* (2011) 62:1097–103. doi: 10.1007/s00284-010-9827-7

21. Persichetti E, De Michele A, Codini M, Traina G. Antioxidative capacity of lactobacillus fermentum LF31 evaluated *in vitro* by oxygen radical absorbance capacity assay. *Nutrition* (2014) 30:936–8. doi: 10.1016/j.nut.2013.12.009

22. Wang Y, Wu Y, Wang Y, Fu A, Gong L, Li W, et al. Bacillus amyloliquefaciens SC06 alleviates the oxidative stress of IPEC-1 *via* modulating Nrf2/Keap1 signaling pathway and decreasing ROS production. *Appl Microbiol Biotechnol* (2017) 101:3015–26. doi: 10.1007/s00253-016-8032-4

23. Knezevic J, Starchl C, Tmava Berisha A, Amrein K. Thyroid-Gut-Axis: How does the microbiota influence thyroid function? *Nutrients* (2020) 12:1769. doi: 10.3390/nu12061769

24. Marrocco I, Altieri F. Measurement and clinical significance of biomarkers of oxidative stress in humans. *Oxid Med Cell Longev* (2017) 2017:6501046. doi: 10.1155/2017/6501046

25. Kochman J, Jakubczyk K. The influence of oxidative stress on thyroid diseases. *Antioxidants (Basel)* (2021) 10:1442. doi: 10.3390/antiox10091442

26. Kang D, Hamasaki N. Mitochondrial oxidative stress and mitochondrial DNA. *Clin Chem Lab Med* (2003) 41:1281–8. doi: 10.1515/cclm.2003.195

27. Le Bras M, Clément MV, Pervaiz S, Brenner C. Reactive oxygen species and the mitochondrial signaling pathway of cell death. *Histol Histopathol* (2005) 20:205–19. doi: 10.14670/hh-20.205

28. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* (2007) 39:44–84. doi: 10.1016/j.biocel.2006.07.001

29. Wink DA, Hines HB, Cheng RY, Switzer CH, Flores-Santana W, Vitek MP, et al. Nitric oxide and redox mechanisms in the immune response. *J Leukoc Biol* (2011) 89:873–91. doi: 10.1189/jlb.1010550

30. Mangge H, Becker K, Fuchs D, Gostner JM. Antioxidants, inflammation and cardiovascular disease. *World J Cardiol* (2014) 6:462–77. doi: 10.4330/wjc.v6.i6.462

31. Lambeth JD. NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol* (2004) 4:181–9. doi: 10.1038/nri1312

32. Quinn MT, Gauss KA. Structure and regulation of the neutrophil respiratory burst oxidase: comparison with nonphagocyte oxidases. *J Leukoc Biol* (2004) 76:760–81. doi: 10.1189/jlb.0404216

33. Cross AR, Jones OT. Enzymic mechanisms of superoxide production. *Biochim Biophys Acta* (1991) 1057:281–98. doi: 10.1016/s0005-2728(05)80140-9

34. Ohye H, Sugawara M. Dual oxidase, hydrogen peroxide and thyroid diseases. *Exp Biol Med (Maywood)* (2010) 235:424–33. doi: 10.1258/ebm.2009.009241

35. Monaghan P, Metcalfe NB, Torres R. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. *Ecol Lett* (2009) 12:75–92. doi: 10.1111/j.1461-0248.2008.01258.x

36. Yin H, Xu L, Porter NA. Free radical lipid peroxidation: mechanisms and analysis. *Chem Rev* (2011) 111:5944–72. doi: 10.1021/cr200084z

37. Yoshida Y, Umeno A, Shichiri M. Lipid peroxidation biomarkers for evaluating oxidative stress and assessing antioxidant capacity *in vivo*. *J Clin Biochem Nutr* (2013) 52:9–16. doi: 10.3164/jcbn.12-112

38. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev* (2014) 2014:360438. doi: 10.1155/2014/360438

39. Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J* (2003) 17:1195–214. doi: 10.1096/fj.02-0752rev

40. Chiorcea-Paquim AM. 8-oxoguanine and 8-oxodeoxyguanosine biomarkers of oxidative DNA damage: A review on HPLC-ECD determination. *Molecules* (2022) 27:1620. doi: 10.3390/molecules27051620

41. Cannan WJ, Tsang BP, Wallace SS, Pederson DS. Nucleosomes suppress the formation of double-strand DNA breaks during attempted base excision repair of clustered oxidative damages. *J Biol Chem* (2014) 289:19881–93. doi: 10.1074/jbc.M114.571588

42. Shokolenko I, Venediktova N, Bochkareva A, Wilson GL, Alexeyev MF. Oxidative stress induces degradation of mitochondrial DNA. *Nucleic Acids Res* (2009) 37:2539–48. doi: 10.1093/nar/gkp100

43. Cecarini V, Gee J, Fioretti E, Amici M, Angeletti M, Eleuteri AM, et al. Protein oxidation and cellular homeostasis: Emphasis on metabolism. *Biochim Biophys Acta* (2007) 1773:93–104. doi: 10.1016/j.bbamcr.2006.08.039

44. Turrens JF, Boveris A. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *Biochem J* (1980) 191:421–7. doi: 10.1042/bj1910421

45. Zimmermann MB, Aeberli I. Dietary determinants of subclinical inflammation, dyslipidemia and components of the metabolic syndrome in overweight children: a review. *Int J Obes (Lond)* (2008) 32 Suppl 6:S11–18. doi: 10.1038/ijo.2008.202

46. Mancini A, Di Segni C. Thyroid hormones, oxidative stress, and inflammation. *Mediators Inflamm* (2016) 2016:6757154. doi: 10.1155/2016/6757154

47. Siti HN, Kamisah Y, Kamsiah J. The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review). *Vascul Pharmacol* (2015) 71:40–56. doi: 10.1016/j.vph.2015.03.005

48. Inoue K, Sakano N, Ogino K, Sato Y, Wang DH, Kubo M, et al. Relationship between ceruloplasmin and oxidative biomarkers including ferritin among healthy Japanese. *J Clin Biochem Nutr* (2013) 52:160–6. doi: 10.3164/jcbn.12-122

49. Kim SH, Kim SH, Lee JH, Lee BH, Yoon HJ, Shin DH, et al. Superoxide dismutase gene (SOD1, SOD2, and SOD3) polymorphisms and antituberculosis drug-induced hepatitis. *Allergy Asthma Immunol Res* (2015) 7:88–91. doi: 10.4168/aair.2015.7.1.88

50. Yasui K, Baba A. Therapeutic potential of superoxide dismutase (SOD) for resolution of inflammation. *Inflammation Res* (2006) 55:359–63. doi: 10.1007/s00011-006-5195-y

51. Nigam S, Schewe T. Phospholipase A(2)s and lipid peroxidation. *Biochim Biophys Acta* (2000) 1488:167–81. doi: 10.1016/s1388-1981(00)00119-0

52. Six DA, Dennis EA. The expanding superfamily of phospholipase A(2) enzymes: classification and characterization. *Biochim Biophys Acta* (2000) 1488:1–19. doi: 10.1016/s1388-1981(00)00105-0

53. Messarah M, Saoudi M, Boumendjel A, Boulakoud MS, Feki AE. Oxidative stress induced by thyroid dysfunction in rat erythrocytes and heart. *Environ Toxicol Pharmacol* (2011) 31:33–41. doi: 10.1016/j.etap.2010.09.003

54. van der Spek AH, Fliers E, Boelen A. Thyroid hormone metabolism in innate immune cells. *J Endocrinol* (2017) 232:R67–r81. doi: 10.1530/joe-16-0462

55. Gluvic ZM, Zafirovic SS, Obradovic MM, Sudar-Milovanovic EM, Rizzo M, Isenovic ER. Hypothyroidism and risk of cardiovascular disease. *Curr Pharm Des* (2022) 28:2065–72. doi: 10.2174/1381612828666220620160516

56. Chakrabarti SK, Ghosh S, Banerjee S, Mukherjee S, Chowdhury S. Oxidative stress in hypothyroid patients and the role of antioxidant supplementation. *Indian J Endocrinol Metab* (2016) 20:674–8. doi: 10.4103/2230-8210.190555

57. Venditti P, Di Meo S. Thyroid hormone-induced oxidative stress. *Cell Mol Life Sci* (2006) 63:414–34. doi: 10.1007/s00018-005-5457-9

58. Chattopadhyay S, Sahoo DK, Roy A, Samanta L, Chainy GB. Thiol redox status critically influences mitochondrial response to thyroid hormone-induced hepatic oxidative injury: A temporal analysis. *Cell Biochem Funct* (2010) 28:126–34. doi: 10.1002/cbf.1631

59. Resch U, Helsel G, Tatzber F, Sinzinger H. Antioxidant status in thyroid dysfunction. *Clin Chem Lab Med* (2002) 40:1132–4. doi: 10.1515/cclm.2002.198

60. Nanda N, Bobby Z, Hamide A. Association of thyroid stimulating hormone and coronary lipid risk factors with lipid peroxidation in hypothyroidism. *Clin Chem Lab Med* (2008) 46:674–9. doi: 10.1515/cclm.2008.139

61. Hak AE, Pols HA, Visser TJ, Drexhage HA, Hofman A, Witteman JC. Subclinical hypothyroidism is an independent risk factor for atherosclerosis and myocardial infarction in elderly women: the Rotterdam study. *Ann Intern Med* (2000) 132:270–8. doi: 10.7326/0003-4819-132-4-200002150-00004

62. Gluvic Z, Sudar E, Tica J, Jovanovic A, Zafirovic S, Tomasevic R, et al. Effects of levothyroxine replacement therapy on parameters of metabolic syndrome and atherosclerosis in hypothyroid patients: a prospective pilot study. *Int J Endocrinol* (2015) 2015:147070. doi: 10.1155/2015/147070

63. Duntas LH. Thyroid disease and lipids. *Thyroid* (2002) 12:287–93. doi: 10.1089/10507250252949405

64. Fernández V, Tapia G, Varela P, Romanque P, Cartier-Ugarte D, Videla LA. Thyroid hormone-induced oxidative stress in rodents and humans: a comparative view and relation to redox regulation of gene expression. *Comp Biochem Physiol C Toxicol Pharmacol* (2006) 142:231–9. doi: 10.1016/j.cbpc.2005.10.007

65. Peppa M, Betsi G, Dimitriadis G. Lipid abnormalities and cardiometabolic risk in patients with overt and subclinical thyroid disease. *J Lipids* (2011) 2011:575840. doi: 10.1155/2011/575840

66. Rizos CV, Elisaf MS, Liberopoulos EN. Effects of thyroid dysfunction on lipid profile. *Open Cardiovasc Med J* (2011) 5:76–84. doi: 10.2174/1874192401105010076

67. Negre-Salvayre A, Coatrieux C, Ingueneau C, Salvayre R. Advanced lipid peroxidation end products in oxidative damage to proteins. potential role in diseases and therapeutic prospects for the inhibitors. *Br J Pharmacol* (2008) 153:6–20. doi: 10.1038/sj.bjp.0707395

68. Gluvic ZM, Obradovic MM, Sudar-Milovanovic EM, Zafirovic SS, Radak DJ, Essack MM, et al. Regulation of nitric oxide production in hypothyroidism. *BioMed Pharmacother* (2020) 124:109881. doi: 10.1016/j.biopha.2020.109881

69. Lerman A, Burnett JCJr. Intact and altered endothelium in regulation of vasomotion. *Circulation* (1992) 86:III12–19.

70. Taddei S, Caraccio N, Virdis A, Dardano A, Versari D, Ghiadoni L, et al. Low-grade systemic inflammation causes endothelial dysfunction in patients with hashimoto's thyroiditis. *J Clin Endocrinol Metab* (2006) 91:5076–82. doi: 10.1210/jc.2006-1075

71. Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado thyroid disease prevalence study. *Arch Intern Med* (2000) 160:526–34. doi: 10.1001/archinte.160.4.526

72. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. Serum TSH, T(4), and thyroid antibodies in the united states population, (1988 to 1994): National health and nutrition examination survey (NHANES III). *J Clin Endocrinol Metab* (2002) 87:489–99. doi: 10.1210/jcem.87.2.8182

73. Berta E, Lengyel I, Halmi S, Zrínyi M, Erdei A, Harangi M, et al. Hypertension in thyroid disorders. *Front Endocrinol (Lausanne)* (2019) 10:482. doi: 10.3389/fendo.2019.00482

74. De Leo S, Lee SY, Braverman LE. Hyperthyroidism. *Lancet* (2016) 388:906–18. doi: 10.1016/s0140-6736(16)00278-6

75. Chaker L, Bianco AC, Jonklaas J, Peeters RP. Hypothyroidism. *Lancet* (2017) 390:1550–62. doi: 10.1016/s0140-6736(17)30703-1

76. Delitala AP. Subclinical hyperthyroidism and the cardiovascular disease. *Horm Metab Res* (2017) 49:723–31. doi: 10.1055/s-0043-117893

77. Stamatouli A, Bedoya P, Yavuz S. Hypothyroidism: Cardiovascular endpoints of thyroid hormone replacement. *Front Endocrinol (Lausanne)* (2019) 10:888. doi: 10.3389/fendo.2019.00888

78. Barreiro Arcos ML. Role of thyroid hormones-induced oxidative stress on cardiovascular physiology. *Biochim Biophys Acta Gen Subj* (2022) 1866:130239. doi: 10.1016/j.bbagen.2022.130239

79. Erdamar H, Cimen B, Gülcemal H, Saraymen R, Yerer B, Demirci H. Increased lipid peroxidation and impaired enzymatic antioxidant defense mechanism in thyroid tissue with multinodular goiter and papillary carcinoma. *Clin Biochem* (2010) 43:650–4. doi: 10.1016/j.clinbiochem.2010.02.005

80. Ramli NSF, Mat Junit S, Leong NK, Razali N. Analyses of antioxidant status and nucleotide alterations in genes encoding antioxidant enzymes in patients with benign and malignant thyroid disorders. *PeerJ* (2017) 5:. doi: 10.7717/peerj.3365

81. Kuzan A, Królewicz E. Contribution of glycation and oxidative stress to thyroid gland pathology-a pilot study. *Biomolecules* (2021) 11:557. doi: 10.3390/biom11040557

82. Piazera BKL, Gomes DV, Vigário P, Salerno VP, Vaisman M. Evaluation of redox profiles in exogenous subclinical hyperthyroidism at two different levels of TSH suppression. *Arch Endocrinol Metab* (2018) 62:545–51. doi: 10.20945/2359-3997000000075

83. Fahim YA, Sharaf NE. Assessment of thyroid function and oxidative stress state in foundry workers exposed to lead. *J Health Pollut* (2020) 10:200903. doi: 10.5696/2156-9614-10.27.200903

84. Torun AN, Kulaksizoglu S, Kulaksizoglu M, Pamuk BO, Isbilen E, Tutuncu NB. Serum total antioxidant status and lipid peroxidation marker malondialdehyde levels in overt and subclinical hypothyroidism. *Clin Endocrinol (Oxf)* (2009) 70:469–74. doi: 10.1111/j.1365-2265.2008.03348.x

85. Baskol G, Atmaca H, Tanriverdi F, Baskol M, Kocer D, Bayram F. Oxidative stress and enzymatic antioxidant status in patients with hypothyroidism before and after treatment. *Exp Clin Endocrinol Diabetes* (2007) 115:522–6. doi: 10.1055/s-2007-981457

86. Fortunato RS, Ferreira AC, Hecht F, Dupuy C, Carvalho DP. Sexual dimorphism and thyroid dysfunction: a matter of oxidative stress? *J Endocrinol* (2014) 221:R31–40. doi: 10.1530/joe-13-0588

87. Marcocci C, Bartalena L. Role of oxidative stress and selenium in graves' hyperthyroidism and orbitopathy. *J Endocrinol Invest* (2013) 36:15–20.

88. Poncin S, Van Eeckoudt S, Humblet K, Colin IM, Gérard AC. Oxidative stress: a required condition for thyroid cell proliferation. *Am J Pathol* (2010) 176:1355–63. doi: 10.2353/ajpath.2010.090682

89. Ruggeri RM, Giovinazzo S, Barbalace MC, Cristani M, Alibrandi A, Vicchio TM, et al. Influence of dietary habits on oxidative stress markers in hashimoto's thyroiditis. *Thyroid* (2021) 31:96–105. doi: 10.1089/thy.2020.0299

90. Faam B, Ghadiri AA, Ghaffari MA, Totonchi M, Khorsandi L. Comparing oxidative stress status among Iranian males and females with malignant and non-malignant thyroid nodules. *Int J Endocrinol Metab* (2021) 19:e105669. doi: 10.5812/ijem.105669

91. Bednarek J, Wysocki H, Sowinski J. Oxidation products and antioxidant markers in plasma of patients with graves' disease and toxic multinodular goiter: effect of methimazole treatment. *Free Radic Res* (2004) 38:659–64. doi: 10.1080/10715760410001701621

92. Nishihara E, Hishinuma A, Kogai T, Takada N, Hirokawa M, Fukata S, et al. A novel germline mutation of KEAP1 (R483H) associated with a non-toxic multinodular goiter. *Front Endocrinol (Lausanne)* (2016) 7:131. doi: 10.3389/fendo.2016.00131

93. Stancioiu F, Mihai D, Papadakis GZ, Tsatsakis A, Spandidos DA, Badiu C. Treatment for benign thyroid nodules with a combination of natural extracts. *Mol Med Rep* (2019) 20:2332–8. doi: 10.3892/mmr.2019.10453

94. Duthoit C, Estienne V, Giraud A, Durand-Gorde JM, Rasmussen AK, Feldt-Rasmussen U, et al. Hydrogen peroxide-induced production of a 40 kDa immunoreactive thyroglobulin fragment in human thyroid cells: the onset of thyroid autoimmunity? *Biochem J* (2001) 360:557–62. doi: 10.1042/0264-6021:3600557

95. Gheorghiu ML, Badiu C. Selenium involvement in mitochondrial function in thyroid disorders. *Hormones (Athens)* (2020) 19:25–30. doi: 10.1007/s42000-020-00173-2

96. Niethammer P, Grabher C, Look AT, Mitchison TJ. A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish. *Nature* (2009) 459:996–9. doi: 10.1038/nature08119

97. Ruggeri RM, Vicchio TM, Cristani M, Certo R, Caccamo D, Alibrandi A, et al. Oxidative stress and advanced glycation end products in hashimoto's thyroiditis. *Thyroid* (2016) 26:504–11. doi: 10.1089/thy.2015.0592

98. Li D, Liang G, Calderone R, Bellanti JA. Vitiligo and hashimoto's thyroiditis: Autoimmune diseases linked by clinical presentation, biochemical commonality, and autoimmune/oxidative stress-mediated toxicity pathogenesis. *Med Hypotheses* (2019) 128:69–75. doi: 10.1016/j.mehy.2019.05.010

99. Ruggeri RM, Campennì A, Giuffrida G, Casciaro M, Barbalace MC, Hrelia S, et al. Oxidative stress as a key feature of autoimmune thyroiditis: an update. *Minerva Endocrinol* (2020) 45:326–44. doi: 10.23736/s0391-1977.20.03268-x

100. Rostami R, Nourooz-Zadeh S, Mohammadi A, Khalkhali HR, Ferns G, Nourooz-Zadeh J. Serum selenium status and its interrelationship with serum biomarkers of thyroid function and antioxidant defense in hashimoto's thyroiditis. *Antioxidants (Basel)* (2020) 9:1070. doi: 10.3390/antiox9111070

101. Rostami R, Aghasi MR, Mohammadi A, Nourooz-Zadeh J. Enhanced oxidative stress in hashimoto's thyroiditis: inter-relationships to biomarkers of thyroid function. *Clin Biochem* (2013) 46:308–12. doi: 10.1016/j.clinbiochem.2012.11.021

102. Ates I, Arikan MF, Altay M, Yilmaz FM, Yilmaz N, Berker D, et al. The effect of oxidative stress on the progression of hashimoto's thyroiditis. *Arch Physiol Biochem* (2018) 124:351–6. doi: 10.1080/13813455.2017.1408660

103. Baser H, Can U, Baser S, Yerlikaya FH, Aslan U, Hidayetoglu BT. Assesment of oxidative status and its association with thyroid autoantibodies in patients with euthyroid autoimmune thyroiditis. *Endocrine* (2015) 48:916–23. doi: 10.1007/s12020-014-0399-3

104. Valea A, Georgescu CE. Selenoproteins in human body: focus on thyroid pathophysiology. *Hormones (Athens)* (2018) 17:183–96. doi: 10.1007/s42000-018-0033-5

105. Chartoumpekis DV, Ziros PG, Habeos IG, Sykiotis GP. Emerging roles of Keap1/Nrf2 signaling in the thyroid gland and perspectives for bench-to-bedside translation. *Free Radic Biol Med* (2022) 190:276–83. doi: 10.1016/j.freeradbiomed.2022.08.021

106. Zarković M. The role of oxidative stress on the pathogenesis of graves' disease. *J Thyroid Res* (2012) 2012:302537. doi: 10.1155/2012/302537

107. Ademoğlu E, Ozbey N, Erbil Y, Tanrikulu S, Barbaros U, Yanik BT, et al. Determination of oxidative stress in thyroid tissue and plasma of patients with graves' disease. *Eur J Intern Med* (2006) 17:545–50. doi: 10.1016/j.ejim.2006.04.013

108. Maouche N, Meskine D, Alamir B, Koceir EA. Trace elements profile is associated with insulin resistance syndrome and oxidative damage in thyroid disorders: Manganese and selenium interest in Algerian participants with dysthyroidism. *J Trace Elem Med Biol* (2015) 32:112–21. doi: 10.1016/j.jtemb.2015.07.002

109. Kocak M, Akarsu E, Korkmaz H, Taysi S. The effect of antithyroid drugs on osteopontin and oxidative stress in graves' disease. *Acta Endocrinol (Buchar)* (2019) 15:221–4. doi: 10.4183/aeb.2019.221

110. Diana T, Daiber A, Oelze M, Neumann S, Olivo PD, Kanitz M, et al. Stimulatory TSH-receptor antibodies and oxidative stress in graves disease. *J Clin Endocrinol Metab* (2018) 103:3668–77. doi: 10.1210/jc.2018-00509

111. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* (2006) 160:1–40. doi: 10.1016/j.cbi.2005.12.009

112. Collery P. Strategies for the development of selenium-based anticancer drugs. *J Trace Elem Med Biol* (2018) 50:498–507. doi: 10.1016/j.jtemb.2018.02.024

113. Stone JR. An assessment of proposed mechanisms for sensing hydrogen peroxide in mammalian systems. *Arch Biochem Biophys* (2004) 422:119–24. doi: 10.1016/j.abb.2003.12.029

114. Nakashima M, Suzuki K, Meirmanov S, Naruke Y, Matsuu-Matsuyama M, Shichijo K, et al. Foci formation of P53-binding protein 1 in thyroid tumors: activation of genomic instability during thyroid carcinogenesis. *Int J Cancer* (2008) 122:1082–8. doi: 10.1002/ijc.23223

115. Krohn K, Maier J, Paschke R. Mechanisms of disease: hydrogen peroxide, DNA damage and mutagenesis in the development of thyroid tumors. *Nat Clin Pract Endocrinol Metab* (2007) 3:713–20. doi: 10.1038/ncpendmet0621

116. Sedelnikova OA, Redon CE, Dickey JS, Nakamura AJ, Georgakilas AG, Bonner WM. Role of oxidatively induced DNA lesions in human pathogenesis. *Mutat Res* (2010) 704:152–9. doi: 10.1016/j.mrrev.2009.12.005

117. Kasai H. Analysis of a form of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutat Res* (1997) 387:147–63. doi: 10.1016/s1383-5742(97)00035-5

118. Karger S, Krause K, Engelhardt C, Weidinger C, Gimm O, Dralle H, et al. Distinct pattern of oxidative DNA damage and DNA repair in follicular thyroid tumours. *J Mol Endocrinol* (2012) 48:193–202. doi: 10.1530/jme-11-0119

119. Wang D, Feng JF, Zeng P, Yang YH, Luo J, Yang YW. Total oxidant/antioxidant status in sera of patients with thyroid cancers. *Endocr Relat Cancer* (2011) 18:773–82. doi: 10.1530/erc-11-0230

120. Gerić M, Domijan AM, Gluščić V, Janušić R, Šarčević B, Garaj-Vrhovac V. Cytogenetic status and oxidative stress parameters in patients with thyroid diseases. *Mutat Res Genet Toxicol Environ Mutagen* (2016) 810:22–9. doi: 10.1016/j.mrgentox.2016.09.010

121. Heydarzadeh S, Kia SK. The cross-talk between polyphenols and the target enzymes related to oxidative stress-induced thyroid cancer. *Oxid Med Cell Longev* (2022) 2022:2724324. doi: 10.1155/2022/2724324

122. Metere A, Frezzotti F, Graves CE, Vergine M, De Luca A, Pietraforte D, et al. A possible role for selenoprotein glutathione peroxidase (GPx1) and thioredoxin reductases (TrxR1) in thyroid cancer: our experience in thyroid surgery. *Cancer Cell Int* (2018) 18:7. doi: 10.1186/s12935-018-0504-4

123. Rovcanin B, Stojsavljevic A, Kekic D, Gopcevic K, Manojlovic D, Jovanovic M, et al. Redox status and antioxidative cofactor metals influence clinical and pathological characteristics of papillary thyroid carcinoma and colloid goiter. *Biol Trace Elem Res* (2020) 197:349–59. doi: 10.1007/s12011-019-01995-x

124. Akinci M, Kosova F, Cetin B, Sepici A, Altan N, Aslan S, et al. Oxidant/antioxidant balance in patients with thyroid cancer. *Acta Cir Bras* (2008) 23:551–4. doi: 10.1590/s0102-86502008000600013

125. Lassoued S, Mseddi M, Mnif F, Abid M, Guermazi F, Masmoudi H, et al. A comparative study of the oxidative profile in graves' disease, hashimoto's thyroiditis, and papillary thyroid cancer. *Biol Trace Elem Res* (2010) 138:107–15. doi: 10.1007/s12011-010-8625-1

126. Matrone A, Ferrari F, Santini F, Elisei R. Obesity as a risk factor for thyroid cancer. *Curr Opin Endocrinol Diabetes Obes* (2020) 27:358–63. doi: 10.1097/med.0000000000000556

127. Shi P, Zhang L, Liu Y, Yang F, Fu K, Li R, et al. Clinicopathological features and prognosis of papillary thyroid cancer patients with type 2 diabetes mellitus. *Gland Surg* (2022) 11:358–68. doi: 10.21037/gs-21-905

128. Ziros PG, Habeos IG, Chartoumpekis DV, Ntalampyra E, Somm E, Renaud CO, et al. NFE2-related transcription factor 2 coordinates antioxidant defense with thyroglobulin production and iodination in the thyroid gland. *Thyroid* (2018) 28:780–98. doi: 10.1089/thy.2018.0018

129. Renaud CO, Ziros PG, Chartoumpekis DV, Bongiovanni M, Sykiotis GP. Keap1/Nrf2 signaling: A new player in thyroid pathophysiology and thyroid cancer. *Front Endocrinol (Lausanne)* (2019) 10:510. doi: 10.3389/fendo.2019.00510

130. Leinonen HM, Kansanen E, Pölönen P, Heinäniemi M, Levonen AL. Role of the Keap1-Nrf2 pathway in cancer. *Adv Cancer Res* (2014) 122:281–320. doi: 10.1016/b978-0-12-420117-0.00008-6

131. Hasegawa Y, Takano T, Miyauchi A, Matsuzuka F, Yoshida H, Kuma K, et al. Decreased expression of glutathione peroxidase mRNA in thyroid anaplastic carcinoma. *Cancer Lett* (2002) 182:69–74. doi: 10.1016/s0304-3835(02)00069-1

132. Cazarin J, Dupuy C, Pires de Carvalho D. Redox homeostasis in thyroid cancer: Implications in Na(+)/I(-) symporter (NIS) regulation. *Int J Mol Sci* (2022) 23:6129. doi: 10.3390/ijms23116129

133. Cena H, Calder PC. Defining a healthy diet: Evidence for the role of contemporary dietary patterns in health and disease. *Nutrients* (2020) 12:334. doi: 10.3390/nu12020334

134. García-García FJ, Monistrol-Mula A, Cardellach F, Garrabou G. Nutrition, bioenergetics, and metabolic syndrome. *Nutrients* (2020) 12:2785. doi: 10.3390/nu12092785

135. Rakhra V, Galappaththy SL, Bulchandani S, Cabandugama PK. Obesity and the Western diet: How we got here. *Mo Med* (2020) 117:536–8.

136. Tan BL, Norhaizan ME, Liew WP. Nutrients and oxidative stress: Friend or foe? *Oxid Med Cell Longev* (2018) 2018:9719584. doi: 10.1155/2018/9719584

137. Haeiwa H, Fujita T, Saitoh Y, Miwa N. Oleic acid promotes adaptability against oxidative stress in 3T3-L1 cells through lipohormesis. *Mol Cell Biochem* (2014) 386:73–83. doi: 10.1007/s11010-013-1846-9

138. Ravaut G, Légiot A, Bergeron KF. Monounsaturated fatty acids in obesity-related inflammation. *Int J Mol Sci* (2020) 22:330. doi: 10.3390/ijms22010330

139. Silva Figueiredo P, Carla Inada A, Marcelino G, Maiara Lopes Cardozo C, de Cássia Freitas K, de Cássia Avellaneda Guimarães R, et al. Fatty acids consumption: The role metabolic aspects involved in obesity and its associated disorders. *Nutrients* (2017) 9:1158. doi: 10.3390/nu9101158

140. Meital LT, Windsor MT, Perissiou M, Schulze K, Magee R, Kuballa A, et al. Omega-3 fatty acids decrease oxidative stress and inflammation in macrophages from patients with small abdominal aortic aneurysm. *Sci Rep* (2019) 9:12978. doi: 10.1038/s41598-019-49362-z

141. Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med (Maywood)* (2008) 233:674–88. doi: 10.3181/0711-mr-311

142. Liu T, Qi H, Ma L, Liu Z, Fu H, Zhu W, et al. Resveratrol attenuates oxidative stress and extends life span in the annual fish nothobranchius guentheri. *Rejuvenation Res* (2015) 18:225–33. doi: 10.1089/rej.2014.1618

143. Bonnefont-Rousselot D. Resveratrol and cardiovascular diseases. *Nutrients* (2016) 8:250. doi: 10.3390/nu8050250

144. Dyck GJB, Raj P, Zieroth S, Dyck JRB, Ezekowitz JA. The effects of resveratrol in patients with cardiovascular disease and heart failure: A narrative review. *Int J Mol Sci* (2019) 20:904. doi: 10.3390/ijms20040904

145. Cheng CK, Luo JY, Lau CW, Chen ZY, Tian XY, Huang Y. Pharmacological basis and new insights of resveratrol action in the cardiovascular system. *Br J Pharmacol* (2020) 177:1258–77. doi: 10.1111/bph.14801

146. Bo S, Ciccone G, Castiglione A, Gambino R, De Michieli F, Villois P, et al. Anti-inflammatory and antioxidant effects of resveratrol in healthy smokers a randomized, double-blind, placebo-controlled, cross-over trial. *Curr Med Chem* (2013) 20:1323–31. doi: 10.2174/0929867311320100009

147. Szkudelska K, Okulicz M, Hertig I, Szkudelski T. Resveratrol ameliorates inflammatory and oxidative stress in type 2 diabetic goto-kakizaki rats. *BioMed Pharmacother* (2020) 125:110026. doi: 10.1016/j.biopha.2020.110026

148. Hu HC, Lei YH, Zhang WH, Luo XQ. Antioxidant and anti-inflammatory properties of resveratrol in diabetic nephropathy: A systematic review and meta-analysis of animal studies. *Front Pharmacol* (2022) 13:841818. doi: 10.3389/fphar.2022.841818

149. Mahjabeen W, Khan DA, Mirza SA. Role of resveratrol supplementation in regulation of glucose hemostasis, inflammation and oxidative stress in patients with diabetes mellitus type 2: A randomized, placebo-controlled trial. *Complement Ther Med* (2022) 66:102819. doi: 10.1016/j.ctim.2022.102819

150. Su M, Zhao W, Xu S. Resveratrol in treating diabetes and its cardiovascular complications: A review of its mechanisms of action. *Antioxidants (Basel)* (2022) 11:1085. doi: 10.3390/antiox11061085

151. Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S, Takada Y. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Res* (2004) 24:2783–840.

152. Rauf A, Imran M, Butt MS, Nadeem M, Peters DG, Mubarak MS. Resveratrol as an anti-cancer agent: A review. *Crit Rev Food Sci Nutr* (2018) 58:1428–47. doi: 10.1080/10408398.2016.1263597

153. Rotondo S, Rajtar G, Manarini S, Celardo A, Rotillo D, de Gaetano G, et al. Effect of trans-resveratrol, a natural polyphenolic compound, on human polymorphonuclear leukocyte function. *Br J Pharmacol* (1998) 123:1691–9. doi: 10.1038/sj.bjp.0701784

154. Mizutani K, Ikeda K, Kawai Y, Yamori Y. Protective effect of resveratrol on oxidative damage in male and female stroke-prone spontaneously hypertensive rats. *Clin Exp Pharmacol Physiol* (2001) 28:55–9. doi: 10.1046/j.1440-1681.2001.03415.x

155. Belguendouz L, Frémont L, Gozzelino MT. Interaction of transresveratrol with plasma lipoproteins. *Biochem Pharmacol* (1998) 55:811–6. doi: 10.1016/s0006-2952(97)00544-3

156. Wenzel E, Soldo T, Erbersdobler H, Somoza V. Bioactivity and metabolism of trans-resveratrol orally administered to wistar rats. *Mol Nutr Food Res* (2005) 49:482–94. doi: 10.1002/mnfr.200500003

157. Delmas D, Jannin B, Latruffe N. Resveratrol: preventing properties against vascular alterations and ageing. *Mol Nutr Food Res* (2005) 49:377–95. doi: 10.1002/mnfr.200400098

158. MacCarrone M, Lorenzon T, Guerrieri P, Agrò AF. Resveratrol prevents apoptosis in K562 cells by inhibiting lipoxygenase and cyclooxygenase activity. *Eur J Biochem* (1999) 265:27–34. doi: 10.1046/j.1432-1327.1999.00630.x

159. Leonard SS, Xia C, Jiang BH, Stinefelt B, Klandorf H, Harris GK, et al. Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses. *Biochem Biophys Res Commun* (2003) 309:1017–26. doi: 10.1016/j.bbrc.2003.08.105

160. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. *Mol Pharm* (2007) 4:807–18. doi: 10.1021/mp700113r

161. Sharifi-Rad J, Rayess YE, Rizk AA, Sadaka C, Zgheib R, Zam W, et al. Turmeric and its major compound curcumin on health: Bioactive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications. *Front Pharmacol* (2020) 11:1021. doi: 10.3389/fphar.2020.01021

162. Jiang T, Ghosh R, Charcosset C. Extraction, purification and applications of curcumin from plant materials-a comprehensive review. *Trends Food Sci Technol* (2021) 112:419–30. doi: 10.1016/j.tifs.2021.04.015

163. Lin X, Bai D, Wei Z, Zhang Y, Huang Y, Deng H, et al. Curcumin attenuates oxidative stress in RAW264.7 cells by increasing the activity of antioxidant enzymes and activating the Nrf2-Keap1 pathway. *PloS One* (2019) 14:e0216711. doi: 10.1371/journal.pone.0216711

164. Menon VP, Sudheer AR. Antioxidant and anti-inflammatory properties of curcumin. *Adv Exp Med Biol* (2007) 595:105–25. doi: 10.1007/978-0-387-46401-5_3

165. Maithili Karpaga Selvi N, Sridhar MG, Swaminathan RP, Sripradha R. Curcumin attenuates oxidative stress and activation of redox-sensitive kinases in high fructose- and high-Fat-Fed Male wistar rats. *Sci Pharm* (2015) 83:159–75. doi: 10.3797/scipharm.1408-16

166. Neag MA, Mocan A, Echeverria J, Pop RM, Bocsan CI, Crisan G, et al. Berberine: Botanical occurrence, traditional uses, extraction methods, and relevance in cardiovascular, metabolic, hepatic, and renal disorders. *Front Pharmacol* (2018) 9:557. doi: 10.3389/fphar.2018.00557

167. Li Z, Geng YN, Jiang JD. Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus. *Evid Based Complement Alternat Med* (2014) 2014:289264. doi: 10.1155/2014/289264

168. Cao RY, Zhang Y, Feng Z, Liu S, Liu Y, Zheng H, et al. The effective role of natural product berberine in modulating oxidative stress and inflammation related atherosclerosis: Novel insights into the gut-heart axis evidenced by genetic sequencing analysis. *Front Pharmacol* (2021) 12:764994. doi: 10.3389/fphar.2021.764994

169. Ma X, Chen Z, Wang L, Wang G, Wang Z, Dong X, et al. The pathogenesis of diabetes mellitus by oxidative stress and inflammation: Its inhibition by berberine. *Front Pharmacol* (2018) 9:782. doi: 10.3389/fphar.2018.00782

170. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: Harms and benefits for human health. *Oxid Med Cell Longev* (2017) 2017:8416763. doi: 10.1155/2017/8416763

171. Aceves C, Anguiano B, Delgado G. The extrathyronine actions of iodine as antioxidant, apoptotic, and differentiation factor in various tissues. *Thyroid* (2013) 23:938–46. doi: 10.1089/thy.2012.0579

172. Zicker S, Schoenherr B. Focus on nutrition: the role of iodine in nutrition and metabolism. *Compend Contin Educ Vet* (2012) 34:E1–4.

173. Luo J, Hendryx M, Dinh P, He K. Association of iodine and iron with thyroid function. *Biol Trace Elem Res* (2017) 179:38–44. doi: 10.1007/s12011-017-0954-x

174. Zimmermann MB, Galetti V. Iodine intake as a risk factor for thyroid cancer: a comprehensive review of animal and human studies. *Thyroid Res* (2015) 8:8. doi: 10.1186/s13044-015-0020-8

175. Wolff J, Chaikoff IL. Plasma inorganic iodide as a homeostatic regulator of thyroid function. *J Biol Chem* (1948) 174:555–64. doi: 10.1016/S0021-9258(18)57335-X

176. Leung AM, Braverman LE. Consequences of excess iodine. *Nat Rev Endocrinol* (2014) 10:136–42. doi: 10.1038/nrendo.2013.251

177. Civitareale D, Saiardi A, Falasca P. Purification and characterization of thyroid transcription factor 2. *Biochem J* (1994) 304(Pt 3):981–5. doi: 10.1042/bj3040981

178. Severo JS, Morais JBS, de Freitas TEC, Andrade ALP, Feitosa MM, Fontenelle LC, et al. The role of zinc in thyroid hormones metabolism. *Int J Vitam Nutr Res* (2019) 89:80–8. doi: 10.1024/0300-9831/a000262

179. Kralik A, Eder K, Kirchgessner M. Influence of zinc and selenium deficiency on parameters relating to thyroid hormone metabolism. *Horm Metab Res* (1996) 28:223–6. doi: 10.1055/s-2007-979169

180. Nishi Y, Kawate R, Usui T. Zinc metabolism in thyroid disease. *Postgrad Med J* (1980) 56:833–7. doi: 10.1136/pgmj.56.662.833

181. Drutel A, Archambeaud F, Caron P. Selenium and the thyroid gland: more good news for clinicians. *Clin Endocrinol (Oxf)* (2013) 78:155–64. doi: 10.1111/cen.12066

182. Köhrle J. Selenium and the thyroid. *Curr Opin Endocrinol Diabetes Obes* (2013) 20:441–8. doi: 10.1097/01.med.0000433066.24541.88

183. Zoidis E, Seremelis I, Kontopoulos N, Danezis GP. Selenium-dependent antioxidant enzymes: Actions and properties of selenoproteins. *Antioxidants (Basel)* (2018) 7:66. doi: 10.3390/antiox7050066

184. Giuliani C, Iezzi M, Ciolli L, Hysi A, Bucci I, Di Santo S, et al. Resveratrol has anti-thyroid effects both in vitro and in vivo. *Food Chem Toxicol* (2017) 107:237–47. doi: 10.1016/j.fct.2017.06.044

185. Han Z, Cen C, Ou Q, Pan Y, Zhang J, Huo D, et al. The potential prebiotic berberine combined with methimazole improved the therapeutic effect of graves' disease patients through regulating the intestinal microbiome. *Front Immunol* (2021) 12:826067. doi: 10.3389/fimmu.2021.826067

186. Benvenga S, Lakshmanan M, Trimarchi F. Carnitine is a naturally occurring inhibitor of thyroid hormone nuclear uptake. *Thyroid* (2000) 10:1043–50. doi: 10.1089/thy.2000.10.1043

187. Benvenga S, Nordio M, Laganà AS, Unfer V. The role of inositol in thyroid physiology and in subclinical hypothyroidism management. *Front Endocrinol (Lausanne)* (2021) 12:662582. doi: 10.3389/fendo.2021.662582

188. Spaggiari G, Brigante G, De Vincentis S, Cattini U, Roli L, De Santis MC, et al. Probiotics ingestion does not directly affect thyroid hormonal parameters in hypothyroid patients on levothyroxine treatment. *Front Endocrinol (Lausanne)* (2017) 8:316. doi: 10.3389/fendo.2017.00316

189. Fröhlich E, Wahl R. Microbiota and thyroid interaction in health and disease. *Trends Endocrinol Metab* (2019) 30:479–90. doi: 10.1016/j.tem.2019.05.008

190. Kiełczykowska M, Kocot J, Paździor M, Musik I. Selenium - a fascinating antioxidant of protective properties. *Adv Clin Exp Med* (2018) 27:245–55. doi: 10.17219/acem/67222

191. Hu S, Rayman MP. Multiple nutritional factors and the risk of hashimoto's thyroiditis. *Thyroid* (2017) 27:597–610. doi: 10.1089/thy.2016.0635

192. Rasmussen LB, Schomburg L, Köhrle J, Pedersen IB, Hollenbach B, Hög A, et al. Selenium status, thyroid volume, and multiple nodule formation in an area with mild iodine deficiency. *Eur J Endocrinol* (2011) 164:585–90. doi: 10.1530/eje-10-1026

193. Huang Z, Rose AH, Hoffmann PR. The role of selenium in inflammation and immunity: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal* (2012) 16:705–43. doi: 10.1089/ars.2011.4145

194. Toulis KA, Anastasilakis AD, Tzellos TG, Goulis DG, Kouvelas D. Selenium supplementation in the treatment of hashimoto's thyroiditis: a systematic review and a meta-analysis. *Thyroid* (2010) 20:1163–73. doi: 10.1089/thy.2009.0351

195. Pizzulli A, Ranjbar A. Selenium deficiency and hypothyroidism: a new etiology in the differential diagnosis of hypothyroidism in children. *Biol Trace Elem Res* (2000) 77:199–208. doi: 10.1385/bter:77:3:199

196. Burk RF, Hill KE. Regulation of selenium metabolism and transport. *Annu Rev Nutr* (2015) 35:109–34. doi: 10.1146/annurev-nutr-071714-034250

197. Sebai H, Hovsépian S, Ristorcelli E, Aouani E, Lombardo D, Fayet G. Resveratrol increases iodide trapping in the rat thyroid cell line FRTL-5. *Thyroid* (2010) 20:195–203. doi: 10.1089/thy.2009.0171

198. Shih A, Davis FB, Lin HY, Davis PJ. Resveratrol induces apoptosis in thyroid cancer cell lines *via* a MAPK- and p53-dependent mechanism. *J Clin Endocrinol Metab* (2002) 87:1223–32. doi: 10.1210/jcem.87.3.8345

199. Truong M, Cook MR, Pinchot SN, Kunnimalaiyaan M, Chen H. Resveratrol induces Notch2-mediated apoptosis and suppression of neuroendocrine markers in medullary thyroid cancer. *Ann Surg Oncol* (2011) 18:1506–11. doi: 10.1245/s10434-010-1488-z

200. Benvenga S, Antonelli A. Inositol(s) in thyroid function, growth and autoimmunity. *Rev Endocr Metab Disord* (2016) 17:471–84. doi: 10.1007/s11154-016-9370-3

201. Nordio M, Pajalich R. Combined treatment with myo-inositol and selenium ensures euthyroidism in subclinical hypothyroidism patients with autoimmune thyroiditis. *J Thyroid Res* (2013) 2013:424163. doi: 10.1155/2013/424163

202. Paparo SR, Ferrari SM, Patrizio A, Elia G, Ragusa F, Botrini C, et al. Myoinositol in autoimmune thyroiditis. *Front Endocrinol (Lausanne)* (2022) 13:930756. doi: 10.3389/fendo.2022.930756

203. Nordio M, Basciani S. Treatment with myo-inositol and selenium ensures euthyroidism in patients with autoimmune thyroiditis. *Int J Endocrinol* (2017) 2017:2549491. doi: 10.1155/2017/2549491

204. Nordio M, Basciani S. Myo-inositol plus selenium supplementation restores euthyroid state in hashimoto's patients with subclinical hypothyroidism. *Eur Rev Med Pharmacol Sci* (2017) 21:51–9.

205. Nordio M, Basciani S. Evaluation of thyroid nodule characteristics in subclinical hypothyroid patients under a myo-inositol plus selenium treatment. *Eur Rev Med Pharmacol Sci* (2018) 22:2153–9. doi: 10.26355/eurrev_201804_14749

206. Pekala J, Patkowska-Sokoła B, Bodkowski R, Jamroz D, Nowakowski P, Lochyński S, et al. L-carnitine–metabolic functions and meaning in humans life. *Curr Drug Metab* (2011) 12:667–78. doi: 10.2174/138920011796504536

207. Gülçin I. Antioxidant and antiradical activities of l-carnitine. *Life Sci* (2006) 78:803–11. doi: 10.1016/j.lfs.2005.05.103

208. Wang ZY, Liu YY, Liu GH, Lu HB, Mao CY. L-carnitine and heart disease. *Life Sci* (2018) 194:88–97. doi: 10.1016/j.lfs.2017.12.015

209. Gnoni A, Longo S, Gnoni GV, Giudetti AM. Carnitine in human muscle bioenergetics: Can carnitine supplementation improve physical exercise? *Molecules* (2020) 25:182. doi: 10.3390/molecules25010182

210. Benvenga S, Ruggeri RM, Russo A, Lapa D, Campenni A, Trimarchi F. Usefulness of l-carnitine, a naturally occurring peripheral antagonist of thyroid hormone action, in iatrogenic hyperthyroidism: a randomized, double-blind, placebo-controlled clinical trial. *J Clin Endocrinol Metab* (2001) 86:3579–94. doi: 10.1210/jcem.86.8.7747

211. Benvenga S, Lapa D, Cannavò S, Trimarchi F. Successive thyroid storms treated with l-carnitine and low doses of methimazole. *Am J Med* (2003) 115:417–8. doi: 10.1016/s0002-9343(03)00399-1

212. Kimmoun A, Munagamage G, Dessalles N, Gerard A, Feillet F, Levy B. Unexpected awakening from comatose thyroid storm after a single intravenous injection of l-carnitine. *Intensive Care Med* (2011) 37:1716–7. doi: 10.1007/s00134-011-2293-2

213. Chee R, Agah R, Vita R, Benvenga S. L-carnitine treatment in a seriously ill cancer patient with severe hyperthyroidism. *Hormones (Athens)* (2014) 13:407–12. doi: 10.14310/horm.2002.1494

214. Maebashi M, Kawamura N, Sato M, Imamura A, Yoshinaga K. Urinary excretion of carnitine in patients with hyperthyroidism and hypothyroidism: augmentation by thyroid hormone. *Metabolism* (1977) 26:351–6. doi: 10.1016/0026-0495(77)90101-9

215. Sinclair C, Gilchrist JM, Hennessey JV, Kandula M. Muscle carnitine in hypo- and hyperthyroidism. *Muscle Nerve* (2005) 32:357–9. doi: 10.1002/mus.20336

216. An JH, Kim YJ, Kim KJ, Kim SH, Kim NH, Kim HY, et al. L-carnitine supplementation for the management of fatigue in patients with hypothyroidism on levothyroxine treatment: a randomized, double-blind, placebo-controlled trial. *Endocr J* (2016) 63:885–95. doi: 10.1507/endocrj.EJ16-0109

217. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. the international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* (2014) 11:506–14. doi: 10.1038/nrgastro.2014.66

218. Mishra V, Shah C, Mokashe N, Chavan R, Yadav H, Prajapati J. Probiotics as potential antioxidants: a systematic review. *J Agric Food Chem* (2015) 63:3615–26. doi: 10.1021/jf506326t

219. Gomes AC, Bueno AA, de Souza RG, Mota JF. Gut microbiota, probiotics and diabetes. *Nutr J* (2014) 13:60. doi: 10.1186/1475-2891-13-60

220. Wang J, Tang H, Zhang C, Zhao Y, Derrien M, Rocher E, et al. Modulation of gut microbiota during probiotic-mediated attenuation of metabolic syndrome in high fat diet-fed mice. *Isme J* (2015) 9:1–15. doi: 10.1038/ismej.2014.99

221. Rad AH, Abbasalizadeh S, Vazifekhah S, Abbasalizadeh F, Hassanalilou T, Bastani P, et al. The future of diabetes management by healthy probiotic microorganisms. *Curr Diabetes Rev* (2017) 13:582–9. doi: 10.2174/1573399812666161014112515

222. Asemi Z, Zare Z, Shakeri H, Sabihi SS, Esmaillzadeh A. Effect of multispecies probiotic supplements on metabolic profiles, hs-CRP, and oxidative stress in patients with type 2 diabetes. *Ann Nutr Metab* (2013) 63:1–9. doi: 10.1159/000349922

223. Wang Y, Wu Y, Wang Y, Xu H, Mei X, Yu D, et al. Antioxidant properties of probiotic bacteria. *Nutrients* (2017) 9:521. doi: 10.3390/nu9050521

224. Wang AN, Yi XW, Yu HF, Dong B, Qiao SY. Free radical scavenging activity of lactobacillus fermentum *in vitro* and its antioxidative effect on growing-finishing pigs. *J Appl Microbiol* (2009) 107:1140–8. doi: 10.1111/j.1365-2672.2009.04294.x

225. Stecchini ML, Del Torre M, Munari M. Determination of peroxy radical-scavenging of lactic acid bacteria. *Int J Food Microbiol* (2001) 64:183–8. doi: 10.1016/s0168-1605(00)00456-6

226. Kullisaar T, Zilmer M, Mikelsaar M, Vihalemm T, Annuk H, Kairane C, et al. Two antioxidative lactobacilli strains as promising probiotics. *Int J Food Microbiol* (2002) 72:215–24. doi: 10.1016/s0168-1605(01)00674-2

227. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutrition* (2012) 28:539–43. doi: 10.1016/j.nut.2011.08.013

228. Martarelli D, Verdenelli MC, Scuri S, Cocchioni M, Silvi S, Cecchini C, et al. Effect of a probiotic intake on oxidant and antioxidant parameters in plasma of athletes during intense exercise training. *Curr Microbiol* (2011) 62:1689–96. doi: 10.1007/s00284-011-9915-3

229. Zilmer M, Soomets U, Rehema A, Langel U. The glutathione system as an attractive therapeutic target. *Drug Design Reviews-Online (Discontinued)* (2005) 2:121–7. doi: 10.2174/1567269053202697

230. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science* (2005) 307:1915–20. doi: 10.1126/science.1104816

231. Berg RD. The indigenous gastrointestinal microflora. *Trends Microbiol* (1996) 4:430–5. doi: 10.1016/0966-842x(96)10057-3

232. Rajilić-Stojanović M, Smidt H, de Vos WM. Diversity of the human gastrointestinal tract microbiota revisited. *Environ Microbiol* (2007) 9:2125–36. doi: 10.1111/j.1462-2920.2007.01369.x

233. Hsiao WW, Metz C, Singh DP, Roth J. The microbes of the intestine: an introduction to their metabolic and signaling capabilities. *Endocrinol Metab Clin North Am* (2008) 37:857–71. doi: 10.1016/j.ecl.2008.08.006

234. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U.S.A.* (2007) 104:13780–5. doi: 10.1073/pnas.0706625104

235. Zhang J, Zhang F, Zhao C, Xu Q, Liang C, Yang Y, et al. Dysbiosis of the gut microbiome is associated with thyroid cancer and thyroid nodules and correlated with clinical index of thyroid function. *Endocrine* (2019) 64:564–74. doi: 10.1007/s12020-018-1831-x

236. Zitvogel L, Ayyoub M, Routy B, Kroemer G. Microbiome and anticancer immunosurveillance. *Cell* (2016) 165:276–87. doi: 10.1016/j.cell.2016.03.001

237. Rajagopala SV, Vashee S, Oldfield LM, Suzuki Y, Venter JC, Telenti A, et al. The human microbiome and cancer. *Cancer Prev Res (Phila)* (2017) 10:226–34. doi: 10.1158/1940-6207.capr-16-0249

238. Benitez AJ, Hoffmann C, Muir AB, Dods KK, Spergel JM, Bushman FD, et al. Inflammation-associated microbiota in pediatric eosinophilic esophagitis. *Microbiome* (2015) 3:23. doi: 10.1186/s40168-015-0085-6

239. Dahmus JD, Kotler DL, Kastenberg DM, Kistler CA. The gut microbiome and colorectal cancer: a review of bacterial pathogenesis. *J Gastrointest Oncol* (2018) 9:769–77. doi: 10.21037/jgo.2018.04.07

240. Trapani KM, Boghossian LJ. Clostridium subterminale septicemia in a patient with metastatic gastrointestinal adenocarcinoma. *Case Rep Infect Dis* (2018) 2018:6031510. doi: 10.1155/2018/6031510

241. Virili C, Fallahi P, Antonelli A, Benvenga S, Centanni M. Gut microbiota and hashimoto's thyroiditis. *Rev Endocr Metab Disord* (2018) 19:293–300. doi: 10.1007/s11154-018-9467-y

242. Wu Q, Rayman MP, Lv H, Schomburg L, Cui B, Gao C, et al. Low population selenium status is associated with increased prevalence of thyroid disease. *J Clin Endocrinol Metab* (2015) 100:4037–47. doi: 10.1210/jc.2015-2222

243. Bülow Pedersen I, Knudsen N, Carlé A, Schomburg L, Köhrle J, Jørgensen T, et al. Serum selenium is low in newly diagnosed graves' disease: a population-based study. *Clin Endocrinol (Oxf)* (2013) 79:584–90. doi: 10.1111/cen.12185

244. Schomburg L. Selenium deficiency due to diet, pregnancy, severe illness, or COVID-19-A preventable trigger for autoimmune disease. *Int J Mol Sci* (2021) 22:8532. doi: 10.3390/ijms22168532

245. Krassas GE, Pontikides N, Tziomalos K, Tzotzas T, Zosin I, Vlad M, et al. Selenium status in patients with autoimmune and non-autoimmune thyroid diseases from four European countries. *Expert Rev Endocrinol Metab* (2014) 9:685–92. doi: 10.1586/17446651.2014.960845

246. Gorini F, Sabatino L. Selenium: An element of life essential for thyroid function. *Molecules* (2021) 26:7084. doi: 10.3390/molecules26237084

10.3389/fendo.2022.1092837

Glossary

8-oxoG	8-oxo guanine
AMPK	adenosine monophosphate-activated protein kinase
BER	base excision repair
CAT	catalase
COX-2	cyclooxygenase-2
CRP	C-reactive protein
GD	Graves' disease
GPR120	G-protein-coupled surface receptor 120
GPx	glutathione peroxidase
GR	glutathione reductase
GSH	glutathione
H2O2	hydrogen peroxide
iNOS	inducible nitric oxide synthase
IP	inositol phosphates
KEAP1	Kelch-like ECH-associated protein 1
LDL-C	low-density lipoprotein-cholesterol
LOX	lipoxygenase
MDA	malondialdehyde
MUFA	monounsaturated fatty acids
Myo	myoinositol
NFE2L2	nuclear factor erythroid 2-related factor 2 gene
NF-kB	nuclear factor-missingb
NIS	sodium/iodide symporter
NO	nitric oxide
NOXs	NADPH-oxidases
OSI	oxidative stress index
OxS	oxidative stress
p53	p53 tumour suppressor protein
PI	phosphatidylinositol
PIP	phosphoinositides
PPARγ	peroxisome proliferator-activated receptor γ
PTC	papillary thyroid carcinoma
PUFA	polyunsaturated fatty acids
RNS	reactive nitrogen species
ROS	reactive oxygen species
SELENOS	selenoprotein S gene
SOD	superoxide dismutase
SOD1	cytoplasmic SOD
SOD2	mitochondrial SOD
SOD3	extracellular SOD
T2DM	type 2 diabetes mellitus
T3	triiodothyronine
T4	thyroxine
TAS	total antioxidant status
TG	thyroglobulin
TNF	tumour necrosis factor
TOS	total oxidative status
TPO	thyroid peroxidase
TRH	thyrotropin-releasing hormone
TSAb	thyroid-stimulating antibodies
TSH	thyroid-stimulating hormone

frontiers | Frontiers in Endocrinology

TYPE Systematic Review
PUBLISHED 30 January 2023
DOI 10.3389/fendo.2023.1119553

OPEN ACCESS

EDITED BY
Luna Samanta,
Ravenshaw University, India

REVIEWED BY
Jun Wang,
Jilin Agriculture University, China
Sergio Minucci,
Università della Campania Luigi Vanvitelli, Italy

*CORRESPONDENCE
Negar Azarpira
negarazarpira@yahoo.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 08 December 2022
ACCEPTED 17 January 2023
PUBLISHED 30 January 2023

CITATION
Dehdari Ebrahimi N, Parsa S, Nozari F, Shahlaee MA, Maktabi A, Sayadi M, Sadeghi A and Azarpira N (2023) Protective effects of melatonin against the toxic effects of environmental pollutants and heavy metals on testicular tissue: A systematic review and meta-analysis of animal studies.
Front. Endocrinol. 14:1119553.
doi: 10.3389/fendo.2023.1119553

Protective effects of melatonin against the toxic effects of environmental pollutants and heavy metals on testicular tissue: A systematic review and meta-analysis of animal studies

Niloofar Dehdari Ebrahimi[1†], Shima Parsa[1], Farnoosh Nozari[1], Mohammad Amin Shahlaee[1], Amirhossein Maktabi[1], Mehrab Sayadi[2], Alireza Sadeghi[3†] and Negar Azarpira[1*]

[1]Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, [2]Cardiovascular research center, Shiraz University of Medical Sciences, Shiraz, Iran, [3]Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Environmental pollution and infertility are two modern global challenges that agonize personal and public health. The causal relationship between these two deserves scientific efforts to intervene. It is believed that melatonin maintains antioxidant properties and may be utilized to protect the testicular tissue from oxidant effects caused by toxic materials.

Methods: A systematic literature search was conducted in PubMed, Scopus, and Web of Science to identify the animal trial studies that evaluated melatonin therapy's effects on rodents' testicular tissue against oxidative stress caused by heavy metal and non-heavy metal environmental pollutants. Data were pooled, and standardized mean difference and 95% confidence intervals were estimated using the random-effect model. Also, the risk of bias was assessed using the Systematic Review Centre for Laboratory animal Experimentation (SYRCLE) tool. (PROSPERO: CRD42022369872)

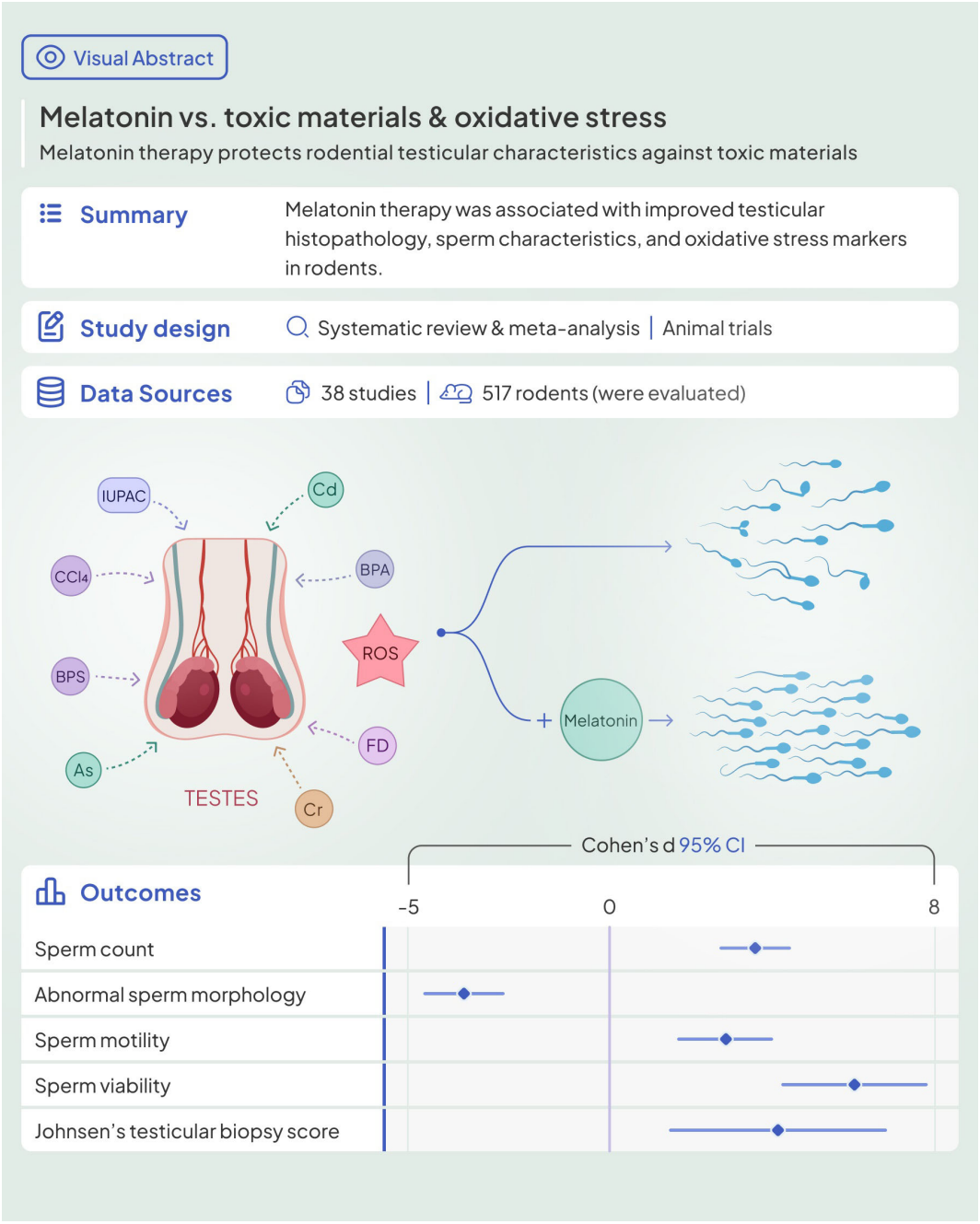
Results: Out of 10039 records, 38 studies were eligible for the review, of which 31 were included in the meta-analysis. Most of them showed beneficial effects of melatonin therapy on testicular tissue histopathology. [20 toxic materials were evaluated in this review, including arsenic, lead, hexavalent chromium, cadmium, potassium dichromate, sodium fluoride, cigarette smoke, formaldehyde, carbon tetrachloride (CCl4), 2-Bromopropane, bisphenol A, thioacetamide, bisphenol S, ochratoxin A, nicotine, diazinon, Bis(2-ethylhexyl) phthalate (DEHP), Chlorpyrifos (CPF), nonylphenol, and acetamiprid.] The pooled results showed that melatonin therapy increased sperm count, motility, viability and body and testicular weights, germinal epithelial height, Johnsen's biopsy score, epididymis weight, seminiferous tubular diameter, serum testosterone, and luteinizing hormone levels, testicular tissue Malondialdehyde, glutathione peroxidase, superoxide dismutase, and glutathione levels. On the other hand, abnormal sperm morphology, apoptotic index, and testicular tissue nitric oxide were lower in the melatonin therapy arms. The included studies presented a high risk of bias in most SYRCLE domains.

Conclusion: In conclusion, our study demonstrated amelioration of testicular histopathological characteristics, reproductive hormonal panel, and tissue markers of oxidative stress. Melatonin deserves scientific attention as a potential therapeutic agent for male infertility.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO, identifier CRD42022369872.

KEYWORDS

melatonin, infertility, rodents, oxidative stress, environmental pollutants, heavy metals



GRAPHICAL ABSTRACT

1 Introduction

Infertility is a universal public health issue with a dramatically increasing prevalence in recent decades (1). About 48 million couples worldwide suffer from fecundity problems (2), of which half have been implicated by male factors (3). Male infertility may result from various factors such as genetic, epigenetic, physical injuries, drugs, and environmental pollutants. Among these, pesticides, plasticizers, refrigerants, dry cleaners, and heavy metals are of great interest as they are widely used in industry. Environmental pollutants impair the function of male fecundity by altering hormonal, molecular, and histological characteristics. These pollutants mostly affect biomechanics through oxidative stress and increasing free radicals causing a shift in the equilibrium between the production of free-radical species and the antioxidant defense system in male reproductive cells (4, 5).

Reactive oxygen species (ROS) are highly reactive molecules generated by cellular metabolism. A physiological level of ROS is essential for the proper development of spermatozoa, including their production, maturation, morphological reshaping, and fertilization process (6). Redox reactions serve as cofactors for the spermatozoa maturation, free radicals also stimulate intracellular pathways resulting acrosome reaction, capacitation, motility, and condensation of chromatin (7–9). ROS concentration increases in excessive exposure to environmental pollutants (10); This excessive ROS overwhelms the cellular antioxidant defense system and accelerates oxidative stress (11). The elevated ROS attacks multiple cellular macromolecules leading to DNA damage, lipid peroxidation, and protein misfolding, resulting in mitochondrial dysfunction and sperms' structural integrity impairment (12–16). The damage worsens by changes in the apoptotic index, cell vacuolization, and diminished capacity to proliferate, followed by a reduction in sperm viability and count (17, 18). Among the different cell types, spermatozoa are highly vulnerable to oxidation due to the abundance of unsaturated fatty acids in the membrane, lack of proper DNA repair mechanisms, and the absence of cytoplasmic antioxidant enzymes, with concomitant negative consequences on sperm quality (19–23).

Antioxidants have gained attention for their role in infertility (24–27). So far, various supplements with antioxidant capabilities, such as ginger, vitamin C, and vitamin E, have been shown to improve hormonal and histological parameters related to male reproduction (28–30). Melatonin also has exhibited potent protective activities against oxidative stress-induced testicular cell damage (31). Melatonin synthetic enzymes and melatonin membrane receptors are identified in testicular cells, indicating the importance of the melatonergic system in male reproduction. It also directly affects testosterone production from Leydig cells (32). Melatonin has recently gained scientific interest due to its protection against oxidative stress-induced testicular cell damage (33, 34). It protects testicular cells from elevated ROS through anti-apoptotic and antioxidant activities (34).

Despite the wealth of evidence, consensus and structured gathering of evidence are still needed. This systematic review and meta-analysis aims to congregate the evidence on melatonin as a protective agent against rodential testicular damages caused by environmental pollutants and toxic materials.

2 Material and methods

This systematic review of relevant studies was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses guideline (PRISMA). The protocol is registered in the International Prospective Register of Systematic Reviews (PROSPERO: CRD42022369872). We systematically searched PubMed, Scopus, and Web of Science from January 1, 1970, until September 9, 2022, for "melatonin" and "reproductive indices" related terms (Supplementary Material 1). Also, we manually searched the reference list of the included papers for additional citations of interest.

2.1 Study selection and eligibility criteria

Firstly, duplicate records were removed automatically. All the records were uploaded to the Rayyan online tool for managing systematic reviews. Three reviewers (NDE, AS, and MAS) screened the records by title and abstract. Then, records were screened for eligibility criteria. Discrepancies were resolved with discussion. Studies were included if they fulfilled the following criteria (1): controlled animal studies (2), the subjects were rodents that were exposed to toxic environmental materials such as environmental pollutants and heavy metals to induce oxidative stress (3), at least one intervention group received melatonin regimen (4), at least one control group with similar oxidative stress that did not receive melatonin (with or without placebo), and (5) the study reported major hallmarks of testicular tissue (histopathologic, biochemical, and sperm analyses). We excluded the studies if they (1): designed as *in-vitro* and ex-vivo, (2) employed non-rodent animals, (3) studied other types of stressors such as physical, ischemic, heat, radiation, chemotherapy, and metabolic agents, (4) melatonin was administered in combination with other drugs or the study employed melatonin derivatives, (5) only evaluated healthy controls without oxidative stress, and (6) they failed to report favorable outcomes. Also, we excluded reviews, letters, and human trials.

2.2 Data extraction and assessment of risk of bias

Two reviewers (FN and NDE) independently extracted the data into Excel spreadsheets, and three reviewers (AS, MAS, and AM) rechecked the data for any mistakes. The following data were extracted from each study: (1) study characteristics (first author, publication year, and country), (2) population characteristics (species, age, and sample size), (3) toxic material, dose, administration route, and duration of exposure, (4) melatonin dose, duration, route, and setting of administration (before, simultaneous, or after oxidative stress), (5) sperm characteristics (count, motility, viability, and abnormal morphology), testicular parameters (height of germinal epithelium, Johnsen's testicular biopsy score (JTBS), seminiferous tubular and luminal diameter, and apoptotic index), hormonal panel (serum testosterone, Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH)), markers of oxidative stress (testicular tissue Superoxide dismutase (SOD), Catalase (CAT) activity,

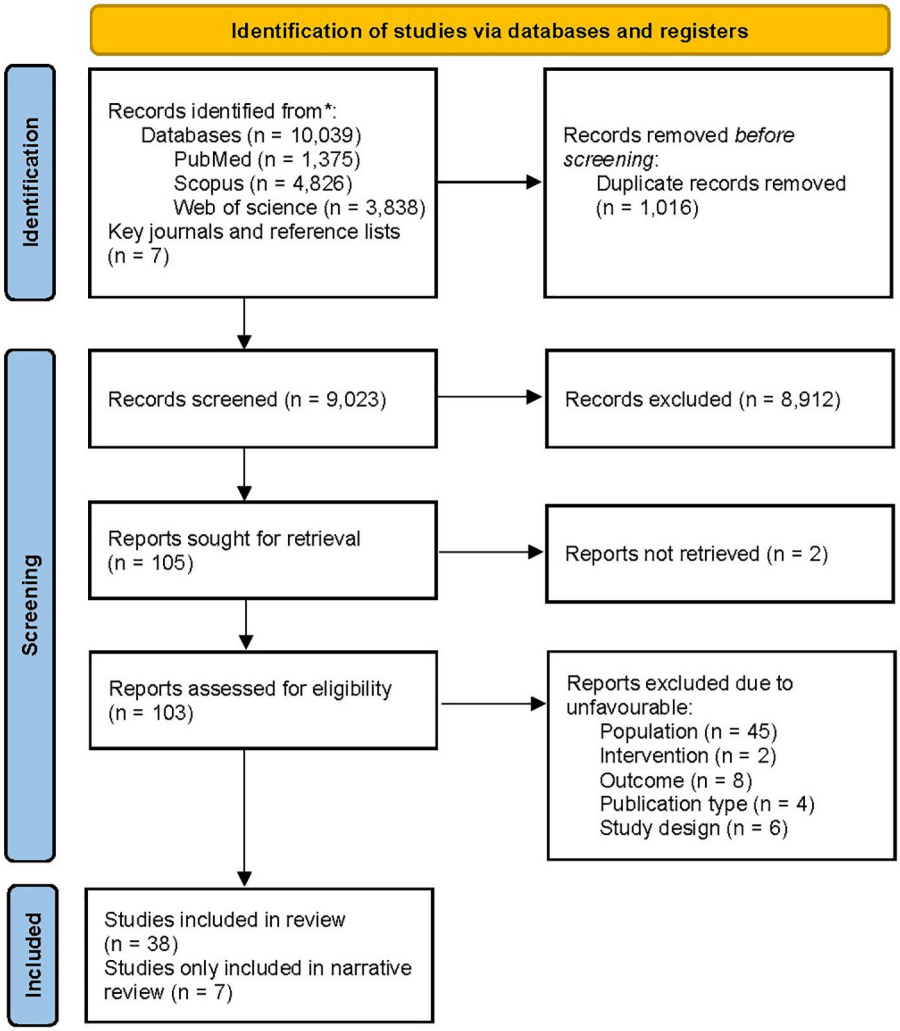


FIGURE 1
PRISMA flow chart of the literature screening. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

Malondialdehyde (MDA), glutathione peroxidase (GPx), glutathione (GSH), and nitric oxide (NO), and somatic characteristics (testis to body relative weight, total testis weight, epididymis weight, body weight, and body weight gain).

Two reviewers (NDE and AS) independently assessed the risk of bias using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool for animal intervention studies.

2.3 Data synthesis and statistical analyses

Meta-analysis was run *via* Stata 13 (College Station, TX, USA) using the DerSimonian-Laird random effect model. Standardized mean difference (SMD) was considered as the effect size for comparing the mean difference of variables between the control and intervention groups. The amount of heterogeneity in the studies was indicated by I-squared. Subgroup analyses were done where at least two studies were available in each subgroup to investigate the differences between heavy metal and non-heavy metal pollutants. Also, the forest plot was provided for each study, and pooled data publication bias was assessed by Egger's test. In addition, sensitivity analysis was done to check for the robustness of our results.

3 Results

3.1 Search results

The PRISMA flow diagram of the literature search is presented in Figure 1. The systematic search resulted in 10,039 records while manual citation searching yielded 7 additional studies. The database searching included PubMed (n=1,375), Web of Science (n=3,838), and Scopus (n=4,826). 1,016 records were removed using automatic duplicate detection. Title and abstract screening was conducted on 9,023 records and 98 studies was sought for retrieval. With exclusion of two studies, which the full-texts were not found and our effort to communicate with the authors failed, 103 articles were assessed for eligibility. A total of 65 articles were excluded due to ineligible population (n=45), design (n=6), intervention (n=2), outcome (n=8), and publication type (n=4). Finally, a total of 38 articles

10.3389/fendo.2023.1119553

TABLE 1 General characteristics of the included studies.

Study name	Rodent	Number of subjects (intervention, control)	Age of subject	Injury agent
Studies that employed heavy metals				
Uygur [2013] (67)	Rats	9, 9	6 weeks	Arsenic
Olayaki [2018] (58)	Rats	5, 5	NA	Lead
Lv [2017] (38)	Mice	6, 6	8 weeks	Hexavalent chromium
Bustos-Obregón [2013] a (37)	Mice	NA, NA	3 months	Arsenic
Sobhani [2015] (Persian) (42)	Rats	8, 8	6-8 weeks	Cadmium
Bashandy [2021] (52)	Rats	8, 8	3 months	Potassium dichromate (PDC)
Bustos-Obregón [2013] b (45)	Mice	22, 22	12 weeks	Arsenic
Venditti [2021] b (68)	Rats	6, 6	8 weeks	Cadmium
Venditti [2021] a (69)	Rats	6, 6	2 months	Cadmium
Ji [2011] (47)	Mice	12, 12	8 weeks	Cadmium
Kara [2007] (55)	Rats	12, 12	14–16 weeks	Cadmium
Li [2015] (48)	Mice	10, 10	8 weeks	Cadmium
Studies that employed environmental pollutants				
Rao [2012] (61)	Rats	15, 15	NA	Sodium Fluoride (NaF)
Aslani [2015] (39)	Rats	5, 5	3-4 months	Cigarette smoke
Abd el salam [2020] (40)	Rats	10, 10	N/A	Formaldehyde
Wang [2018] (41)	Rats	8, 8	N/A	Carbon tetrachloride (CCl4)
Huang [2009] (54)	Rats	6, 6	8 weeks	2-Bromopropane
Kadir [2021] (63)	Rats	6, 6	1 day	Bisphenol A
Karabulut [2020] (56)	Rats	7, 7	NA	Thioacetamide
Kumar [2020] (71)	Hamsters	6, 6	90-100 days	Bisphenol S
Malekinejad [2014] (57)	Rats	8, 8	8 weeks	Ochratoxin A (OTA)
Mohammadghasemi [2018] (49)	Mice	8, 8	10–12 weeks	Nicotine
Sarabia [2011] (50)	Mice	6, 6	12 weeks	Diazinon
Othman [2014] (17)	Rats	8, 8	8 weeks	Bisphenol A
Ozen [2008] (60)	Rats	7, 7	NA	Formaldehyde
Anjum [2011] (43)	Mice	6, 6	NA	Bisphenol A
Bahrami [2018] (44)	Mice	8, 8	4 weeks	Bis(2-ethylhexyl) phthalate (DEHP)
Sarabia [2009] a (36)	Mice	NA, NA	12 weeks	Diazinon
Dunjić [2022] (53)	Rats	6, 6	NA	Carbon tetrachloride (CCl4)
Ajani [2019] (thesis) (59)	Rats	10, 10	NA	Bisphenol A
Umosen [2014] (66)	Rats	6, 6	7-8 weeks	Chlorpyrifos (CPF)
Elwakeel [2018] (46)	Mice	6, 6	9-12 months	Bisphenol A
Rashad [2021] (62)	Rats	7, 7	8 weeks	Bisphenol A
Sarabia [2009] b (35)	Mice	NA, NA	12 weeks	Diazinon
Tabassum [2016] (64)	Rats	8, 8	NA	Nonylphenol
Umosen [2012] (65)	Rats	10, 10	NA	Chlorpyrifos (CPF)
Wu [2013] (70)	Rats	10, 10	8 weeks	Bisphenol A
Zayman [2022] (51)	Mice	6, 7	32 weeks	Acetamiprid

NA, Not Available.

10.3389/fendo.2023.1119553

TABLE 2 Details on experimental designs of each included study.

Study name	Injury agent					Melatonin				
	Name	Route	Each dose (mg/kg)	Duration	Cumulative dose (mg/kg)	Route	Each dose (mg/kg)	Duration	Cumulative dose (mg/kg)	Mode of intervention
Studies that employed heavy metals										
Uygur [2013] (67)	Arsenic	Oral	5	30 days	150	IP	25	30 days	750	Therapeutic
Olayaki [2018] (58)	Lead	Oral	50	28 days	1400	Oral	4 and 10	2 and 4 weeks	56, 112, 140, and 280	Therapeutic
Lv [2017] (38)	Hexavalent chromium	IP	16.2	7 days	113.4	IP	25	7 days	175	Simultaneous
Bustos-Obregón [2013] a (37)	Arsenic	Oral	7	8, 32, and 66 days	58.1, 225.4, and 464.8	Oral	10	8, 32, and 66 days	80, 320, and 660	Therapeutic
Sobhani [2015] (Persian) (42)	Cadmium	IP	2	30 days	60	IP	10, 15, and 20	30 days	300, 450, and 600	Simultaneous
Bashandy [2021] (52)	Potassium dichromate (PDC)	Oral	10	56 days	560	IP	2.5 and 5	8 weeks	140 and 280	Preventive
Bustos-Obregón [2013] b (45)	Arsenic	Oral	7	33 days	231	Oral	10	33 days	330	Simultaneous
Venditti [2021] b (68)	Cadmium	Oral	50	NA	NA	Oral	NA	NA	NA	Simultaneous
Venditti [2021] a (69)	Cadmium	Oral	50 mg CdCl2/L	40 days	2000 mg CdCl2/L	NA	3 mg/L	40 days	120 mg/L	Therapeutic
Ji [2011] (47)	Cadmium	IP	2	1 day	2	IP	5	1-2 days	20	Preventive
Kara [2007] (55)	Cadmium	SQ	1	30 days	30	SQ	10	1 month	300	NA
Li [2015] (48)	Cadmium	IP	2	7 days	14	IP	10	7 days	70	Preventive
Studies that employed environmental pollutants										
Rao [2012] (61)	Sodium Fluoride (NaF)	Oral	10	60 days	600	IP	10	60 days	600	Preventive
Aslani [2015] (39)	Cigarette smoke	Inhaled	30 minutes	3 days	90 minutes	IP	25	5 days	125	Therapeutic
Abd el salam [2020] (40)	Formaldehyde	IP	10	30 days	150	IP	25	30 days	375	Simultaneous
Wang [2018] (41)	Carbon tetrachloride (CCl4)	IP	8 g/kg	1 day	8 g/kg	IP	10	2 days	20	Preventive
Huang [2009] (54)	2-Bromopropane	IP	1000	7 days	7000	IP	5	1 day	5	Preventive
Kadir [2021] (63)	Bisphenol A	SQ and oral	25 and 50	4 and 49 days	100 and 200	SQ and oral	10	4 and 49 days	40 and 490	Therapeutic
Karabulut [2020] (73)	Thioacetamide	IP	300	1 day	600	IP	10	1 and 2 day	10 and 20	Therapeutic and preventive
Kumar [2020] (71)	Bisphenol S	Oral	75	28 days	2100	IP	10	28 days	140	Preventive
Malekinejad [2014] (57)	Ochratoxin A (OTA)	Oral	0.2	28 days	5.6	Oral	15	28 days	420	Therapeutic
Mohammadghasemi [2018] (49)	Nicotine	IP	1	30 days	3	IP	10	30 days	300	Therapeutic
Sarabia [2011] (50)	Diazinon	IP	21.6 and 43.3	1 day	21.6 and 43.3	IP	10	Single dose	10	Preventive
Othman [2014] (17)	Bisphenol A	NA	50	21 and 42 days	450 and 900	NA	10	3 and 6 weeks	90 and 180	Preventive

(Continued)

TABLE 2 Continued

Study name	Injury agent					Melatonin				
	Name	Route	Each dose (mg/kg)	Duration	Cumulative dose (mg/kg)	Route	Each dose (mg/kg)	Duration	Cumulative dose (mg/kg)	Mode of intervention
Ozen [2008] (60)	Formaldehyde	IP	10	30 days	150	IP	25	30 days	375	Therapeutic
Anjum [2011] (43)	Bisphenol A	NA	10	14 days	140	NA	10	14 days	140	Therapeutic
Bahrami [2018] (44)	Bis(2-ethylhexyl) phthalate (DEHP)	Oral	2000	14 days	28000	IP	10	14 days	140	NA
Sarabia [2009] a (36)	Diazinon	IP	21.6 and 43.3	1 day	21.6 and 43.3	IP	10	Single dose	10	Preventive
Dunjić [2022] (53)	Carbon tetrachloride (CCl4)	NA	1 ml/kg	1 day	1 ml/kg	NA	50	Single dose	50	Therapeutic
Ajani [2019] (thesis) (59)	Bisphenol A	Oral	10	45 days	450	IP	10	45 days	450	Simultaneous
Umosen [2014] (66)	Chlorpyrifos (CPF)	Oral	8.5	28 days	238	Oral	0.5	28 days	14	Preventive
Elwakeel [2018] (46)	Bisphenol A	Oral	50 and 100	48 days	900 and 1800	Oral	100	48 days	1800	Preventive
Rashad [2021] (62)	Bisphenol A	IP	50	21 days	450	IP	10	21 days	90	Preventive
Sarabia [2009] b (35)	Diazinon	IP	21.6 and 43.3	1 day	21.6 and 43.3	IP	10	Single dose	10	Preventive
Tabassum [2016] (64)	Nonylphenol	Oral	25	45 days	1125	IP	10	45 days	450	Preventive
Umosen [2012] (65)	Chlorpyrifos (CPF)	Oral	8.5	28 days	238	Oral	0.5	28 days	14	Preventive
Wu [2013] (70)	Bisphenol A	Oral	200	10 days	2000	IP	10	10 days	100	Preventive
Zayman [2022] (51)	Acetamiprid	Oral	25	21 days	525	IP	20	21 days	420	NA

IP, Intraperitoneal; SQ, Subcutaneous; CdCl2, Cadmium chloride; NA, Not Available.

were eligible for the study; among them, 7 (35–41) were only included in the narrative data synthesis.

3.2 Study characteristics

Studies were published between 2007 and 2022 and all were published in English except one which was in Persian (42). Mice, rats, and hamsters were the subjects of 13 (35–38, 43–51), 24 (17, 39–42, 52–70), and one (71) studies, respectively. Studies have utilized heavy metals (n= 12) including arsenic (37, 45, 67), lead (58), cadmium (42, 47, 48, 55, 68, 69), Hexavalent chromium (38), and Potassium dichromate (52) and toxic materials (n= 26) including sodium fluoride (NaF) (61), 2-Bromopropane (54), Bisphenol A (17, 43, 46, 59, 62, 63, 70), thioacetamide (56), Bisphenol S (71), ochratoxin A (57), nicotine (49), Cigarette smoke (39), diazinon (35, 36, 50), formaldehyde (40, 60), bis(2-ethylhexyl) phthalate (DEHP) (44), carbon tetrachloride (CCl4) (41, 53), Chlorpyrifos (65, 66), nonylphenol (64), and acetamiprid (51) to induce oxidative stress. To administer the stressors, oral (n= 19) (37, 44–46, 51, 52, 57–59, 63–72), intraperitoneal (n= 14, IP) (35, 36, 38, 40–42, 47–50, 54, 56, 60, 62), and subcutaneous (n= 2, SQ) (55, 63) routes were used. To administer melatonin, oral (n= 9) (37, 45, 46, 57, 58, 63, 65, 66, 68), IP (n= 24) (35, 36, 38–42, 44, 47–52, 54, 56, 59, 60, 62, 64, 67, 70–72), and SQ (n= 2) (55, 63) routes were used. Melatonin was administered prior to (n= 18, preventive) (17, 35, 36, 41, 46–48, 50, 52, 54, 56, 62, 64–66, 70–72), simultaneously (n= 6) (38, 40, 42, 45, 59, 68), and after (n= 12, therapeutic) (37, 39, 43, 49, 53, 56–58, 60, 63, 67, 69) the start of stressor. The characteristics of the included studies are summarized in the Tables 1, 2.

3.3 Sperm and somatic characteristics

Sperm characteristics were reported in the included studies as abnormal morphology, count, motility, and viability. Melatonin therapy significantly improved all these parameters: abnormal morphology (SMD -3.59 with 95% CI -4.60, -2.59), count (SMD 3.56 with 95% CI 2.7, 4.42), motility (SMD 2.92 with 95% CI 2.16, 3.69), and viability (SMD 5.99 with 95% CI 4.19, 7.78), all with p-values <0.001.

Between-study heterogeneity was substantial for all these outcomes with I-squared ranging between 81% and 88% and p-values <0.001 for all outcomes. Also, Egger's test showed statistically significant publication bias in all the outcomes with p-values <0.001. Forest plots of analyses for sperm parameter outcomes are presented in Figure 2.

We categorized relative testis to body, total testicular, and body weight and body weight gain as somatic indices. The meta-analyses showed a significant impact of melatonin therapy on total testicular and body weight (SMD 1.15 with 95%CI 0.56, 1.73 and p-value <0.001

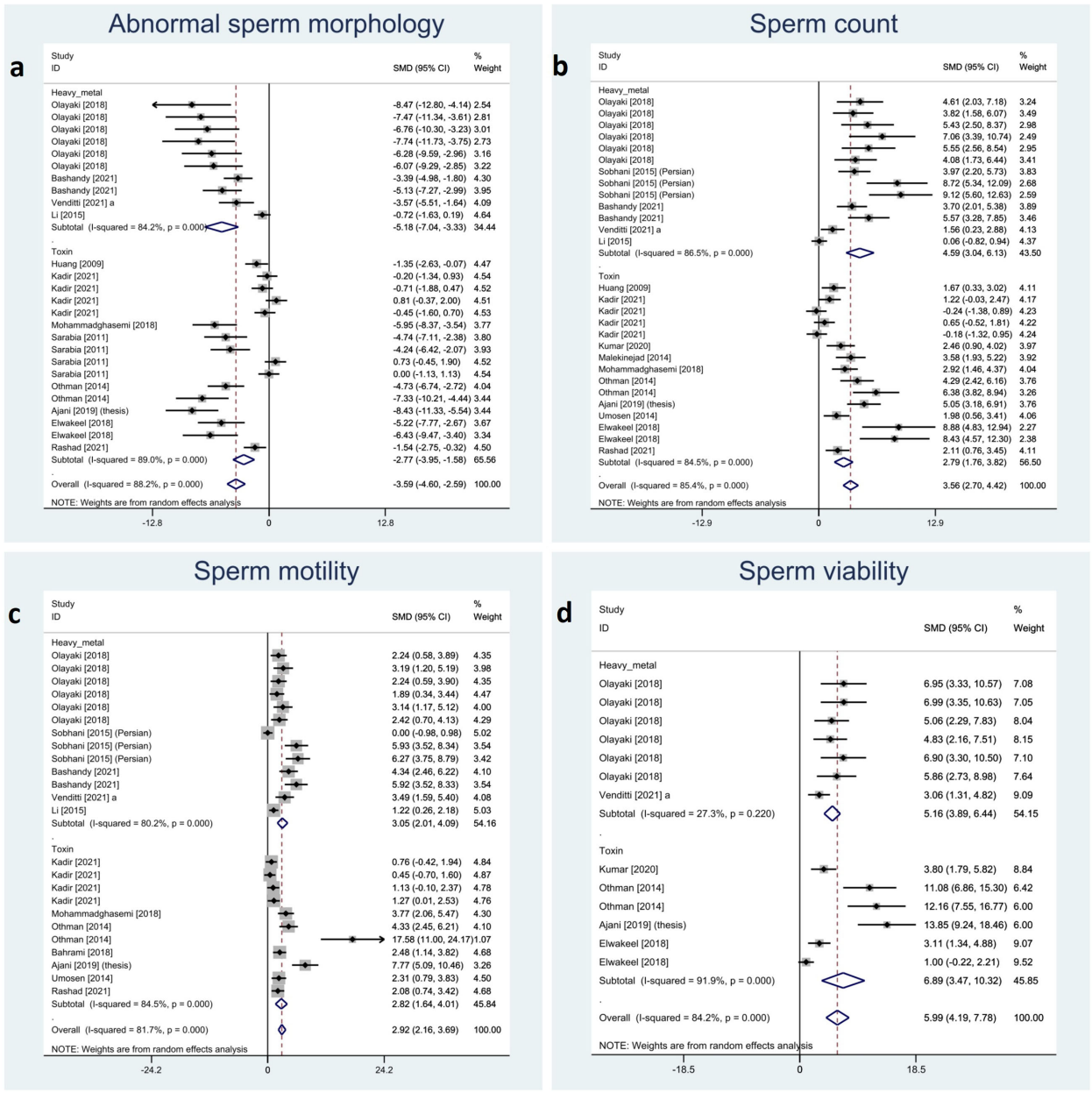


FIGURE 2
Forest plots for overall and subgroup effect measures on the impact of melatonin therapy on sperm characteristics including **(A)** abnormal sperm morphology, **(B)** count, **(C)** motility, and **(D)** viability.

and SMD 1.11 with 95%CI 0.42, 1.80 and p-value 0.002, respectively). Although, testis to body relative weight and body weight gain were not significantly affected by melatonin therapy (SMD 0.85 with 95% CI -0.05, 1.74 and p-value 0.064 and SMD -0.18 with 95%CI -1.62, 1.25 and p-value 0.803, respectively). Investigation of between-study variation revealed substantial heterogeneity with I-squared ranging between 72% and 83% (p-values <0.001 and 0.002). Assessment of publication bias was not feasible for body weight gain due to low sample size. Egger's test showed statistically significant publication bias in testis to body relative weight and total testicular weight (p-value 0.003 and 0.012, respectively). Forest plots and detailed results of Egger's test for sperm parameter outcomes is presented in Figure 3 and Supplementary Material 2.

3.4 Testicular tissue parameters

Testicular parameters were reported in the included studies as height of germinal epithelium, JTBS, tubular diameter, luminal diameter, epididymis weight, and apoptotic index. Meta-analyses on these variables showed that melatonin therapy significantly increased height in germinal epithelium (SMD 3.63 with 95% CI 2.05, 5.21 and p-value <0.001), JTBS (SMD 4.13 with 95% CI 1.44, 6.81 and p-value <0.001), tubular diameter (SMD 2.44 with 95% CI 1.41, 3.47 and p-value <0.001), and epididymis weight (SMD 1.03 with 95% CI.014, 1.93 and p-value 0.024) and decreased apoptotic index (SMD -4.07 with 95% CI -7.23, -0.91 and p-value 0.012). Although not statistically significant, melatonin therapy increased luminal diameter (SMD 0.45 with 95% CI -0.90, 1.79 and p-value 0.515).

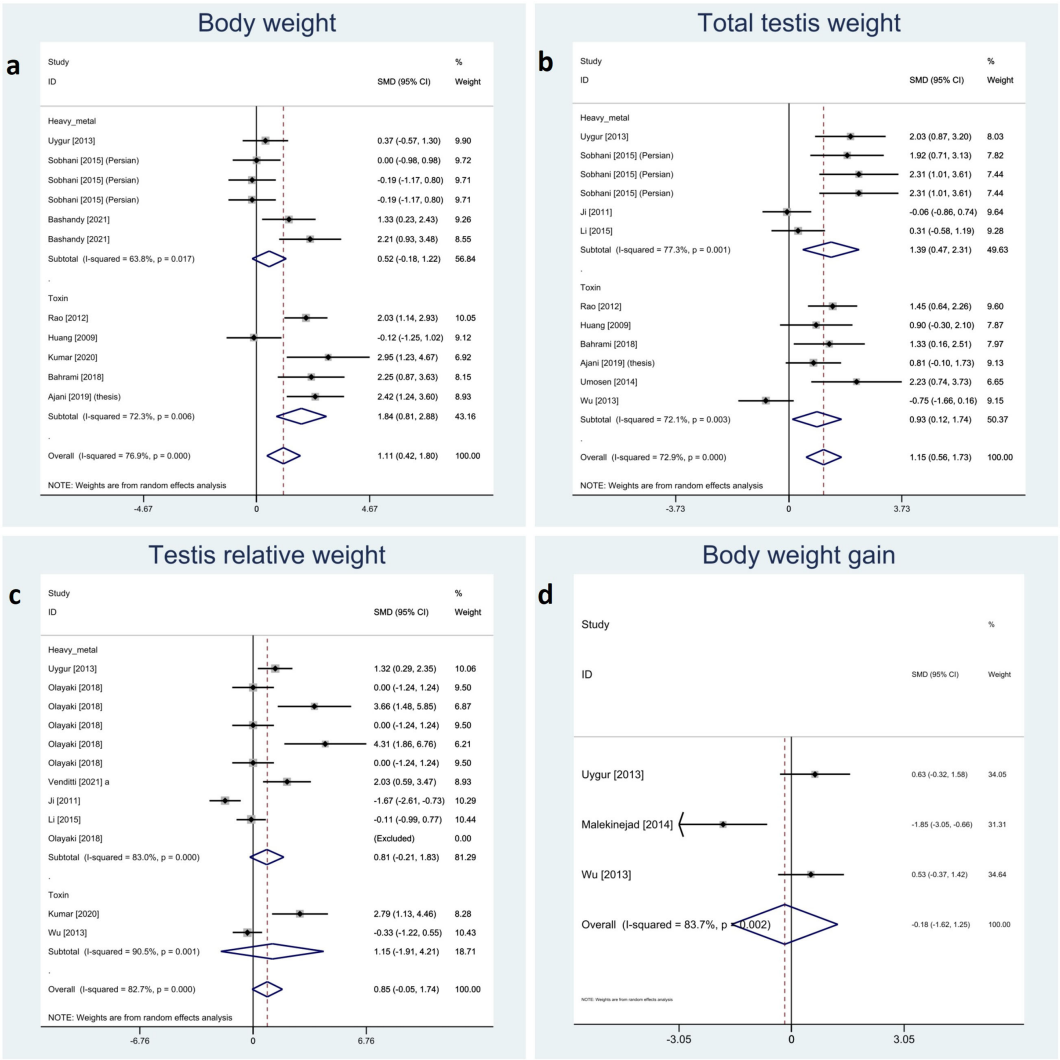


FIGURE 3
Forest plots for overall and subgroup effect measures on the impact of melatonin therapy on the **(A)** body, **(B)** total and **(C)** relative testicular weights and **(D)** body weight gain.

Between-study heterogeneity was considerable for all these outcomes with I-squared ranging between 76% and 91% and p-values <0.001 for all outcomes. Egger's test showed statistically significant publication bias in all the outcomes with p-values <0.001 for tubular diameter, and epithelial height and 0.011, 0.026 and 0.008 for JTBS, luminal diameter, and epididymis, respectively. Forest plots and detailed results of Egger's test for sperm parameter outcomes are presented in Figure 4 and Supplementary Material 2.

3.5 Reproductive hormones

Included studies reported serum FSH, LH, and testosterone; among them, melatonin therapy increased serum LH and testosterone significantly (SMD 1.61 with 95% CI 0.59, 2.63 and p-value 0.002 and SMD 1.87 with 95% CI 1.14, 2.60 and p-value <0.001, respectively). On the other hand, changes in serum FSH were not statistically significant (SMD 0.55 with 95% CI -0.49, 1.60 and p-value 0.299). Between-study heterogeneity was substantial or considerable for reproductive hormones I-squared ranging between 85% and 88% with p-value <0.001. Egger's test showed significant publication bias for serum LH with p-value <0.001. Forest plots and detailed results of Egger's test for reproductive hormones outcomes are presented in Figure 5 and Supplementary Material 2.

3.6 Oxidative markers

All the reported oxidative markers showed significant changes with melatonin therapy: testicular tissue CAT (SMD 2.34 with 95%CI 1.51, 3.17), GSH (SMD 2.82 with 95%CI 1.46, 4.18), GPx (SMD 1.26 with 95%CI 0.51, 2.02), MDA (SMD -4.83 with 95%CI -6.05, -3.61), SOD (SMD 1.62 with 95%CI 0.81, 2.44), and NO (SMD -1.93 with 95%CI -2.97, -0.90) with all p-values <0.001. Between-study heterogeneity was substantial to considerable I-squared ranging between 60% to 90% with all p-values <0.001 except for NO (p-value 0.054). Using Egger's regression model, all these outcomes suffered from publication bias (p-values <0.001) except GPx (p-value 0.992). Forest plots and detailed

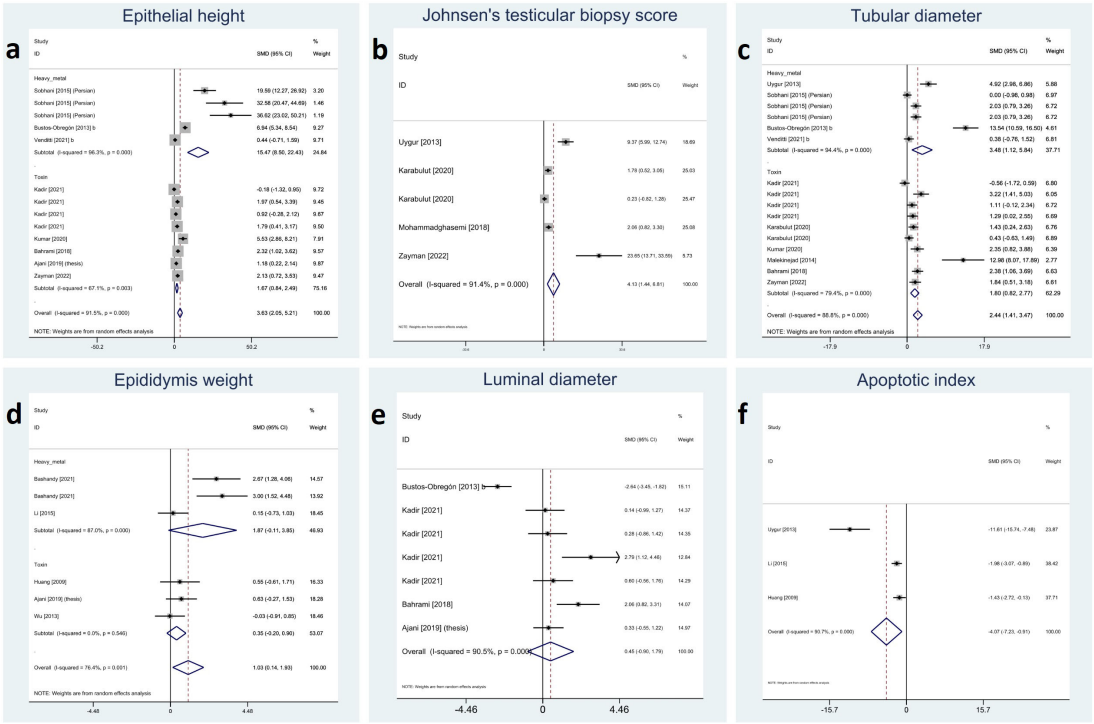


FIGURE 4
Forest plots for overall and subgroup effect measures on the impact of melatonin therapy on the parameters of testicular tissue including **(A)** epithelial height, **(B)** Johnsen's biopsy score, **(C)** seminiferous tubular diameter, **(D)** epididymis weight, **(E)** seminiferous luminal diameter, and **(F)** apoptotic index.

results of Egger's test for sperm parameter outcomes is presented in Figure 6 Supplementary Material 2.

3.7 Sensitivity analyses and risk of bias assessment

Sensitivity analyses were done with omitting one study each time to investigate robustness of our results. The leave-one-out plots are provided in the Supplementary Materials 3. After removing studies from the analyses individually, none substantially affected the pooled SMD estimates in the study.

For each domain, studies scored 1 if they were assessed as low risk. Studies scored between 2 and 4 for risk of bias assessment by SYRCKLE checklist. All the studies were labeled as unclear risk on random sequence generation, allocation concealment, random housing, blinding of investigators and outcome assessors, and random outcome assessment. For other sources of bias, all the studies were assessed as low risk. 21, 12, and 37 studies were labeled as low risk on baseline characteristics, incomplete outcome data, and selective outcome reporting, respectively. All the details are presented in the Figure 7 and Supplementary Material 4.

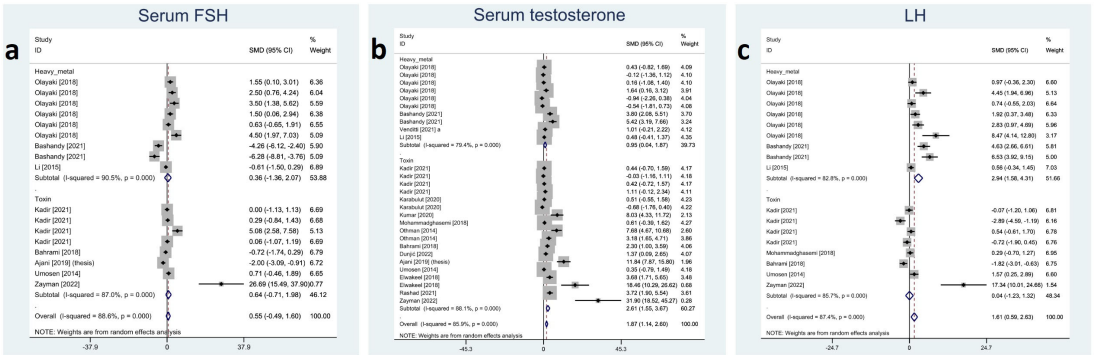


FIGURE 5
Forest plots for overall and subgroup effect measures on the impact of melatonin therapy on the serum level of reproductive hormones including **(A)** FSH, **(B)** testosterone, and **(C)** LH. FSH, Follicle-Stimulating Hormone; LH, Luteinizing Hormone.

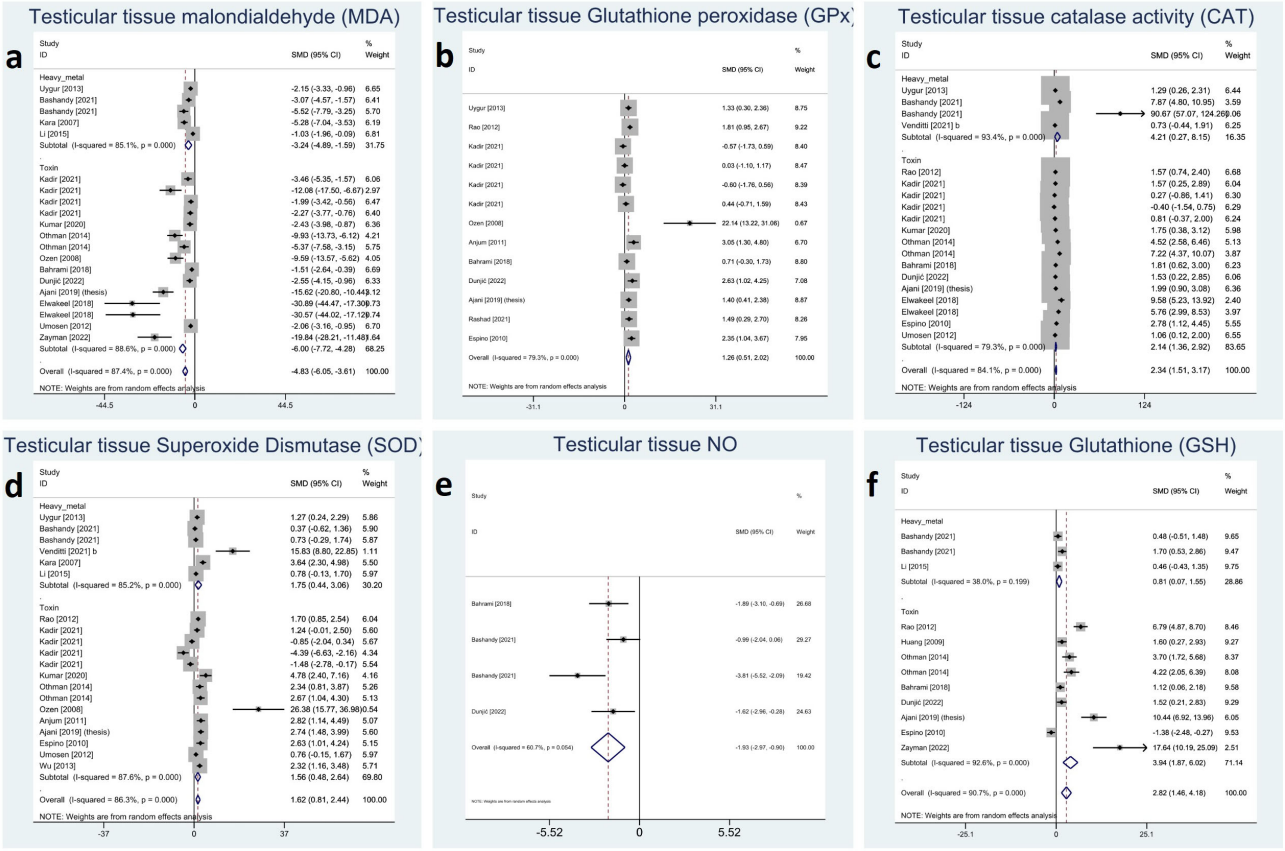


FIGURE 6
Forest plots for overall and subgroup effect measures on the impact of melatonin therapy on the testicular tissue level of oxidative stress markers including **(A)** MDA, **(B)** GPx, **(C)** CAT, **(D)** SOD, **(E)** NO, **(F)** GSH. MDA, Malondialdehyde; GPx, Glutathione Peroxidase; CAT, Catalase; SOD, Superoxide Dismutase; NO, Nitric Oxide; GSH, Glutathione.

3.8 Subgroup analyses

To investigate between study heterogeneity, subgroup analyses were done by categorizing the stressors as heavy metals and non-heavy metals. Abnormal sperm morphology, body and epididymis weight, epithelial height, serum LH, FSH, testosterone, sperm count, motility, viability, CAT, GSH, GPx, SOD, MDA, total and relative testicular weights, and tubular diameter were eligible for subgroup analysis. There was no significant difference between the protective effect of melatonin therapy against heavy metals and other toxins (non-heavy metals). Nonetheless, this method failed to reduce the heterogeneity within subgroups. Whenever feasible, subgroup analyses are demonstrated in the Figures 1-6.

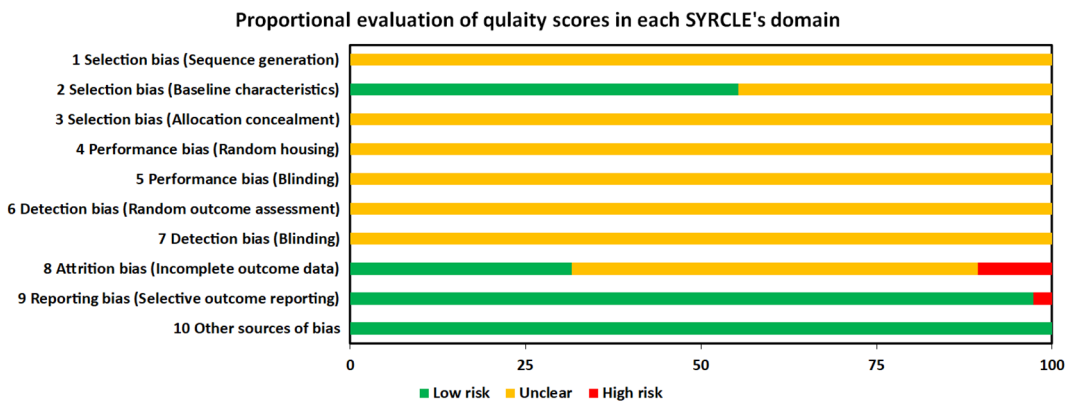


FIGURE 7
Proportional evaluation of quality score in each SYRCLE's domain. SYRCLE, Systematic Review Centre for Laboratory Animal Experimentation.

4 Discussion

To the best of our knowledge, this is the first systematic review and meta-analysis examining how melatonin intake protects rodents' male reproductive system in exposure to environmental pollutants. Environmental toxins, as potent oxidative stressors, damage male infertility by causing an imbalance between the cells' free radical levels and the antioxidant defensive system. The following sections have gone through melatonin's potential effects and related mechanisms to investigate its protective role on the male reproductive system.

4.1 Hormone parameters

The testosterone hormone mainly controls the spermatogenesis process in Sertoli cells, and LH regulates testosterone synthesis in Leydig cells (74, 75). As it is revealed by our data, melatonin intake increases serum testosterone and LH levels in male rodents injured by toxic components. These findings can be interpreted by antioxidants' effects on reproductive hormones previously reported in reviews by Vecchio et al. and Banihani (28, 76).

Spermatogenesis, a process carried out in Sertoli cells in the testes, is mainly under testosterone control (74). Testosterone is synthesized in Leydig cells and is regulated by LH (75). Environmental pollutants may exert their effect as an endocrine disruption chemical in addition to their anti-oxidant effect (77). Some of the substances included in our study, such as Bisphenol A, Arsenic, and Cadmium, have an endocrine-disrupting effect (77, 78). Arsenic, for example, may interfere with gonadotropins' function by suppressing their release and decreasing the transcription of androgen receptors, besides arsenic especially affects testosterone by decreasing its synthesis (79–81). Moreover, environmental pollutants by the accumulation of ROS could be accompanied by an over generation of reactive nitrogen species such as NO (82). High levels of ROS and NO generation in the testicles decrease the expression of biosynthetic enzymes, i.e., suppressing the steroidogenic acute regulatory protein (StAR) and cytochrome P450 side chain cleavage in Leydig cells (83). These cause a decrease in testosterone secretion, which is the primary hormone needed for optimal spermatogenesis (84).

Although it remains controversial, melatonin's effect is likely to be reducing on serum testosterone levels in preclinical studies (32, 85–90). Melatonin acts directly on Leydig cells to reduce steroidogenesis and spermatogenic activity in the testes (86, 91). In our study, melatonin showed protective properties and relatively prevented the toxic effects of stressors on rodents' serum testosterone levels in treatment arms. This effect can be explained by the protective effect of melatonin on Leydig cells against oxidative stress, increased NO, and pro-inflammatory factors (92). However, conducting more meticulous investigations in this regard is needed.

4.2 Oxidative stress parameters

Antioxidant defense system plays a crucial role in cells responding to environmental stresses (93). Numerous antioxidant responses are involved in antioxidant mechanisms. These responses include both non-enzymatic molecules (such as GSH) and enzymes (such as CAT, SOD, and GPx) (94). This system defends tissues and cells by scavenging free radicals against oxidative stress-related harm (72); however, it is not completely immune to free radicals (65).

As suggested by the results of this analysis, melatonin has been demonstrated to be generally essential in buffering oxidative stress. Regarding the effect of melatonin on MDA, GSH, and GPx levels, these results agree with recent meta-analyses conducted by Morvaridzade et al. and Sumsuzzman et al. (94, 95).

Environmental hazard components activate oxidative stress in testicular cells, causing damage to macromolecules involving membranes' lipids. The testicular tissue MDA and NO levels increase by lipid peroxidation and endothelial damage, respectively. These environmental stressors also harm the pathways essential to GSH, CAT, and SOD synthesis as members of the antioxidant defense system. These changes in oxidative markers can be justified by ROS activity. ROS directly damages the macromolecules necessary for antioxidant production and overwhelms its capacity.

Melatonin plays its role by eliminating free and lipid peroxyl radicals before they act to damage macromolecules and membrane lipids (96, 97). Furthermore, it can improve CAT, GPx, SOD, and GSH expression and activity, possibly by interacting with nuclear or membrane receptors (98). Moreover, melatonin works complementary with CAT and GPx to keep the steady-state levels of intracellular H2O2, a more destructive form of free radicals with a longer half-life (96).

There are inconsistencies between our results and Sumsuzzman et al. reports regarding CAT and SOD levels (95), which are probably due to the varying types and numbers of melatonin receptors, bioavailability and concentration in different tissues, and the insufficient number of studies to support the results. Nevertheless, this concept remains controversial.

4.3 Sperm and somatic parameters

The results of this meta-analysis shows that melatonin significantly improves sperm parameters, including sperm count, viability, motility, and morphology. These findings align with the previous reviews regarding the ameliorating effects of antioxidants on semen qualities (28). Likewise, a systematic review by Wang et al. revealed that antioxidant treatment after varicocelectomy could significantly enhance the quality of sperm parameters (29).

In addition, findings from this systematic review and meta-analysis confirm that melatonin intake makes a marked enhancement in testicle tissue parameters, including histo-architecture, seminiferous tubular diameter, epithelial height, epididymis and total testis weight, and JTBS. In this regard, our data align with another systematic review by Tatar et al. showing the protective role of antioxidants on the weights of testes and epididymis (30).

Normal spermatogenesis is a specific determinant of semen quality (99). Toxic pollutants affect spermatogenesis by diminishing the cellular ability to proliferate and altering the apoptotic index (100). Elevated apoptosis causes a decrease in cell viability and count. Also, pollutants affect sperm motility by disturbing the function of proteins

serving sperm movement as well as deterioration of the mitochondrial function to support sperm's motion energy. Lowered testosterone levels, poor sperm quality, vacuolization in seminiferous tubules, disordered germinal epithelium, and high apoptotic index cause testicular dysfunction, leading to testicular atrophy and weight loss. As demonstrated by our data, melatonin decreases the apoptotic index, which can be justified by the free radical scavenging characteristics of melatonin. As a direct and indirect free radical scavenger, melatonin protects testis tissue/cells from dysfunctions and abnormal apoptosis.

Despite the effects of melatonin on the apoptosis index, germ cell maturation, and testosterone levels, the factors that total testis weight depends on, we did not observe any correlations in the relative testis weight. This might be due to a simultaneous modulation of body weight in melatonin-treated individuals.

The adverse effect of environmental pollutants on body weight is probably associated with their action as enzymatic toxins, eventually leading to disruption in metabolic processes that could be well modulated by melatonin administration. In this review, melatonin intervention is shown to be essential in buffering body weight against toxicity damage. This is in accordance with two other systematic reviews by Mostafavi et al. and Loloei et al. (101, 102). This observation is further supported by an earlier meta-analysis by Delpino et al., suggesting that supplemental melatonin highlighted a considerable decline in body weight after individuals experienced obesity (103).

5 Conclusions and future research directions

Melatonin had beneficial protective effects against oxidative stress caused by toxic materials in rodent animal models. Although included studies crucially suffered from low quality and methodological heterogeneity. Melatonin and stressor agents' dose and duration of administration, rodents' characteristics, and assessment strategies varied significantly across the studies. For more literature consolidation, meticulous future studies with less difference in methodology are needed.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

NA and NDE conceptualized the study. AS and NDE designed the study. NE and AS searched databases. NDE and AS screened the records. NDE, FN, MS, AM, and AS extracted the data. NDE and AS performed quality assessment. AS and MS performed meta-analysis. NDE, AS, and SP provided the draft of the manuscript. NA supervised the work. All authors contributed to the article and approved the final version. AS and NDE have contributed equally to this work and share first authorship. All authors contributed to the article and approved the submitted version.

Acknowledgments

The present study was supported the Vice-chancellor for Research (code: 27117), Shiraz University of Medical Sciences, Shiraz, Iran. This study is a part of the thesis by the first author, Niloofar Dehdari Ebrahimi, for obtaining a medical doctor degree in Shiraz University of Medical Sciences. The authors also wish to express their sincere gratitude to Hossein Noroozpoor for sketching the visual abstract.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1119553/full#supplementary-material

References

1. Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, Toppari J, Andersson AM, Eisenberg ML, et al. Male Reproductive disorders and fertility trends: Influences of environment and genetic susceptibility. *Physiol Rev* (2016) 96(1):55–97. doi: 10.1152/physrev.00017.2015

2. Organization WH. *Infertility* (2020). Available at: https://www.who.int/news-room/fact-sheets/detail/infertility.

3. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. *Reprod Biol endocrinol: RB&E.* (2015) 13:37. doi: 10.1186/s12958-015-0032-1

4. Sonne C, Torjesen PA, Fuglei E, Muir DC, Jenssen BM, Jørgensen EH, et al. Exposure to persistent organic pollutants reduces testosterone concentrations and affects sperm viability and morphology during the mating peak period in a controlled experiment on farmed Arctic foxes (Vulpes lagopus). *Environ Sci Technol* (2017) 51 (8):4673–80. doi: 10.1021/acs.est.7b00289

5. Tvrdá E, Massanyi P, Lukáč N. Physiological and pathological roles of free radicals in male reproduction. In: *Spermatozoa-facts and perspectives*. IntechOpen London, UK (2017).

6. Du Plessis SS, Agarwal A, Halabi J, Tvrda E. Contemporary evidence on the physiological role of reactive oxygen species in human sperm function. *J assist Reprod Genet* (2015) 32(4):509–20. doi: 10.1007/s10815-014-0425-7

7. Leclerc P, de Lamirande E, Gagnon C. Regulation of protein-tyrosine phosphorylation and human sperm capacitation by reactive oxygen derivatives. *Free Radical Biol Med* (1997) 22(4):643–56. doi: 10.1016/S0891-5849(96)00379-6

8. Donà G, Fiore C, Tibaldi E, Frezzato F, Andrisani A, Ambrosini G, et al. Endogenous reactive oxygen species content and modulation of tyrosine phosphorylation during sperm capacitation. *Int J androl* (2011) 34(5 Pt 1):411–9. doi: 10.1111/j.1365-2605.2010.01097.x

9. Aitken RJ, Paterson M, Fisher H, Buckingham DW, van Duin M. Redox regulation of tyrosine phosphorylation in human spermatozoa and its role in the control of human sperm function. *J Cell Sci* (1995) 108(Pt 5):2017–25. doi: 10.1242/jcs.108.5.2017

10. Tipoe GL, Leung T-M, Hung M-W, Fung M-L. Green tea polyphenols as an anti-oxidant and anti-inflammatory agent for cardiovascular protection. *Cardiovasc Haematol Disorders-Drug Targets (Formerly Curr Drug Targets-Cardiovascular Hematol Disorders).* (2007) 7(2):135–44. doi: 10.2174/187152907780830905

11. Dias TR, Martin-Hidalgo D, Silva BM, Oliveira PF, Alves MG. Endogenous and exogenous antioxidants as a tool to ameliorate Male infertility induced by reactive oxygen species. *Antioxid Redox Signaling* (2020). doi: 10.1089/ars.2019.7977

12. Adwas AA, Elsayed A, Azab A, Quwaydir F. Oxidative stress and antioxidant mechanisms in human body. *J Appl Biotechnol Bioeng* (2019) 6(1):43–7. doi: 10.15406/jabb.2019.06.00173

13. De Lamirande E, Gagnon C. Reactive oxygen species and human spermatozoa: I. effects on the motility of intact spermatozoa and on sperm axonemes. *J androl* (1992) 13 (5):368–78.

14. Su X. Lipid peroxidation: Types and its determination. *Oxid Antioxid Med Sci* (2022) 11(6).

15. Li X, Fang EF, Scheibye-Knudsen M, Cui H, Qiu L, Li J, et al. Di-(2-ethylhexyl) phthalate inhibits DNA replication leading to hyperPARylation, SIRT1 attenuation and mitochondrial dysfunction in the testis. *Sci Rep* (2014) 4(1):1–9. doi: 10.1038/srep06434

16. Paoli D, Gallo M, Rizzo F, Baldi E, Francavilla S, Lenzi A, et al. Mitochondrial membrane potential profile and its correlation with increasing sperm motility. *Fertil steril* (2011) 95(7):2315–9. doi: 10.1016/j.fertnstert.2011.03.059

17. Othman AI, Edrees GM, El-Missiry MA, Ali DA, Aboel-Nour M, Dabdoub BR. Melatonin controlled apoptosis and protected the testes and sperm quality against bisphenol a-induced oxidative toxicity. *Toxicol Ind Health* (2016) 32(9):1537–49. doi: 10.1177/0748233714561286

18. Singh S, Singh SK. Chronic exposure to perfluorononanoic acid impairs spermatogenesis, steroidogenesis and fertility in male mice. *J Appl Toxicol* (2019) 39 (3):420–31. doi: 10.1002/jat.3733

19. Agarwal A, Roychoudhury S, Sharma R, Gupta S, Majzoub A, Sabanegh E. Diagnostic application of oxidation-reduction potential assay for measurement of oxidative stress: clinical utility in male factor infertility. *Reprod biomed online* (2017) 34(1):48–57. doi: 10.1016/j.rbmo.2016.10.008

20. Aitken RJ, Baker MA. Oxidative stress, sperm survival and fertility control. *Mol Cell Endocrinol* (2006) 250(1-2):66–9. doi: 10.1016/j.mce.2005.12.026

21. Venkatesh S, Shamsi MB, Deka D, Saxena V, Kumar R, Dada R. Clinical implications of oxidative stress & sperm DNA damage in normozoospermic infertile men. *Indian J Med Res* (2011) 134(3):396–8.

22. Barati E, Nikzad H, Karimian M. Oxidative stress and male infertility: current knowledge of pathophysiology and role of antioxidant therapy in disease management. *Cell Mol Life sci: CMLS* (2020) 77(1):93–113. doi: 10.1007/s00018-019-03253-8

23. Koppers AJ, Mitchell LA, Wang P, Lin M, Aitken RJ. Phosphoinositide 3-kinase signalling pathway involvement in a truncated apoptotic cascade associated with motility loss and oxidative DNA damage in human spermatozoa. *Biochem J* (2011) 436(3):687–98. doi: 10.1042/BJ20110114

24. Martins da Silva SJ. Male Infertility and antioxidants: one small step for man, no giant leap for andrology? *Reprod biomed Online* (2019) 39(6):879–83. doi: 10.1016/j.rbmo.2019.08.008

25. Agarwal A, Leisegang K, Majzoub A, Henkel R, Finelli R, Panner Selvam MK, et al. Utility of antioxidants in the treatment of Male infertility: Clinical guidelines based on a systematic review and analysis of evidence. *World J men's Health* (2021) 39(2):233–90. doi: 10.5534/wjmh.200196

26. Ross C, Morriss A, Khairy M, Khalaf Y, Braude P, Coomarasamy A, et al. A systematic review of the effect of oral antioxidants on male infertility. *Reprod biomed online* (2010) 20(6):711–23. doi: 10.1016/j.rbmo.2010.03.008

27. Busetto GM, Agarwal A, Virmani A, Antonini G, Ragonesi G, Del Giudice F, et al. Effect of metabolic and antioxidant supplementation on sperm parameters in oligo-astheno-teratozoospermia, with and without varicocele: A double-blind placebo-controlled study. *Andrologia* (2018) 50(3). doi: 10.1111/and.12927

28. Banihani SA. Effect of ginger (Zingiber officinale) on semen quality. *Andrologia* (2019) 51(6):e13296. doi: 10.1111/and.13296

29. Wang J, Wang T, Ding W, Wu J, Wu G, Wang Y, et al. Efficacy of antioxidant therapy on sperm quality measurements after varicocelectomy: A systematic review and meta-analysis. *Andrologia* (2019) 51(10):e13396. doi: 10.1111/and.13396

30. Tatar T, Akdevelioğlu Y. Effect of pollen, pit powder, and gemmule extract of date palm on male infertility: A systematic review. *J Am Coll Nutr* (2018) 37(2):154–60. doi: 10.1080/07315724.2017.1364183

31. Haghi-Aminjan H, Asghari MH, Farhood B, Rahimifard M, Hashemi Goradel N, Abdollahi M. The role of melatonin on chemotherapy-induced reproductive toxicity. *J Pharm Pharmacol* (2018) 70(3):291–306. doi: 10.1111/jphp.12855

32. Yang M, Guan S, Tao J, Zhu K, Lv D, Wang J, et al. Melatonin promotes male reproductive performance and increases testosterone synthesis in mammalian leydig cells†. *Biol reprod* (2021) 104(6):1322–36. doi: 10.1093/biolre/ioab046

33. Reiter RJ, Tan D-X, Fuentes-Broto L. Melatonin: a multitasking molecule. *Prog Brain Res* (2010) 181:127–51. doi: 10.1016/S0079-6123(08)81008-4

34. Guo Y, Sun J, Li T, Zhang Q, Bu S, Wang Q, et al. Melatonin ameliorates restraint stress-induced oxidative stress and apoptosis in testicular cells *via* NF-κB/iNOS and Nrf2/HO-1 signaling pathway. *Sci Rep* (2017) 7(1):1–13. doi: 10.1038/s41598-017-09943-2

35. Sarabia L, Maurer I, Bustos-Obregón E. Melatonin prevents damage elicited by the organophosphorous pesticide diazinon on mouse sperm DNA. *Ecotoxicol Environ safety* (2009) 72(2):663–8. doi: 10.1016/j.ecoenv.2008.04.023

36. Sarabia L, Maurer I, Bustos-Obregón E. Melatonin prevents damage elicited by the organophosphorous pesticide diazinon on the mouse testis. *Ecotoxicol Environ safety* (2009) 72(3):938–42. doi: 10.1016/j.ecoenv.2008.04.022

37. Bustos-Obregón E, Poblete D, Catriao R, del Sol M, Fernandes FH. Melatonin protective role in mouse cauda epipidymal spermatozoa damage induced by sodium arsenite. *Int J Morphol* (2013) 31:1251–6. doi: 10.4067/S0717-95022013000400017

38. Lv Y, Zhang P, Guo J, Zhu Z, Li X, Xu D, et al. Melatonin protects mouse spermatogonial stem cells against hexavalent chromium-induced apoptosis and epigenetic histone modification. *Toxicol Appl Pharmacol* (2018) 340:30–8. doi: 10.1016/j.taap.2017.12.017

39. Aslani H, Kesıcı H, Karaca Z, Özyurt B, Taş U, Ekıcı F, et al. Beneficial effects of melatonin and BQ-123 on the rat testis damage caused by cigarette smoke. *Turkish J Med Sci* (2015) 45(1):11–7. doi: 10.3906/sag-1312-66

40. abd el salam L. Light and electron microscopic study on the possible protective effect of melatonin on formaldehyde induced testicular damage in adult albino rats. *Egyptian J Histol* (2020) 43(4):1047–58. doi: 10.21608/ejh.2020.19282.1199

41. Wang T, Li Y, Wu X, Yang X, Wang Y, Wang DJ. Protective effects of melatonin on CCl4-induced acute liver damage and testicular toxicity in rats. *Indian J Pharm Sci* (2018) 80:1100–7. doi: 10.4172/pharmaceutical-sciences.1000461

42. Sobhani M, Rouzbehi A, Mahmoodi R, Sobhani Z. The protective effect of melatonin on sperm morphology and histology of the rat testis damage induced by cadmium chloride. *J Mazandaran Univ Med Sci* (2015) 25(123):32–44.

43. Anjum S, Rahman S, Kaur M, Ahmad F, Rashid H, Ansari RA, et al. Melatonin ameliorates bisphenol a-induced biochemical toxicity in testicular mitochondria of mouse. *Food Chem Toxicol* (2011) 49(11):2849–54. doi: 10.1016/j.fct.2011.07.062

44. Bahrami N, Goudarzi M, Hosseinzadeh A, Sabbagh S, Reiter RJ, Mehrzadi S. Evaluating the protective effects of melatonin on di(2-ethylhexyl) phthalate-induced testicular injury in adult mice. *Biomed Pharmacother* (2018) 108:515–23. doi: 10.1016/j.biopha.2018.09.044

45. Bustos-Obregón E, Poblete D, Catriao R, Fernandes FH. Protective role of melatonin in mouse spermatogenesis induced by sodium arsenite. *Int J Morphol Int J Morphol* (2013) 31:849. doi: 10.4067/S0717-95022013000300012

46. Elwakeel S, Abd El-Monem D. AMELIORATIVE EFFECT OF MELATONIN AND QUERCETIN AGAINST BISPHENOL a INDUCED REPRODUCTIVE TOXICITY IN MALE ALBINO MICE. *Ciec e Técnica Vitivinícola.* (2018) 33:2018:31–64.

47. Ji Y-L, Wang H, Meng C, Zhao X-F, Zhang C, Zhang Y, et al. Melatonin alleviates cadmium-induced cellular stress and germ cell apoptosis in testes. *J Pineal Res* (2012) 52 (1):71–9. doi: 10.1111/j.1600-079X.2011.00921.x

48. Li R, Luo X, Li L, Peng Q, Yang Y, Zhao L, et al. The protective effects of melatonin against oxidative stress and inflammation induced by acute cadmium exposure in mice testis. *Biol Trace Elem Res* (2016) 170(1):152–64. doi: 10.1007/s12011-015-0449-6

49. Mohammadghasemi F, Jahromi SK. Melatonin ameliorates testicular damages induced by nicotine in mice. *Iranian J Basic Med Sci* (2018) 21(6):639–44. doi: 10.22038/IJBMS.2018.28111.6829

50. Sarabia L, Espinoza-Navarro O, Maurer I, Ponce C, Bustos-Obregón E. Protective effect of melatonin on damage in the sperm parameters of mice exposed to diazinon. *Int J Morphol* (2011) 29:1241–7. doi: 10.4067/S0717-95022011000400029

51. Zayman E, Gül M, Erdemli ME, Gül S, Bağ HG, Taşlıdere E. Biochemical and histopathological investigation of the protective effects of melatonin and vitamin e against the damage caused by acetamiprid in balb-c mouse testicles at light and electron microscopic level. *Environ Sci pollut Res* (2022) 29(31):47571–84. doi: 10.1007/s11356-022-19143-9

52. Bashandy SAE, Ebaid H, Al-Tamimi J, Ahmed-Farid OAH, Omara EA, Alhazza IM. Melatonin alleviated potassium dichromate-induced oxidative stress and reprotoxicity in Male rats. *BioMed Res Int* (2021) 2021:3565360. doi: 10.1155/2021/3565360

53. Dunjic M, Krstić D, Zivkovic J, Cvetković S, Dunjić K, Mirković M, et al. Acutely applied melatonin prevents CCl4-induced testicular lesions in rats: the involvement of the oxidative capacity and arginine metabolism. *Braz J Pharm Sci* (2022) 58. doi: 10.1590/s2175-97902022e19745

54. Huang F, Ning H, Xin Q-Q, Huang Y, Wang H, Zhang Z-H, et al. Melatonin pretreatment attenuates 2-bromopropane-induced testicular toxicity in rats. *Toxicology* (2009) 256(1):75–82. doi: 10.1016/j.tox.2008.11.005

55. Kara H, Cevik A, Konar V, Dayangac A, Yilmaz M. Protective effects of antioxidants against cadmium-induced oxidative damage in rat testes. *Biol Trace Elem Res* (2007) 120(1):205–11. doi: 10.1007/s12011-007-8019-1

56. Karabulut D, Akin AT, Sayan M, Kaymak E, Ozturk E, Yakan B. Effects of melatonin against thioacetamide-induced testicular toxicity in rats. *Int J Morphol* (2020) 38:1455–62. doi: 10.4067/S0717-95022020000501455

57. Malekinejad H, Mirzakhani N, Razi M, Cheraghi H, Alizadeh A, Dardmeh F. Protective effects of melatonin and glycyrrhiza glabra extract on ochratoxin a–induced damages on testes in mature rats. *Hum Exp Toxicol* (2011) 30(2):110–23. doi: 10.1177/0960327110368416

58. Olayaki LA, Alagbonsi IA, Abdulrahim AH, Adeyemi WJ, Bakare M, Omeiza N. Melatonin prevents and ameliorates lead-induced gonadotoxicity through antioxidative and hormonal mechanisms. *Toxicol Ind Health* (2018) 34(9):596–608. doi: 10.1177/0748233718773508

59. Olumide SA. *Protective effects of melatonin on bisphenol a-induced reproductive toxicity in Male wistar rats*. University of Ibadan (2019).

60. Ozen OA, Kus MA, Kus I, Alkoc OA, Songur A. Protective effects of melatonin against formaldehyde-induced oxidative damage and apoptosis in rat testes: An immunohistochemical and biochemical study. *Syst Biol Reprod Med* (2008) 54(4-5):169–76. doi: 10.1080/19396360802422402

61. Rao MV, Bhatt RN. Protective effect of melatonin on fluoride-induced oxidative stress and testicular dysfunction in rats. *Fluoride* (2012) 45(2):116–24.

62. Rashad S, Ahmed S, El-Sayed M, Ahmed D. The toxic effect of bisphenol a on albino rat testicles and the possible protective value of vitamin e and melatonin. *Egyptian Soc Clin Toxicol J* (2021) 9(2):1–12. doi: 10.21608/esctj.2021.63294.1001

63. Risikat Kadir E, Sheriff Ojulari L, Abdullah Gegele T, Adetayo Lawal I, Sulu-Gambari L, Ajoke Sulaimon F, et al. Altered testicular histomorphometric and antioxidant levels following *In vivo* bisphenol-a administration. *IJT* (2021) 15(3):165–74. doi: 10.32598/IJT.15.3.796.1

64. Tabassum H, Parvez S, Raisuddin S. Melatonin abrogates nonylphenol-induced testicular dysfunction in wistar rats. *Andrologia* (2017) 49(5). doi: 10.1111/and.12648

65. Umosen AJ, Ambali SF, Ayo JO, Mohammed B, Uchendu C. Alleviating effects of melatonin on oxidative changes in the testes and pituitary glands evoked by subacute chlorpyrifos administration in wistar rats. *Asian Pacific J Trop biomed* (2012) 2(8):645–50. doi: 10.1016/S2221-1691(12)60113-0

66. Umosen AJ, Chidiebere U. Effect of melatonin on chlorpyrifos-induced alterations in reproductive hormones and semen characteristics in wistar rats. *Am J Phytomed Clin Ther* (2014) 2:742–53.

67. Uygur R, Aktas C, Caglar V, Uygur E, Erdogan H, Ozen OA. Protective effects of melatonin against arsenic-induced apoptosis and oxidative stress in rat testes. *Toxicol Ind Health* (2016) 32(5):848–59. doi: 10.1177/0748233713512891

68. Venditti M, Ben Rhouma M, Romano MZ, Messaoudi I, Reiter RJ, Minucci S. Altered expression of DAAM1 and PREP induced by cadmium toxicity is counteracted by melatonin in the rat testis. *Genes* (2021) 12(7). doi: 10.3390/genes12071016

69. Venditti M, Ben Rhouma M, Romano MZ, Messaoudi I, Reiter RJ, Minucci S. Evidence of melatonin ameliorative effects on the blood-testis barrier and sperm quality alterations induced by cadmium in the rat testis. *Ecotoxicol Environ safety* (2021) 226:112878. doi: 10.1016/j.ecoenv.2021.112878

70. Wu HJ, Liu C, Duan WX, Xu SC, He MD, Chen CH, et al. Melatonin ameliorates bisphenol a-induced DNA damage in the germ cells of adult male rats. *Mutat Res* (2013) 752(1-2):57–67. doi: 10.1016/j.mrgentox.2013.01.005

71. Kumar J, Verma R, Haldar C. Melatonin ameliorates bisphenol s induced testicular damages by modulating nrf-2/HO-1 and SIRT-1/FOXO-1 expressions. *Environ Toxicol* (2021) 36(3):396–407. doi: 10.1002/tox.23045

72. Rao PS, Kalva S, Yerramilli A, Mamidi S. Free radicals and tissue damage: Role of antioxidants. *Free radic antioxid* (2011) 1(4):2–7.

73. Karbownik M, Gitto E, Lewinski A, Reiter RJ. Induction of lipid peroxidation in hamster organs by the carcinogen cadmium: Amelioration by melatonin. *Cell Biol Toxicol* (2001) 17(1):33–40. doi: 10.1023/A:1010903130693

74. Griswold MD. The central role of sertoli cells in spermatogenesis. *Semin Cell Dev Biol* (1998). 9(4):411–6. doi: 10.1006/scdb.1998.0203

75. Santi D, Spaggiari G, Casarini L, Fanelli F, Mezzullo M, Pagotto U, et al. Central hypogonadism due to a giant,"silent" FSH-secreting, atypical pituitary adenoma: effects of adenoma dissection and short-term leydig cell stimulation by luteinizing hormone (LH) and human chorionic gonadotropin (hCG). *Aging Male* (2017) 20(2):96–101. doi: 10.1080/13685538.2016.1276161

76. Vecchio M, Navaneethan SD, Johnson DW, Lucisano G, Graziano G, Saglimbene V, et al. Interventions for treating sexual dysfunction in patients with chronic kidney disease. *Cochrane Database Syst Rev* (2010) 12). doi: 10.1002/14651858.CD007747.pub2

77. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-disrupting chemicals: An endocrine society scientific statement. *Endocr Rev* (2009) 30(4):293–342. doi: 10.1210/er.2009-0002

78. Anne B, Raphael R. Endocrine disruptor chemicals. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, et al, editors. *Endotext*. South Dartmouth (MA): MDText.com, Inc. (2000).

79. Jana K, Jana S, Samanta PK. Effects of chronic exposure to sodium arsenite on hypothalamo-pituitary-testicular activities in adult rats: possible an estrogenic mode of action. *Reprod Biol endocrinol: RB&E.* (2006) 4:9. doi: 10.1186/1477-7827-4-9

80. Rosenblatt AE, Burnstein KL. Inhibition of androgen receptor transcriptional activity as a novel mechanism of action of arsenic. *Mol Endocrinol (Baltimore Md).* (2009) 23(3):412–21. doi: 10.1210/me.2008-0235

81. Chiou TJ, Chu ST, Tzeng WF, Huang YC, Liao CJ. Arsenic trioxide impairs spermatogenesis *via* reducing gene expression levels in testosterone synthesis pathway. *Chem Res toxicol* (2008) 21(8):1562–9. doi: 10.1021/tx700366x

82. Lubos E, Handy DE, Loscalzo J. Role of oxidative stress and nitric oxide in atherothrombosis. *Front biosci: J virtual library* (2008) 13:5323. doi: 10.2741/3084

83. Kumar N, Singh AK. Impact of environmental factors on human semen quality and male fertility: a narrative review. *Environ Sci Europe* (2022) 34(1):1–13. doi: 10.1186/s12302-021-00585-w

84. Oduwole OO, Huhtaniemi IT, Misrahi M. The roles of luteinizing hormone, follicle-stimulating hormone and testosterone in spermatogenesis and folliculogenesis revisited. *Int J Mol Sci* (2021) 22(23). doi: 10.3390/ijms222312735

85. Qin F, Zhang J, Zan L, Guo W, Wang J, Chen L, et al. Inhibitory effect of melatonin on testosterone synthesis is mediated *via* GATA-4/SF-1 transcription factors. *Reprod biomed online* (2015) 31(5):638–46. doi: 10.1016/j.rbmo.2015.07.009

86. Ahmad R, Haldar C. Effect of intra-testicular melatonin injection on testicular functions, local and general immunity of a tropical rodent funambulus pennanti. *Endocrine* (2010) 37(3):479–88. doi: 10.1007/s12020-010-9331-7

87. Reiter RJ. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. *Endocr Rev* (1991) 12(2):151–80. doi: 10.1210/edrv-12-2-151

88. Frungieri MB, Mayerhofer A, Zitta K, Pignataro OP, Calandra RS, Gonzalez-Calvar SI. Direct effect of melatonin on Syrian hamster testes: melatonin subtype 1a receptors, inhibition of androgen production, and interaction with the local corticotropin-releasing hormone system. *Endocrinology* (2005) 146(3):1541–52. doi: 10.1210/en.2004-0990

89. Maitra SK, Ray AK. Role of light in the mediation of acute effects of a single afternoon melatonin injection on steroidogenic activity of testis in the rat. *J biosci* (2000) 25(3):253–6. doi: 10.1007/BF02703932

90. Valenti S, Giusti M. Melatonin participates in the control of testosterone secretion from rat testis: an overview of our experience. *Ann New York Acad Sci* (2002) 966:284–9. doi: 10.1111/j.1749-6632.2002.tb04228.x

91. da Costa CF, Gobbo MG, Taboga SR, Pinto-Fochi ME, Góes RM. Melatonin intake since weaning ameliorates steroidogenic function and sperm motility of streptozotocin-induced diabetic rats. *Andrology* (2016) 4(3):526–41. doi: 10.1111/andr.12158

92. Olszowski T, Baranowska-Bosiacka I, Gutowska I, Chlubek D. Pro-inflammatory properties of cadmium. *Acta Biochim Polonica* (2012) 59(4):475–82. doi: 10.18388/abp.2012_2080

93. Li D, Ding Z, Du K, Ye X, Cheng S. Reactive oxygen species as a link between antioxidant pathways and autophagy. *Oxid Med Cell longevity* (2021) 2021:5583215. doi: 10.1155/2021/5583215

94. Morvaridzadeh M, Sadeghi E, Agah S, Nachvak SM, Fazelian S, Moradi F, et al. Effect of melatonin supplementation on oxidative stress parameters: a systematic review and meta-analysis. *Pharmacol Res* (2020) 161:105210. doi: 10.1016/j.phrs.2020.105210

95. Sumsuzzman DM, Khan ZA, Choi J, Hong Y. Differential role of melatonin in healthy brain aging: a systematic review and meta-analysis of the SAMP8 model. *Aging* (2021) 13(7):9373–97. doi: 10.18632/aging.202894

96. Tan D-X, Manchester LC, Reiter RJ, Plummer BF, Limson J, Weintraub ST, et al. Melatonin directly scavenges hydrogen peroxide: a potentially new metabolic pathway of melatonin biotransformation. *Free Radical Biol Med* (2000) 29(11):1177–85. doi: 10.1016/S0891-5849(00)00435-4

97. Reiter RJ, Tan D-X, Galano A. Melatonin reduces lipid peroxidation and membrane viscosity. *Front Media SA;* (2014) p:377. doi: 10.3389/fphys.2014.00377

98. Tomás-Zapico C, Coto-Montes A. A proposed mechanism to explain the stimulatory effect of melatonin on antioxidative enzymes. *J pineal Res* (2005) 39(2):99–104. doi: 10.1111/j.1600-079X.2005.00248.x

99. Sharpe RM. Environmental/lifestyle effects on spermatogenesis. philosophical transactions of the royal society of London series b. *Biol Sci* (2010) 365(1546):1697–712. doi: 10.1098/rstb.2009.0206

100. Shaha C, Tripathi R, Mishra DP. Male Germ cell apoptosis: Regulation and biology. *Philos Trans R Soc London Ser B Biol Sci* (2010) 365(1546):1501–15. doi: 10.1098/rstb.2009.0124

101. Mostafavi S-A, Akhondzadeh S, Reza Mohammadi M, Keshtkar A-A, Hosseini S, Reza Eshraghian M, et al. Role of melatonin in body weight: A systematic review and meta-analysis. *Curr Pharm Design* (2017) 23(23):3445–52. doi: 10.2174/1381612822666161129145618

102. Loloei S, Sepidarkish M, Heydarian A, Tahvilian N, Khazdouz M, Heshmati J, et al. The effect of melatonin supplementation on lipid profile and anthropometric indices: A systematic review and meta-analysis of clinical trials. *Diabetes Metab Syndr: Clin Res Rev* (2019) 13(3):1901–10. doi: 10.1016/j.dsx.2019.04.043

103. Delpino FM, Figueiredo LM. Melatonin supplementation and anthropometric indicators of obesity: A systematic review and meta-analysis. *Nutrition* (2021) 91:111399. doi: 10.1016/j.nut.2021.111399

frontiers | Frontiers in Endocrinology

TYPE Systematic Review
PUBLISHED 31 January 2023
DOI 10.3389/fendo.2023.1123999

OPEN ACCESS

EDITED BY
Dipak Kumar Sahoo,
Iowa State University, United States

REVIEWED BY
Chandana Haldar,
Department of Zoology, Banaras Hindu University, India
Christopher Zdyrski,
Iowa State University, United States

*CORRESPONDENCE
Negar Azarpira
negarazarpira@gmail.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Cellular Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 14 December 2022
ACCEPTED 17 January 2023
PUBLISHED 31 January 2023

CITATION
Dehdari Ebrahimi N, Shojaei-Zarghani S, Taherifard E, Dastghaib S, Parsa S, Mohammadi N, Sabet Sarvestani F, Moayedfard Z, Hosseini N, Safarpour H, Sadeghi A, Azarpira N and Safarpour AR (2023) Protective effects of melatonin against physical injuries to testicular tissue: A systematic review and meta-analysis of animal models.
Front. Endocrinol. 14:1123999.
doi: 10.3389/fendo.2023.1123999

Protective effects of melatonin against physical injuries to testicular tissue: A systematic review and meta-analysis of animal models

Niloofar Dehdari Ebrahimi[1†], Sara Shojaei-Zarghani[2], Ehsan Taherifard[1], Sanaz Dastghaib[3], Shima Parsa[1], Nasim Mohammadi[1], Fatemeh Sabet Sarvestani[1], Zahra Moayedfard[4], Nima Hosseini[1], Heidar Safarpour[5], Alireza Sadeghi[2†], Negar Azarpira[1*] and Ali Reza Safarpour[2]

[1]Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, [2]Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, [3]Endocrinology and Metabolism Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, [4]Department of Tissue Engineering and Cell Therapy, School of Advanced Technologies in Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, [5]Health Policy Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Modern societies face infertility as a global challenge. There are certain environmental conditions and disorders that damage testicular tissue and may cause male infertility. Melatonin, as a potential antioxidant, may protect testicular tissue. Therefore, we conducted this systematic review and meta-analysis to evaluate the effects of melatonin in animal models against physical, heat, and ischemic damage to the testicular tissue.

Methods: PubMed, Scopus, and Web of Science were systematically searched to identify animal trials evaluating the protective effect of melatonin therapy on rodent testicular tissue when it is exposed to physical, thermal, ischemic, or hypobaric oxygen stress. Random-effect modeling was used to estimate the standardized mean difference and 95% confidence intervals based on the pooled data. Additionally, the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool was used to assess the risk of bias. The study protocol was prospectively registered in PROSPERO (CRD42022354599).

Results: A total of 41 studies were eligible for review out of 10039 records. Studies employed direct heat, cryptorchidism, varicocele, torsion-detorsion, testicular vascular occlusion, hypobaric hypoxia, ischemia-reperfusion, stress by excessive or restraint activity, spinal cord injury, and trauma to induce stress in the subjects. The histopathological characteristics of testicular tissue were generally improved in rodents by melatonin therapy. Based on the pooled data, sperm count, morphology, forward motility, viability, Johnsen's biopsy score, testicular tissue glutathione peroxidase, and superoxide dismutase levels were higher in the melatonin treatment rodent arms. In contrast, the malondialdehyde level in

testicular tissue was lower in the treatment rodent arms. The included studies suffered from a high risk of bias in most of the SYRCLE domains.

Conclusion: This study concludes that melatonin therapy was associated with improved testicular histopathological characteristics, reproductive hormonal panel, and tissue markers of oxidative stress in male rodents with physical, ischemic, and thermal testicular injuries. In this regard, melatonin deserves scientific investigations as a potential protective drug against rodent male infertility.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42022354599.

KEYWORDS

infertility, melatonin, rodents, oxidative stress, ischemia, reperfusion, heat

1 Introduction

The pathophysiology of male infertility is caused by a number of variables, including genetics and epigenetic changes, hormonal imbalances, environmental influences, and physical injuries like varicocele, cryptorchidism, and testicular torsion (1, 2). Some of these factors change the balance between the generation of free-radical species and the antioxidant defense system, which in turn disrupts functional male fecundity. Reactive oxygen species (ROS) are generally crucial for some common physiological processes like spermatogenesis, sperm capacitation, and the acrosome reaction; however, an increase in ROS production leads to "oxidative stress" (3, 4), which harms cells by inducing oxidative damages like lipid peroxidation, DNA damage, and protein misfolding resulting to abnormal semen parameters (5). Utilizing antioxidant supplements, such as melatonin, zinc, coenzyme Q10 (CoQ10), omega-3 fatty acids, vitamin E, and L-carnitine, as novel treatment strategies to address male infertility diseases has recently drawn increasing attention (6–8).

Melatonin, the sleep-wakefulness hormone, was initially discovered by Aaron Lerner in the pineal gland of a bovine in 1958 (9). Once believed that it only is secreted by the pineal gland, melatonin is now understood to be produced throughout the body in various tissues, including the cardiovascular, endocrine, immunological, male reproductive, skin, and gastrointestinal tract systems (10, 11). As an endogenous indole amine, melatonin plays a crucial role in many biological processes, like circadian rhythm, redox homeostasis, epigenetic regulation, body temperature regulation, fetal development, local and general immunity, and reproductive physiology (5, 12, 13). Melatonin has been extensively explored for its antioxidant properties (14–17). Melatonin can pass through the blood-testis barrier and is capable of entering testis cells (18). Melatonin has direct and indirect antioxidant properties. As an electron-rich molecule, it can negate free radicals, making stable products that are able to be excreted in urine. Melatonin's antioxidant mechanism involves free-radical scavenger cascade indicating efficiency of secondary and tertiary metabolites. Furthermore, melatonin indirectly acts by stimulating antioxidant enzymes (19–21). In fact, it can modulate the mRNA levels and activity of some well-known antioxidants such as Glutathione Peroxidase (GPx), ascorbate, and superoxide dismutase (SOD) (15, 22). It plays a role in the production and secretion of testosterone by Leydig cells and increases the responsiveness of Sertoli cells to Follicle-Stimulating Hormone (FSH) during testis development and growth (23, 24). The detrimental disorders of unilateral testicular damages (such as undescended testis and torsion), which influence morphometric, spermatogenesis, and oxidative parameters, could be reversed by melatonin as an antioxidant (25, 26). Up to now, it's widely recognized that melatonin has different effective roles in the male reproductive system, which exerts these effects directly (non-receptor mediated) or indirectly (receptor-mediated pathways) (27, 28). In humans, melatonin has two types of high-affinity G-protein-coupled receptors called melatonin type 1 and 2 receptors (MT1 and MT2) (29). In the male reproductive system, melatonin, through the effect of MT1, MT2, and retinoic acid receptor-related orphan receptor/retinoid Z receptor (ROR/RZR) implicates in proliferation (such as spermatogonial stem cells-SSCs), differentiation (such as spermatogonia to spermatids) and metabolic functions (23, 30, 31). On the other hand, melatonin acts directly as ROS and reactive nitrogen scavenger (RNS) according to its structure that had been suggested to be an electron donor (32, 33). Melatonin is believed to have an anti-apoptotic effect in the testes by decreasing mitochondrial-related apoptosis and ROS-related mitochondrial damage (34, 35).

Animal models have gained interest due to the limitations of human studies in reproductive research, such as ethical limitations in administering drugs and performing biopsies, and the long and ambiguous period of disease development and progression. As a result, we decided to systematically review the literature for rodent animal studies that used, evaluated, and reported melatonin and its protective effects on male genital systems against physical injuries.

2 Materials and method

This systematic review and meta-analysis was conducted following The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement (36), and the protocol is registered in the International Prospective Register of Systematic Reviews (PROSPERO: CRD42022354599).

2.1 Key question

This review aimed to investigate the protective effects of exogenous melatonin on the parameters of male reproductive function against physical, heat, or ischemic injuries compared to placebo in rodent subjects. We asked if melatonin therapy (whether before or after the induction of stress) prevents oxidative pathways in testicular cells.

2.2 Data sources and searches

Two reviewers (AS and SP) conducted a comprehensive search in PubMed, Scopus, and Web of Science for records from January 1, 1970, until September 9, 2022, for "melatonin", "testicular function", and their equivalents as keywords. The search strategies comprised a combination of Medical Subject Headings (MeSH) or their equivalent (where available), keywords, truncations, and boolean operators. A manual backward and forward citation search was also done for all the included studies. The detailed search strategy is provided in Supplementary Material 1.

2.3 Study selection and eligibility criteria

Firstly, duplicate records were removed electronically. Then, using the Rayyan online tool for managing systematic reviews (37), four reviewers (NDE, SP, NM, and AS) independently screened titles and abstracts, followed by the full-text screening of the identified records for eligibility criteria. Disagreements were resolved with discussion. Studies were included if they satisfied the following criteria: (1) controlled animal studies, (2) the population was rodents that were exposed to physical, electrical, ischemic, or thermal injuries as oxidative stress to the testicular tissue, (3) at least one intervention group received melatonin regimen, (4) at least one control group with similar stress, and (5) reported major hallmarks of testicular tissue (histopathologic, biochemical, and sperm analyses).

The exclusion criteria were: (1) *in-vitro* and ex-vivo studies, (2) non-rodent animals with other types of stresses (radiation, chemotherapy, toxins, and metabolic), (3) combination therapy of melatonin with other drugs, (4) treatment using derivatives of melatonin, (5) healthy controls without stress, and (6) reported irrelevant outcomes. Also, reviews, letters, and human trails were excluded from the review. Our search was not restricted by language.

2.4 Data extraction and risk of bias assessment

Two reviewers (ET and FSS) independently extracted the favorable data into Excel spreadsheets. Any disagreements were resolved by discussion and involvement of a third author (AS). Structured forms were used for data extraction of the following contents: (1) study characteristics (first author, publication year, and country), (2) population characteristics (species, age, sample size, and type of stress), (3) melatonin dose, duration, and route, (4) time of assessment of outcome, (5) tissue and plasma biochemical indices, and (6) histopathological characteristics. For missing data, we contacted the first or corresponding author and waited for a response for at least one month.

We assessed the risk of bias in the studies using the Systematic Review Centre for Laboratory animal Experimentation (SYRCLE) tool for animal intervention studies (38). Two authors (NDE and ET) independently reviewed each article and classified it as high, low, or unclear for each bias domain. Disagreements were resolved *via* consensus or by a third (AS) reviewer if necessary.

2.5 Data synthesis and statistical analysis

Data were analyzed using Stata MP Version 16 (StataCorp, College Station, TX, USA), and a p-value < 0.05 was considered statistically significant. Under a random-effects model (DerSimonian-Laird method), the pooled effect sizes were reported as standardized mean difference (Glass's Δ) and 95% confidence interval (39). The statistical heterogeneity was examined using Cochran's Q statistic, p-value, and I-squared. I-squared was employed to qualify heterogeneity as "perhaps not important", "moderate heterogeneity", "substantial heterogeneity", and "considerable heterogeneity" if I-squared values were 0-40%, 30-60%, 50-90%, and 75-100%, respectively (40). Subgroup analyses were implemented only if three or more studies were available for each subgroup to identify possible sources of heterogeneity. In case of missing data, if crucial, studies were removed from the analysis. Also, funnel plots were used to detect visual asymmetry in publications when at least ten studies were available for the outcome (40).

3 Results

3.1 Search results

The PRISMA flow diagram of the literature search is presented in Figure 1. The systematic search yielded 10,039 records, while manual citation searching yielded no additional studies. The database searching included PubMed (n=1,375), Web of Science (n=3,838), and Scopus (n=4,826). 1,016 records were removed using automatic duplicate detection. Title and abstract screening was conducted on 9,023 records, and 110 studies were sought for retrieval. With the exclusion of 5 studies that we failed to retrieve (41–45), 105 articles were assessed for eligibility. A total of 64 articles were excluded due to ineligible population (n=53), design (n=3), intervention (n=4), outcome (n=1), and publication type (n=3). Finally, 41 articles were eligible for the study; 12 (46–57) were only included in narrative data synthesis. Two studies were on the same subject population; thus, for data synthesis, they were treated as one (47, 51). Despite our effort to contact the authors, 8 studies contained missing values; therefore, they were excluded from the analysis (47, 52–55, 57–59).

3.2 Study characteristics

Included studies were published between 2000 and 2022 in English. Rats (n=36) (25, 46–54, 56, 58–82) and mice (n=5) (55, 57, 83–85) were the subjects in the included studies. Studies employed direct heat (n=3) (82, 84, 85), cryptorchidism (n=2) (58, 72), varicocele (n=3) (60, 73, 77), torsion-detorsion (n=18) (25, 46, 48, 49, 54, 56, 59, 62–66, 74, 75, 78–81), testicular vascular occlusion (n=4) (52, 53, 61, 67), hypobaric hypoxia (n=3) (47, 51, 55), ischemia-reperfusion (n=2) (50, 76), stress by excessive

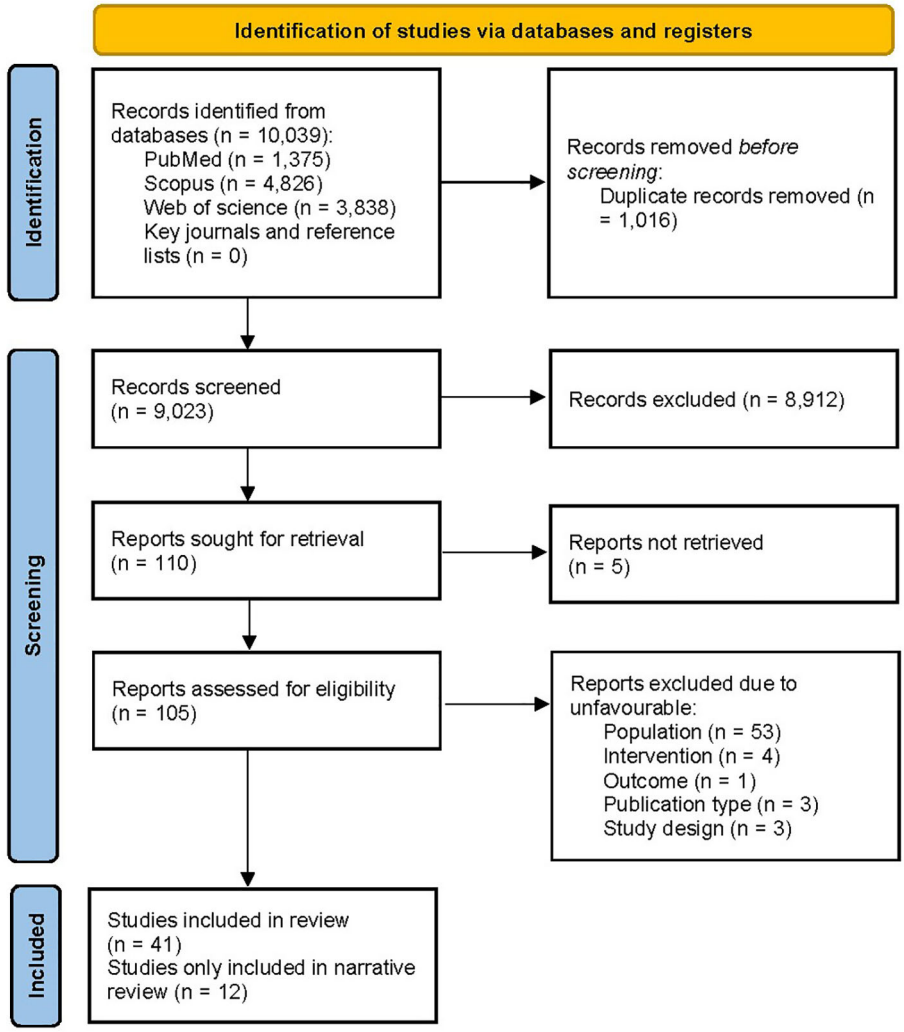


FIGURE 1
PRISMA flow diagram illustrating the process of selection of the studies.

(n=3) (68, 69, 71) or restraint (n=1) (83) activity, spinal cord injury (n=1) (57), and trauma (n=1) (70). Studies induced the stress mechanisms bilaterally (n=16) (47, 48, 50, 51, 55, 57, 58, 68, 69, 71, 72, 75, 76, 82–85) and unilaterally (n=25) (25, 46, 49, 52–54, 56, 58–67, 70, 73, 74, 77–81). Mice aged between 6 - 12 weeks and rats aged between 6 – 24 weeks. Studies administered melatonin intraperitoneal (n=31) (25, 46, 48, 49, 52–54, 56–60, 62, 63, 65–71, 73, 75, 77–81, 83–85), oral (n=5) (47, 51, 55, 61, 72, 82), intravenous (n=3) (50, 64, 76), and intramuscular (n=1) (74). Characteristics of the included articles are summarized in Figure 2. The dose of melatonin therapy, descriptive histopathological findings, duration of stress induction, and outcomes assessment ranged significantly between the studies presented in Table 1 and Supplementary Materials 2.

3.3 Outcomes

18 outcomes were pooled from the included studies which were classified into five groups: (a) sperm parameters, (b) reproductive hormone profile, (c) markers of oxidative stress in testicular tissue, (d) body weight and testicular somatic indices, and (e) exploratory outcomes. Pooled outcomes included: total sperm count, forward progressive motility, normal sperm morphology, sperm viability, Johnson tubular biopsy score, serum testosterone and Inhibin-B levels, testicular tissue SOD, malondialdehyde (MDA), GPx, and catalase (CAT) activity, final body and testes weight, testis to body weight ratio, seminiferous tubular diameter, percentage of tubules with TUNEL-positive cells (TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling), and number of TUNEL-positive cells per tubule. The forest plots of the overall pooled effects sizes are presented in the Figure 3– 7.

3.3.1 Sperm parameters

The combined SMDs for the effect of melatonin therapy on total sperm count (SMD = 2.358, 95% CI: 0.285 to 4.431, p-value = 0.026), forward progressive motility (SMD = 5.907, 95% CI: 4 to 7.814, p-value <0.001), normal sperm morphology (SMD = 3.312, 95% CI: 1.516 to 5.108, p-value <0.001), and sperm viability (SMD = 2.116, 95% CI: 0.291 to 3.941, p-value = 0.023) were statistically significant. On the other hand, total sperm motility was not significantly affected by melatonin therapy (SMD = -1.893, 95% CI: -9.076 to 5.29, p-value = 0.605). Between study

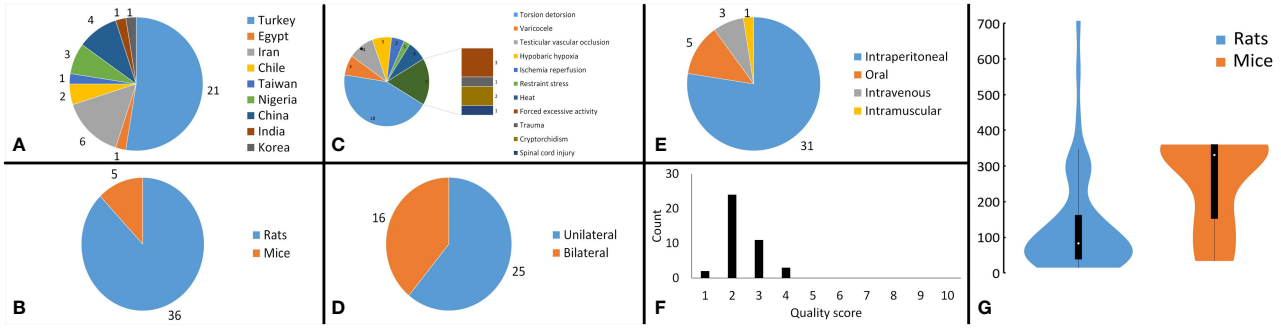


FIGURE 2
Analysis of study characteristics. Pie chart of **(A)** the countries that studies were published from, **(B)** the subject rodents across the studies, **(C)** the stress mechanism used in the studies, **(D)** the side that stresses were induced in the subjects, **(E)** the route of melatonin administration across the studies, **(F)** bar chart, illustrating the distribution of the methodological quality scores across the studies, and **(G)** violin plot demonstrating the distribution of overall cumulative dosages that were administered to the rodents (mg/kg).

TABLE 1 Basic characteristics of the included studies.

First author [year]	Country	Rodent species	Injury (location)	n/ intervention, control	Age of subjects	Melatonin per dose	Route of intervention	SYRCLE score
Abasiyanik [2004] (80)	Turkey	Rats	Torsion detorsion (Unilateral)	17, 12	N/M	N/M	IP	2
Abo El Gheit [2021] (60)	Egypt	Rats	Varicocele (Unilateral)	13, 13	12 weeks	10 mg/kg	IP	3
Aktas [2011] (46)	Turkey	Rats	Torsion detorsion (Unilateral)	21, 7	N/M	N/M	IP	1
Asghari [2016] (61)	Iran	Rats	Testicular vasculature occlusion (Unilateral)	10, 10	N/M	3 mg/kg	Oral	3
Bustos-Obregón [2010] & Hartley [2009] (47, 51)	Chile	Rats	Intermittent hypobaric hypoxia (Bilateral)	48, 48	8-12 weeks	10 mg/kg	Oral	2
Chen [2021] (48)	Taiwan	Rats	Torsion detorsion (Bilateral)	6, 6	N/M	50 mg/kg	IP	2
Duru [2007] (62)	Nigeria	Rats	Torsion detorsion (Unilateral)	60, 30	N/M	N/M	IP	2
Ekici [2012] (63)	Turkey	Rats	Torsion detorsion (Unilateral)	6, 6	12 weeks	10 mg/kg	IP	3
Erdemir [2008] (49)	Turkey	Rats	Torsion detorsion (Unilateral)	10, 10	22- 24 weeks	N/M	IP	3
Esrefoglu [2004] (50)	Turkey	Rats	Ischemia reperfusion (Bilateral)	8, 8	N/M	10 mg/kg	IV	2
Gul [2018] (64)	Turkey	Rats	Torsion detorsion (Unilateral)	12, 12	90 days	40 mg/kg	IV	3
Guo [2017] (83)	China	Mice	Restraint stress (Bilateral)	10, 10	6 weeks	10 mg/kg	IP	2
Gürbilek [2000] (81)	Turkey	Rats	Torsion detorsion (Unilateral)	12, 12	N/M	50 mg/kg	IP	2
Haldera [2020] (82)	India	Rats	Heat (Bilateral)	6, 6	11-12 weeks	10 mg/kg	Oral	3
Jeong [2010] (65)	Korea	Rats	Torsion detorsion (Unilateral)	16, 12	6 and 10 weeks	N/M	IP	2
Kanter [2010] (66)	Turkey	Rats	Torsion detorsion (Unilateral)	8, 8	16 weeks	N/M	IP	3
Koksal [2012] (67)	Turkey	Rats	Testicular vasculature occlusion (Unilateral)	16, 16	N/M	N/M	IP	2

(Continued)

TABLE 1 Continued

First author [year]	Country	Rodent species	Injury (location)	n/ intervention, control	Age of subjects	Melatonin per dose	Route of intervention	SYRCLE score
Kurcer [2008] (53)	Turkey	Rats	Testicular vasculature occlusion (Unilateral)	N/M	8 weeks	N/M	IP	2
Kurcer [2010] (52)	Turkey	Rats	Testicular vasculature occlusion (Unilateral)	N/M	8 weeks	N/M	IP	3
Mahmudi [2022] (68)	Iran	Rats	Forced treadmill exercise (Bilateral)	8, 8	N/M	10 mg/kg/ week	IP	1
Minaii [2013] (69)	Iran	Rats	Forced swimming test (Bilateral)	12, 12	N/M	10 mg/kg/ week	IP	3
Mirhoseini [2017] (25)	Iran	Rats	Torsion detorsion (Unilateral)	8, 8	N/M	N/M	IP	2
Mirhoseini [2019] (70)	Iran	Rats	Trauma (Unilateral)	16, 8	N/M	N/M	IP	2
Moayeri [2017] (71)	Iran	Rats	Forced swimming test (Bilateral)	10, 10	N/M	10 mg/kg/ week	IP	2
Olayaki [2017] (72)	Nigeria	Rats	Cryptorchidism (Bilateral)	12, 6	N/M	4 and 10 mg/ kg	Oral	2
Onur [2004] (73)	Turkey	Rats	Varicocele (Unilateral)	20, 10	12-14 weeks	5 and 10 mg/ kg	IP	2
Ozturk [2003] (74)	Turkey	Rats	Torsion detorsion (Unilateral)	10, 10	N/M	N/M	IM	2
Parlaktas [2014] (75)	Turkey	Rats	Torsion detorsion (Bilateral)	7, 7	20-24 weeks	N/M	IP	3
Qin [2021] (84)	China	Mice	Heat (Bilateral)	5, 5	9 weeks	20 mg/kg	IP	2
Saalu [2006] (58)	Nigeria	Rats	Cryptorchidism (Bilateral and unilateral)	16, 16	N/M	0.7 mg/kg	IP	2
Sahna [2006] (76)	Turkey	Rats	Ischemia reperfusion (Bilateral)	6, 6	8 weeks	10 mg/kg	IV	4
Sekmenli [2016] (54)	Turkey	Rats	Torsion detorsion (Unilateral)	7, 7	12 weeks	17 mg/kg	IP	4
Semercioz [2003] (77)	Turkey	Rats	Varicocele (Unilateral)	10, 10	12-14 weeks	10 mg/kg	IP	3
Semercioz [2017] (59)	Turkey	Rats	Torsion detorsion (Unilateral)	10, 10	N/M	3 mg/kg	IP	2
Vargas [2011] (55)	Chile	Mice	Intermittent and continuous hypobaric hypoxia (Bilateral)	16, 16	12 weeks	10 mg/kg	Oral	2
Yildirim [2006] (56)	Turkey	Rats	Torsion detorsion (Unilateral)	14, 7	N/M	N/M	IP	2
Yuan [2016] (57)	China	Mice	Spinal cord injury (Bilateral)	N/M	8 weeks	N/M	IP	2
Yurtçu [2008] (78)	Turkey	Rats	Torsion detorsion (Unilateral)	10, 10	7.5 weeks	17 mg/kg	IP	4
Yurtçu [2009] (79)	Turkey	Rats	Torsion detorsion (Unilateral)	20, 10	N/M	17 mg/kg	IP	2
Zhang [2020] (85)	China	Mice	Heat (Bilateral)	60, 30	10-12 weeks	20 mg/kg	IP	2

N/M, not mentioned; IP, Intraperitoneal; IV, Intravenous; IM, Intramuscular; SYRCLE, Systematic Review Centre for Laboratory Animal Experimentation.

heterogeneity was considerable for sperm viability (I^2 = 82.5% and p-value for Q test <0.001), total sperm count (I^2 = 87.55% and p-value for Q test <0.001), total sperm motility (I2 = 92.45% and p-value for Q test <0.001), and normal sperm morphology (I^2 = 84.78% and p-value for Q test <0.001) and moderate for forward progressive sperm motility (I^2 = 45.57% and p-value for Q test = 0.138).

Johnsen's score was examined and reported in 14 studies, of which, 11 were included in the meta-analysis. The combined SMD for

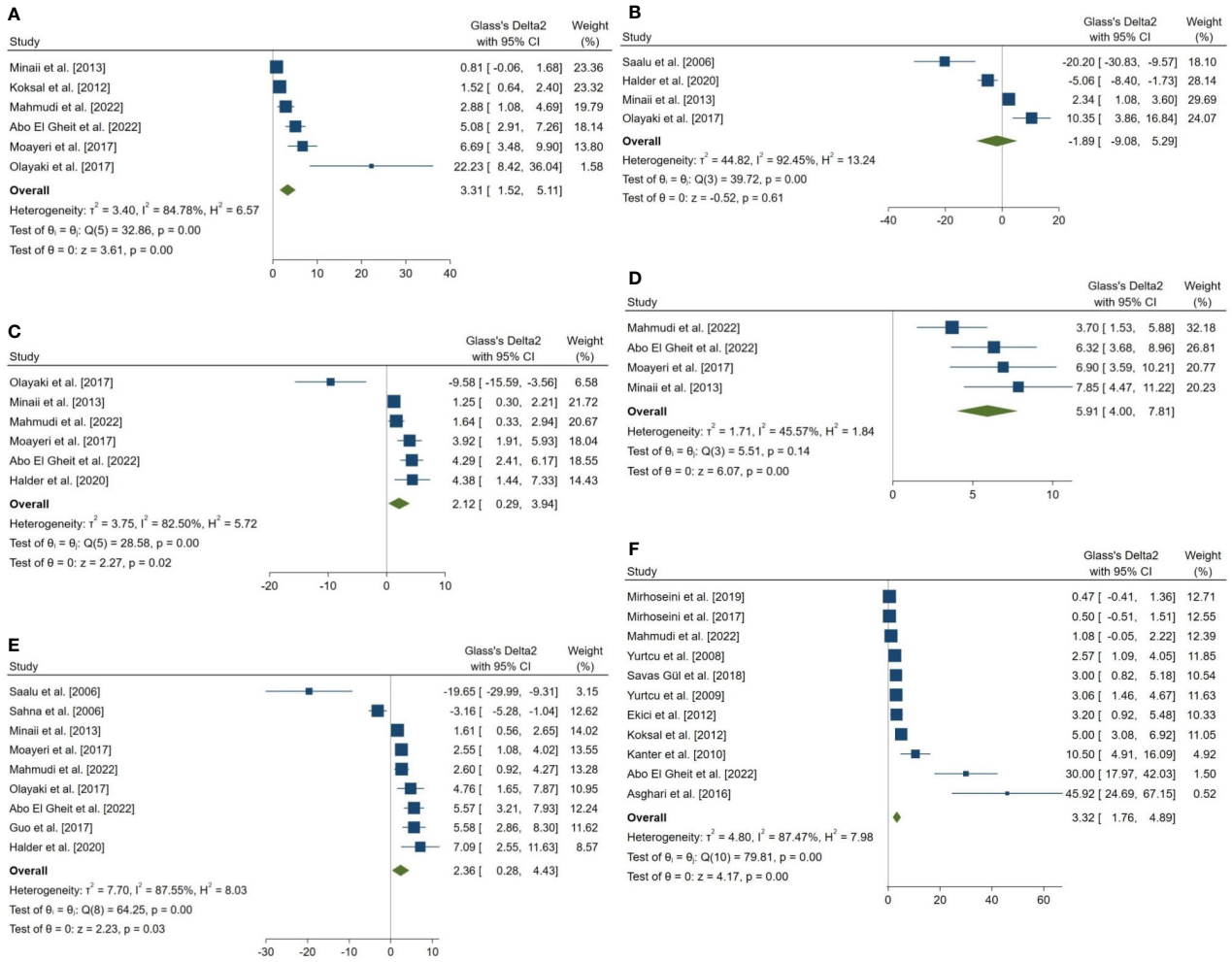


FIGURE 3
Forest plots for the overall pooled effects sizes of sperm parameters. **(A)** normal morphology (n=6), **(B)** total motility (n=4), **(C)** viability (n=6), **(D)** forward progressive motility (n=4), **(E)** total count (n=9), **(F)** Johnsen's biopsy score (n=11).

the effect of melatonin therapy on Johnsen's mean testicular biopsy score was (SMD = 3.322, 95% CI: 1.759 to 4.885, p-value <0.001). Substantial between study heterogeneity was observed in the analysis.

3.3.2 Reproductive hormone profile

The overall pooled SMDs for the effect of melatonin therapy on rodents' reproductive hormones were (SMD = 1.012, 95% CI: -1.991 to 4.015, p-value = 0.509) and (SMD = 2.659, 95% CI: 1.296 to 4.022, p-value <0.001) for serum testosterone and Inhibin-B levels, respectively. Substantial between study heterogeneity was observed in both analyses (I^2 = 89.03% and p-value for Q test <0.001 and I^2 = 65.95% and p-value for Q test = 0.032 for serum testosterone and Inhibin-B levels, respectively).

3.3.3 Markers of oxidative stress in testicular tissue

The pooled SMDs for the effect of melatonin therapy on testicular tissue antioxidant activity were for SOD (SMD = 7.698, 95% CI: 3.863 to 11.533, p-value <0.001), malondialdehyde (SMD = -2.738, 95% CI: -3.795 to -1.681, p-value <0.001), GPx (SMD = 4.927, 95% CI: 1.197 to 8.658, p-value = 0.005), and catalase (CAT, SMD = 2.323, 95% CI: 0.42 to 4.226, p-value = 0.017) were. However, considerable between-study heterogeneity (I^2 = 90.2% and p-value for Q test <0.001 for SOD, I^2 = 88.43% and p-value for Q test <0.001 for MDA, I^2 = 93.39% and p-value for Q test <0.001 for GPx, and I^2 = 83.72% and p-value for Q test <0.001 for CAT) was observed in testicular tissue antioxidant activities.

3.3.4 Body weight and testicular somatic indices

The pooled SMDs for the effect of melatonin therapy on body weight and testicular somatic indices were not statistically significant; for final body weight (SMD = 2.076, 95% CI: -1.438 to 5.59, p-value = 0.247), final total testis weight (SMD = 3.745, 95% CI: -6.905 to 14.396, p-value = 0.491), testis to body weight ratio (SMD = 0.036, 95% CI: -1.089 to 1.162, p-value = 0.95), and seminiferous tubular diameter (SMD = 0.818, 95% CI: -0.018 to 1.655, p-value = 0.055). The between-study heterogeneity was substantial to considerable for body weight and testicular somatic indices; for final body weight (I^2 = 92.57% and p-value for Q test <0.001), final total testis weight (I^2 = 93.88% and p-value for Q test <0.001), testis to body weight ratio (I^2 = 73.12% and p-value for Q test = 0.005), and seminiferous tubular diameter (I^2 = 62% and p-value for Q test 0.048).

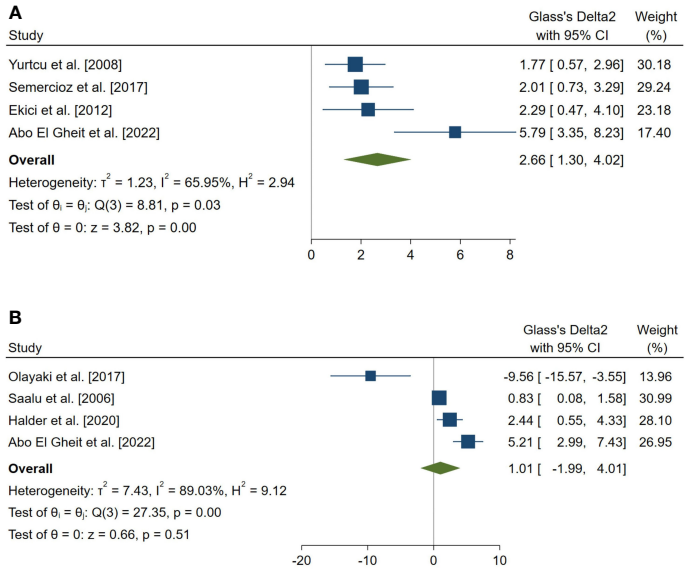


FIGURE 4
Forest plots for the overall pooled effects sizes of serum level of reproductive hormones. **(A)** Serum Inhibin-B (n=4), **(B)** testosterone (n=4).

3.4 Exploratory outcomes

In a complementary analysis, we assessed the effect of melatonin therapy on TUNEL assay of seminiferous tubular cells (54, 65, 66, 68, 83, 85). The pooled SMD for the effect of melatonin therapy on the percentage of tubules with TUNEL-positive cells (SMD = -3.886, 95% CI: -6.365 to -1.406, p-value = 0.002) was statistically significant. On the other hand, this measure was not statistically significant for the number of TUNEL-positive cells per tubule (SMD = -5.636, 95% CI: -11.495 to 0.222, p-value = 0.059).

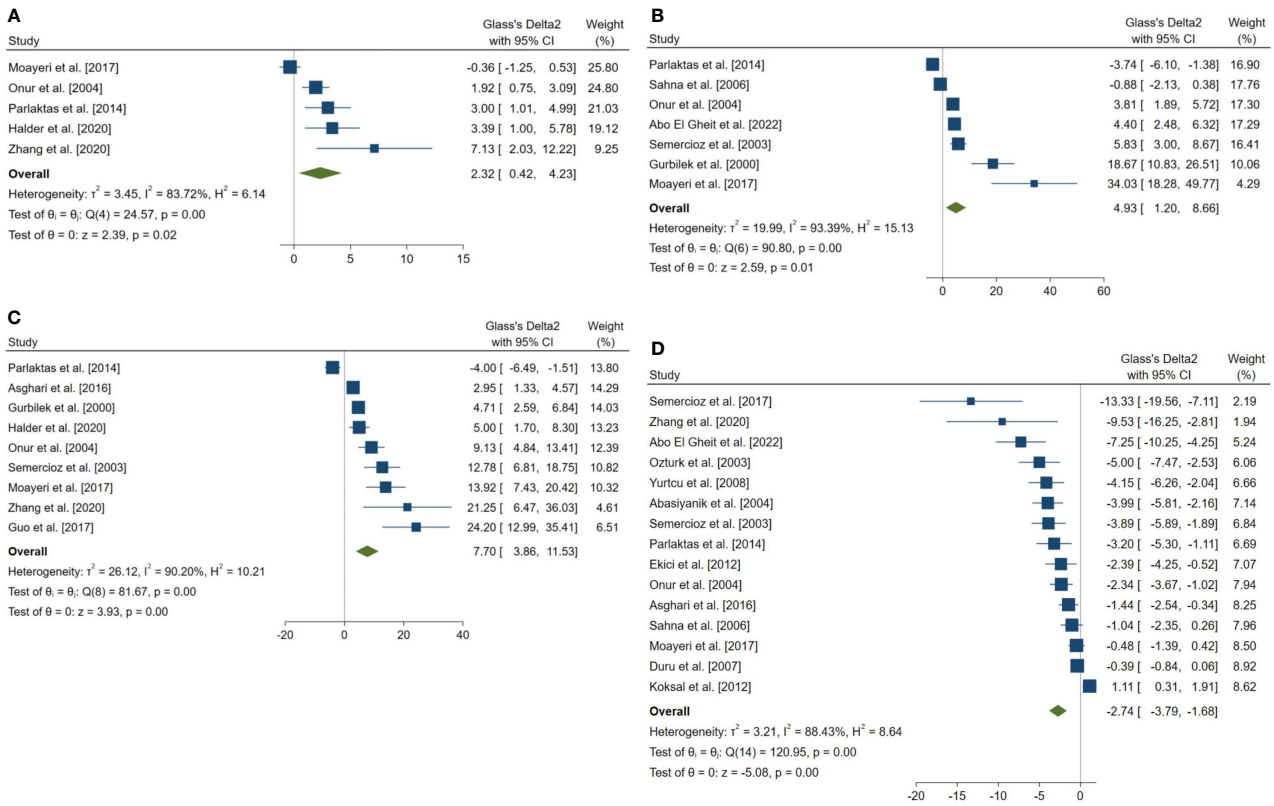


FIGURE 5
Forest plots for the overall pooled effects sizes of the testicular tissue oxidative stress markers. **(A)** CAT (n=5), **(B)** GPx (n=7), **(C)** SOD (n=9), **(D)** MDA (n=15). MDA, Malondialdehyde; GPx, Glutathione Peroxidase; CAT, Catalase; SOD, Superoxide Dismutase.

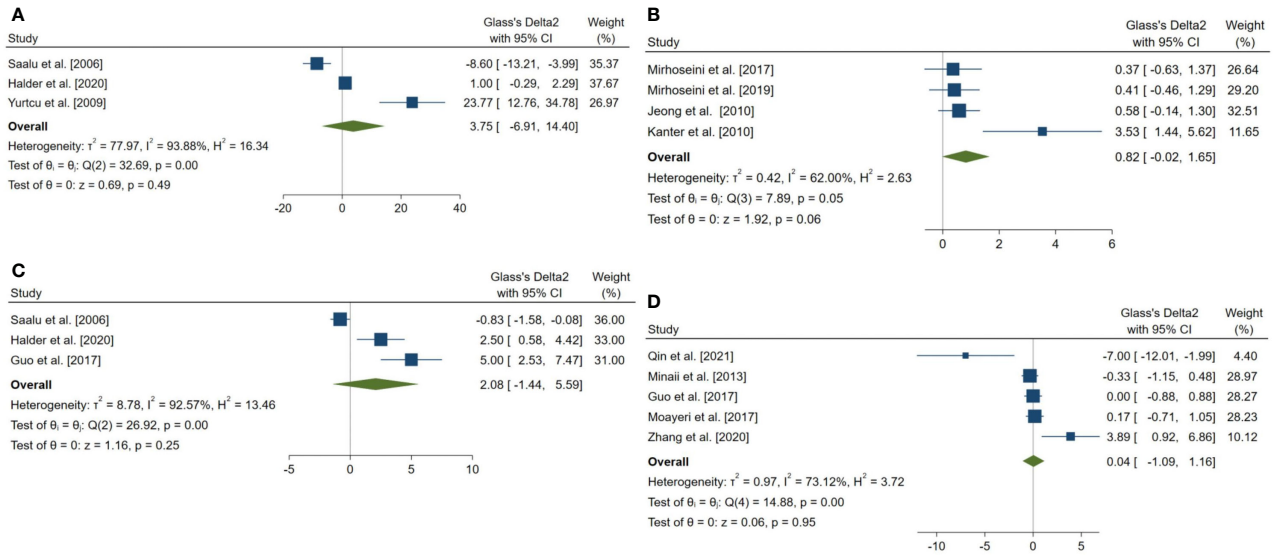


FIGURE 6
Forest plots for the overall pooled effects sizes of body weight and testicular somatic indices. **(A)** total testicular weight (n=3), **(B)** seminiferous tubular diameter (n=4), **(C)** final body weight (n=3), **(D)** testis to body relative weight (n=5).

3.5 Publication bias

Two outcomes were eligible for analysis of publication bias. The funnel plots regarding the effects of melatonin therapy on testicular tissue MDA activity and Johnsen have been analyzed for publication bias. Both plots lack symmetry on visual inspection, suggesting a high risk of publication bias. Egger's regression and Begg's tests also showed consistent results: p-value <0.001 for both tests in both outcomes. The funnel plots are presented in Supplementary Material 4.

3.6 Subgroup analyses

Subgroup analyses were done on the side of induction of stress (unilateral vs. bilateral), mechanism of stress (ischemic vs. non-ischemic injuries), and duration of melatonin therapy (> 2 weeks vs. ≤ 2 weeks). Subgroup analyses revealed significant between-group differences for SOD based on the mechanism of stress and duration of melatonin therapy (p-value <0.001 and 0.01, respectively). All the meta-analyses are stratified in Table 2.

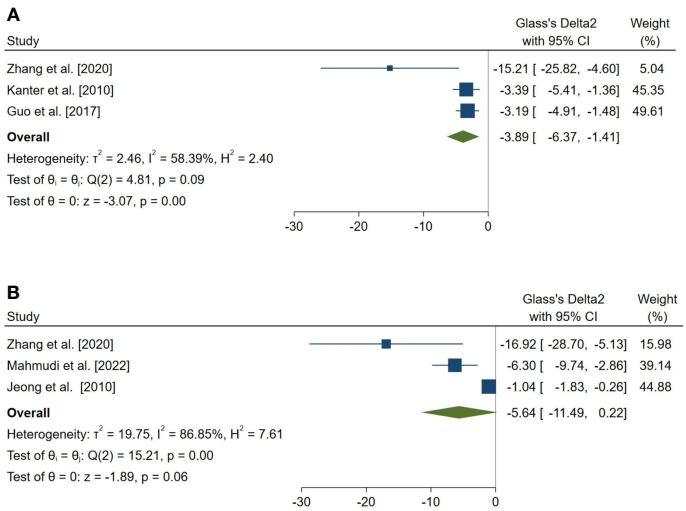


FIGURE 7
Forest plots for the overall pooled effects sizes of exploratory outcomes, including **(A)** the percentage of tubules with TUNEL-positive cells (n=3) and **(B)** the number of TUNEL-positive cells per tubule (n=3). TUNEL, Terminal deoxynucleotidyl transferase dUTP nick end labeling.

3.7 Risk of bias assessment

For each domain, studies scored 1 if they were assessed as low risk using SYRCLE tool. Studies scored between 1 and 4. All the studies were labeled as unclear risk on random sequence generation, allocation concealment, random housing, blinding, and random outcome assessment. For other sources of bias, all the studies were assessed as low risk. Studies were low risk based on baseline characteristics (n=16), incomplete outcome data (n=4), and selective outcome reporting (n= 35). All the details are presented in Figure 8 and Supplementary Material 5.

4 Discussion

Our results suggest the beneficial effects of melatonin on male rodents' infertility caused by physical testicular injuries. We hypothesized that melatonin might exert this favorable effect by influencing the reproductive system, inducing antioxidant defense, and suppressing apoptosis. Furthermore, subgroup analysis revealed a stronger impact of melatonin on testicular SOD in non-ischemia-induced injuries and studies with longer intervention duration compared to the comparison groups. Treatment duration and mechanism of stress were detected as possible sources of heterogeneity for testicular SOD and GPx analysis. In the following, we discussed the possible related mechanisms.

4.1 Effects on sperm and testis parameters

Male infertility is commonly caused by ejaculatory dysfunction, no or low sperm count, or abnormal morphology or motility of the sperms (86). In the present study, melatonin improved spermatogenesis, total sperm count, sperm viability and morphology, and forward progression sperm motility in animals with infertility induced by physical injuries. However, total testicular weight, testis to body weight ratio, sperm motility, and seminiferous tubule diameter were not affected by melatonin administration. Our results were partially in line with previous studies on other models of male infertility. Melatonin protects the reproductive system against the toxicity of chemotherapeutic agents (87). Zi et al. reported the favorable effects of melatonin on doxorubicin-induced impaired spermatogenesis and sperm quality, except for sperm motility (88). Melatonin also suppressed bleomycin, etoposide, and cisplatin-induced testicular damage by ameliorating histopathological alterations of testes, testicular weight, and sperm motility, viability, and morphology, but not sperm count and its progressive motility (89). These discrepancies may be due to the differences in the type of animals, experimental models of male infertility, and dose, duration, and route of melatonin administration. Melatonin may directly affect the male reproductive system by interacting with its receptors on the testes, epididymis, or spermatozoa (90, 91). Melatonin increased the expression of spermatogenesis-related genes and led bovine Sertoli cells to transit from G1 to S phase (92). In addition to the experimental studies, lower serum and seminal melatonin levels in patients with idiopathic oligoasthenoteratozoospermia compared to the fertile men and a significant positive correlation between serum melatonin and sperm motility were detected in a previous case-control study (93). Due to the conflicting evidence (55, 91), more studies should be performed to clarify the effects of melatonin on sperm and testis parameters.

4.2 Effects on reproductive hormone levels

Testosterone has fundamental roles in male reproductive system development and spermatogenesis (94). Our results revealed that melatonin does not affect plasma testosterone levels, similar to some reports on other animal models of male infertility (89, 95). Nonetheless, transgenic mammals with endogenously elevated melatonin levels revealed elevated testosterone production (96). Evidence also suggests that melatonin induces testosterone activation and inhibits its destruction (97). Although, the low number of included studies may conceal the possible influence of melatonin on testosterone; consequently, further studies are needed to address the issue.

The serum level of inhibin-B, a hormone produced primarily by the Sertoli cells of the testes, is a potential marker of testicular function and spermatogenesis (98, 99). Our findings demonstrated the improvement of inhibin-B levels following melatonin administration, which may be because of melatonin's beneficial effects on preserving Sertoli cells against injuries. In this regard, melatonin upregulated inhibin-B expression in bovine Sertoli cells *via* its MT1 and MT2 receptors (92). In a randomized controlled trial by Lu et al., melatonin supplementation increased the peripheral blood inhibin B levels following varicocelectomy that could be attributed to its effects on spermatogenesis function (100).

4.3 Effects on oxidant/antioxidant balance

Testicular oxidative stress could be derived from intrinsic etiologies, including testicular torsion, varicocele, cryptorchidism, infection, inflammation, or aging (101). Some extrinsic factors, such as intense exercise, can also raise oxidative stress levels in the testes (69). Sperms are extremely sensitive to oxidative damage due to the high levels of polyunsaturated fatty acids in their membranes and the low content of enzymatic antioxidants. Oxidative stress is directly correlated with increased apoptosis in germ cells and mature spermatozoa by changing caspase activity and disrupting mitochondrial membrane (101). Therefore, multiple studies indicated the association between oxidative stress and abnormal sperm count, motility, viability, morphology, DNA integrity, and fertilization ability (5, 101, 102). According to our results, melatonin administration significantly increased the SOD, GPx, and CAT activities, essential enzymatic antioxidants, and reduced MDA levels, as a lipid peroxidation product, in the testicular tissue of animals. Antioxidant properties of melatonin are reported in previous literature. Melatonin could directly scavenge oxidants and indirectly increase enzymatic antioxidant levels (103). Morvaridzadeh et al. conducted a meta-analysis of randomized controlled trials on patients with a different health condition. They reported increased total

TABLE 2 Subgroup analyses.

Outcome		Subgroup	Number of effect sizes	Pooled SMD [95% CI]	P-for-difference	P-for-heterogeneity	I^2 (%)
GPx	All		7	4.927 [1.197, 8.658]	0.005	<0.001	93.39
	Mechanism of stress	Ischemia-induced stress	3	2.650 [-3.300, 8.610]	0.370	<0.001	93.18
		Non-ischemia-induced stress	4	5.750 [2.510, 8.980]		<0.001	79.79
	Treatment duration	More than 2 weeks	4	5.748 [2.514, 8.983]	0.370	<0.001	79.79
		2 weeks and less	3	2.652 [-3.303, 8.607]		<0.001	93.18
Johnsen score	All		11	3.322 [1.759, 4.885]	<0.001	<0.001	87.47
	Mechanism of stress	Ischemia-induced stress	8	3.694 [1.804, 5.585]	0.59	<0.001	84.84
		Non-ischemia-induced stress	3	2.660 [-0.620, 5.930]		<0.001	91.46
MDA	All		15	-2.738 [-3.795, -1.681]	<0.001	<0.001	88.43
	Mechanism of stress	Ischemia-induced stress	10	-2.408 [-3.665, -1.151]	0.33	<0.001	89.09
		Non-ischemia-induced stress	5	-3.73 [-6.070, -1.380]		<0.001	87.00
	Treatment duration	More than 2 weeks	5	-4.297 [-6.897, -1.697]	0.16	<0.001	89.71
		2 weeks and less	10	-2.224 [-3.417, -1.030]		<0.001	87.72
SOD	All		9	7.698 [3.863, 11.533]	<0.001	<0.001	90.2
	Mechanism of stress	Ischemia-induced stress	3	1.286 [-3.299, 5.870]	<0.001	<0.001	93.29
		Non-ischemia-induced stress	6	12.31 [7.390, 17.240]		<0.001	73.78
	Treatment duration	More than 2 weeks	5	11.495 [6.524, 16.467]	0.014	0.002	75.69
		2 weeks and less	4	2.848 [-1.909, 7.605]		<0.001	91.74
	Stress side	Bilateral	4	14.660 [5.528, 23.791]	0.05	0.001	82.75
		Unilateral	5	4.575 [0.270, 8.879]		<0.001	92.13

GPx, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase.

antioxidant capacity, glutathione levels, SOD, GPx, and glutathione reductase, but not CAT activities, and reduced MDA levels following melatonin supplementation (104). Melatonin could upregulate the antioxidant nuclear factor erythroid 2-related factor 2 (Nrf2)/heme oxygenase-1 (HO-1) signaling pathway in the damaged testes (83). This pathway induces the transcription of antioxidant proteins and leads to ROS clearance (105). Melatonin may also exert its beneficial effects through upregulating micro-RNA-34a/silent information regulator 1 (SIRT1)/forkhead transcription factors-class O (type1) (FOXO1) epigenetic axis (60). This pathway stimulates antioxidants' expression, inhibits pro-inflammatory pathways, decreases apoptosis, improves mitochondrial biogenesis, repairs cell damage, and prevents cells' dysfunction and infertility (106). Therefore, the effects of melatonin on other oxidative stress-related conditions should be investigated.

4.4 Effects on apoptosis

Apoptosis, the programmed cell death, takes place during normal spermatogenesis. However, physical testicular injuries could elevate germ cell apoptosis and reduce seminiferous tubule diameter and sperm count (60, 68, 107, 108). We observed that melatonin decreases apoptotic germ cells and ameliorates the detrimental impact of physical injuries on the testes. Consistently, melatonin had protective effects against apoptotic cell damage caused by radiation (109, 110) or drugs (89) in the testes in other studies. Melatonin also inhibited endoplasmic reticulum stress-induced apoptosis in reproductive tissues, and therefore exerted protective effects on diabetes-related reproductive impairment (111). The anti-apoptotic effect of melatonin could be mediated *via* increasing Bcl-2 gene expression, an anti-apoptotic gene marker, and lowering

mitochondrial membrane potentials and pro-apoptotic Bcl-2-associated X protein (BAX), P53, caspase 3, and nuclear factor-κB (NF-κB) expression (83, 89, 112, 113). Melatonin also activated the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway in frozen-thawed human sperms (114). Activation of this pathway causes increased sperm motility, suppressed apoptotic cascade and caspase, and decreased membrane permeability and ROS production in spermatozoa (115). Furthermore, melatonin protected human spermatozoa from H_2O_2-induced DNA fragmentation and apoptosis *via* MT1 and extracellular signal–regulated kinase signaling (116). Some other studies also support the anti-apoptotic effects of melatonin on injuries to the female reproductive system (117, 118), heart (113), kidney (119), liver (120), and nervous tissue (121). Due to the limited evidence, we recommend that future studies assess the mechanisms related to the anti-apoptotic effects of melatonin in physical injuries to male testicular tissue.

4.5 Strengths and limitations

To the best of our knowledge, this is the first systematic review and meta-analysis on the protective effects of melatonin against physical injuries to rodents' testicular tissue. Animal models are indispensable tools for assessing new agents' effectiveness and side effects for disease management. However, they do not completely imitate human models. Therefore, the interpretation of our findings should be conducted with caution. High statistical heterogeneity, publication bias, and low quality of the eligible studies are other limitations of our meta-analysis. Between-study methodological heterogeneity was also found in our study due to the differences between animals' characteristics, the dose of melatonin, the intervention schedule, and model of infertility induction. Furthermore, the low number of the included studies prohibited us from doing subgroup analysis for some variables to detect other sources of heterogeneity. Almost all the available studies that have utilized animal models to evaluate the effects of melatonin therapy against male infertility were on rodent subjects. None of the included studies have evaluated and reported possible adverse effects of melatonin therapy. Finally, there are other outcomes that could have been helpful in explaining the mechanisms behind the effects of exogenous melatonin on male rodents' reproductive system such as dihydrotestosterone, corticosterone, testicular and general immunity which were not investigated by the included studies.

5 Conclusion and future direction

In conclusion, melatonin protects against male infertility caused by physical injuries through direct effects on rodent male reproductive system cells, inducing antioxidant defense, and inhibiting apoptosis. More well-designed animal studies should be performed to clarify other mechanisms underlying these effects. To avoid methodological variations, future studies should be more harmonized regarding the mechanism of injury and design of treatment to develop consensus on definitions and methods in this field of research. Also, we recommend employing non-rodent subjects to assess generalizability of the results of this review. Discussions should be made about concerns on melatonin therapy with doses that are needed for anti-infertility effects. Future studies should consider detecting possible adverse effects and other outcomes such as dihydrotestosterone, corticosterone in their protocols.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

NA and NDE conceptualized the study. AS, NDE, SS-Z, and ARS designed the study. NDE, AS, and SS-Z searched databases. NDE, NM, and SP screened the records. NDE, ET, FSS, and AS extracted the data. NDE and AS performed quality assessment. AS, SS-Z, and ARS performed meta-analysis. AS, SS-Z, SD, NH, and ZM provided the draft of the manuscript. AS visualized the data. NA and ARS supervised the work. All authors contributed to the article and approved the final version. AS and NDE have contributed equally to this work and share first authorship. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1123999/full#supplementary-material

References

1. Babakhanzadeh E, Nazari M, Ghasemifar S, Khodadadian A. Some of the factors involved in male infertility: a prospective review. *Int J Gen Med* (2020) 13:29. doi: 10.2147/IJGM.S241099

2. Sahoo DK, Roy A. Compromised rat testicular antioxidant defence system by hypothyroidism before puberty. *Int J Endocrinol* (2012) 2012:637825. doi: 10.1155/2012/637825

3. Aitken RJ, Baker MA, Nixon B. Are sperm capacitation and apoptosis the opposite ends of a continuum driven by oxidative stress? *Asian J Androl* (2015) 17(4):633. doi: 10.4103/1008-682X.153850

4. Shim E, Lee JW, Park H, Zuccarello GC, Kim GH. Hydrogen peroxide signalling mediates fertilization and post-fertilization development in the red alga bostrychia moritziana. *J Exper Botany* (2022) 73(3):727–41. doi: 10.1093/jxb/erab453

5. Agarwal A, Virk G, Ong C, Du Plessis SS. Effect of oxidative stress on male reproduction. *wjomsh* (2014) 32(1):1–17. doi: 10.5534/wjmh.2014.32.1.1

6. Torres-Arce E, Vizmanos B, Babio N, Marquez-Sandoval F, Salas-Huetos AJB. Dietary antioxidants in the treatment of male infertility: Counteracting oxidative stress. *Biology* (2021) 10(3):241. doi: 10.3390/biology10030241

7. Agarwal A, Selvam MKP, Baskaran S, Finelli R, Leisegang K , Barbăroşie C, et al. Highly cited articles in the field of male infertility and antioxidants: A scientometric analysis. *World J Mens World* (2021) 39(4):760. doi: 10.5534/wjmh.200181

8. Sahoo DK, Roy A, Chainy GB. Protective effects of vitamin e and curcumin on l-thyroxine-induced rat testicular oxidative stress. *J Am Chem Soc* (2008) 176(2-3):121–8. doi: 10.1016/j.cbi.2008.07.009

9. Lerner AB, Case JD, Takahashi Y, Lee TH, Mori W. Isolation of melatonin, the pineal gland factor that lightens melanocytes[1]. *J Am Chem Soc* (1958) 80(10):2587–. doi: 10.1021/ja01543a060

10. Slominski RM, Reiter RJ, Schlabritz-Loutsevitch N, Ostrom RS, Slominski AT. Melatonin membrane receptors in peripheral tissues: distribution and functions. *Endocrinol c.* (2012) 351(2):152–66. doi: 10.1016/j.mce.2012.01.004

11. Singh M, Jadhav HR. Melatonin: functions and ligands. *Drug Discov Today* (2014) 19(9):1410–8. doi: 10.1016/j.drudis.2014.04.014

12. Bhattacharya K, Sengupta P, Dutta S. Role of melatonin in male reproduction. *Asian Pacific J Reprod* (2019) 8(5):211. doi: 10.4103/2305-0500.268142

13. Ahmad R, Haldar C. Effect of intra-testicular melatonin injection on testicular functions, local and general immunity of a tropical rodent funambulus pennanti. *Endocrine* (2010) 37(3):479–88. doi: 10.1007/s12020-010-9331-7

14. Hacışevki A, Baba B. An overview of melatonin as an antioxidant molecule: a biochemical approach. *clin approaches p* (2018) 5:59–85. doi: 10.5772/intechopen.79421

15. Rodriguez C, Mayo JC, Sainz RM, Antolín I, Herrera F, Martín V, et al. Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res* (2004) 36(1):1–9. doi: 10.1046/j.1600-079X.2003.00092.x

16. Chainy GBN, Sahoo DK. Hormones and oxidative stress: an overview. *Free Radic Res* (2020) 54(1):1–26. doi: 10.1080/10715762.2019.1702656

17. Reiter RJ, Mayo JC, Tan DX, Sainz RM, Alatorre-Jimenez M, Qin L. Melatonin as an antioxidant: under promises but over delivers. *J Pineal Res* (2016) 61(3):253–78. doi: 10.1111/jpi.12360

18. Sun T, Song L, Ma J, Yu H, Zhou S, Wang S, et al. Melatonin and its protective role against male reproductive toxicity induced by heavy metals, environmental pollutants, and chemotherapy: A review. *BIOCELL* (2020) 44(4):479. doi: 10.32604/biocell.2020.011675

19. Sahoo DK, Chainy GBN. Hormone-linked redox status and its modulation by antioxidants. *Vitamins and Hormones*. Academic Press (2023). doi: 10.1016/bs.vh.2022.10.007

20. Ma X, Idle JR, Krausz KW, Gonzalez FJ. Metabolism of melatonin by human cytochromes p450. *Drug Metab Dispos* (2005) 33(4):489–94. doi: 10.1124/dmd.104.002410

21. Rodriguez C, Mayo JC, Sainz RM, Antolín I, Herrera F, Martín V, et al. Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res* (2004) 36(1):1–9. doi: 10.1046/j.1600-079X.2003.00092.x

22. Milczarek R, Hallmann A, Sokołowska E, Kaletha K, Klimek J. Melatonin enhances antioxidant action of α-tocopherol and ascorbate against NADPH-and iron-dependent lipid peroxidation in human placental mitochondria. *J Pineal Res* (2010) 49(2):149–55. doi: 10.1111/j.1600-079X.2010.00779.x

23. Deng S-L, Wang Z-P, Jin C, Kang X-L, Batool A, Zhang Y, et al. Melatonin promotes sheep leydig cell testosterone secretion in a co-culture with sertoli cells. *Theriogenology* (2018) 106:170–7. doi: 10.1016/j.theriogenology.2017.10.025

24. Yu K, Deng S-L, Sun T-C, Li Y-Y, Liu Y-X. Melatonin regulates the synthesis of steroid hormones on male reproduction: a review. *Molecules* (2018) 23(2):447. doi: 10.3390/molecules23020447

25. Mirhoseini M, Talebpour Amiri F, Karimpour Malekshah AA, Rezanejad Gatabi Z, Ghaffari E. Protective effects of melatonin on testis histology following acute torsion-detorsion in rats. *Int J Reprod Biomed* (2017) 15(3):141–6. doi: 10.29252/ijrm.15.3.141

26. Mirhoseini M, Gatabi ZR, Saeedi M, Morteza-Semnani K, Amiri FT, Kelidari HR, et al. Protective effects of melatonin solid lipid nanoparticles on testis histology after testicular trauma in rats. *Res Pharma Sci* (2019) 14(3):201. doi: 10.4103/1735-5362.258486

27. Rocha C, Rato L, Martins A, Alves M, Oliveira P. Melatonin and male reproductive health: relevance of darkness and antioxidant properties. *Current Mol Med* (2015) 15 (4):299–311. doi: 10.2174/1566524015666150505155530

28. Sun T-C, Li H-Y, Li X-Y, Yu K, Deng S-L, Tian LJC. Protective effects of melatonin on male fertility preservation and reproductive system. *Cryobiology* (2020) 95:1–8. doi: 10.1016/j.cryobiol.2020.01.018

29. Liu J, Clough SJ, Hutchinson AJ, Adamah-Biassi EB, Popovska-Gorevski M, Dubocovich ML, et al. MT1 and MT2 melatonin receptors: a therapeutic perspective. *Ann Rev Pharmacol Toxicol* (2016) 56:361. doi: 10.1146/annurev-pharmtox-010814-124742

30. Navid S, Abbasi M, Hoshino Y. The effects of melatonin on colonization of neonate spermatogonial mouse stem cells in a three-dimensional soft agar culture system. *Stem Cell Res Ther* (2017) 8(1):1–10. doi: 10.1186/s13287-017-0687-y

31. Navid S, Rastegar T, Baazm M, Alizadeh R, Talebi A, Gholami K, et al. *In vitro* effects of melatonin on colonization of neonate mouse spermatogonial stem cells. *Stem Cell Res Ther* (2017) 63(6):370–81. doi: 10.1186/s13287-017-0687-y

32. Zhang G, Yang W, Jiang F, Zou P, Zeng Y, Ling X, et al. PERK regulates Nrf2/ARE antioxidant pathway against dibutyl phthalate-induced mitochondrial damage and apoptosis dependent of reactive oxygen species in mouse spermatocyte-derived cells. *Toxicol Let* (2019) 308:24–33. doi: 10.1016/j.toxlet.2019.03.007

33. Jockers R, Delagrange P, Dubocovich ML, Markus RP, Renault N, Tosini G, et al. Update on melatonin receptors: IUPHAR review 20. *Bri J Pharmacol* (2016) 173 (18):2702–25. doi: 10.1111/bph.13536

34. Reiter RJ, Rosales-Corral S, Tan DX, Jou MJ, Galano A, Xu BJC, et al. Melatonin as a mitochondria-targeted antioxidant: one of evolution's best ideas. *Cell Mol Life Sci* (2017) 74(21):3863–81. doi: 10.1007/s00018-017-2609-7

35. Reiter RJ, Tan DX, Rosales-Corral S, Galano A, Zhou XJ, Xu BJM. Mitochondria: central organelles for melatonin' s antioxidant and anti-aging actions. *Molecules* (2018) 23 (2):509. doi: 10.3390/molecules23020509

36. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* (2021) 372:n71. doi: 10.1136/bmj.n71

37. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst. Rev* (2016) 5(1):210. doi: 10.1186/s13643-016-0384-4

38. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res methodol* (2014) 14:43. doi: 10.1186/1471-2288-14-43

39. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Controlled Clin Trials.* (1986) 7(3):177–88. doi: 10.1016/0197-2456(86)90046-2

40. Higgins J, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, Welch VA eds. *Cochrane handbook for systematic reviews of interventions. 2nd ed*. Chichester (UK: John Wiley & Sons (2019).

41. Liu ZM, Zhu WP, Huang DP, Bai PM. [Effects of melatonin on oxidative stress and apoptosis-related gene signaling pathways following testicular torsion in rats]. *Zhonghua Nan Ke Xue.* (2019) 25(5):309–14.

42. Yurtçu M, Abasiyanik A, Avunduk M, Karagözoğlu E, Abasiyanik F. The effects of one dose and seven days' managements of melatonin and steroid to prevent ischemia-reperfusion injury in testicular torsion. *Turkiye Klinikleri J Med Sci* (2005) 25:496–500.

43. Yurtçu M, Abasiyanik A, Gökçe R, Avinduk MC, Özdamar MY. Investigation of the effects of melatonin and steroid (st) in preventing of testicular atrophy after surgical management performed with fowler-stephens procedure of intraabdominal testis in late period. *Cocuk Cerrahisi Dergisi* (2008) 22:33–8.

44. Duru FIO, Oshiozokhai Y, Noronha CC, Okanlawon A. Alterations in morphometry and malondialdehyde levels in adult sprague-dawley rat testes in three obstructive vasectomy models: Effect of melatonin. *Asian J Pharm Clin Res* (2011) 4:27–30.

45. Duru FI, Olabiyi O, Noronha CC, Akinwande AI, Okanlawon AO. Brief ischaemia reduces testicular lipid peroxidation following subsequent ischaemia: an evidence for ischaemic preconditioning. *Nig Q J Hosp Med* (2008) 18(3):149–52. doi: 10.4314/nqjhm.v18i3.45016

46. Aktaş A, Tuncer M, Yıldırım A, Nergiz Y, Akkus M. Protective effects of melatonin on testicular torsion and detorsion damage in sprague-dawley rats. *Int J Morphol* (2011) 29:7–15. doi: 10.4067/S0717-95022011000100001

47. Bustos-Obregon E, Sánchez R, Ramos B, Torres-Diaz L. Rat spermatogenesis damage in intermittent hypobaric hypoxia and the protective role of melatonin. II: Testicular parameters. *Int J Morphol* (2010) 28:537–47. doi: 10.4067/S0717-95022010000200034

48. Chen YT, Chuang FC, Yang CC, Chiang JY, Sung PH, Chu YC, et al. Combined melatonin-adipose derived mesenchymal stem cells therapy effectively protected the testis from testicular torsion-induced ischemia-reperfusion injury. *Stem Cell Res Ther* (2021) 12 (1):370. doi: 10.1186/s13287-021-02439-x

49. Erdemir F, Parlaktaş BS, Özyurt H, Boztepe Ö, Atiş Ö, Şahin Ş. Antioxidant effect of melatonin in systemic circulation of rats after unilateral testicular torsion. *Turkish J Med Sci* (2008) 38:1–6.

50. Eşrefoğlu M, Gül M, Parlakpinar H, Acet A. Effects of melatonin and caffeic acid phenethyl ester on testicular injury induced by myocardial ischemia/reperfusion in rats. *Fundam Clin Pharmacol* (2005) 19(3):365–72. doi: 10.1111/j.1472-8206.2005.00331.x

51. Hartley R, Castro-Sánchez R, Ramos-Gonzalez B, Bustos-Obregón E. Rat spermatogenesis damage in intermittent hypobaric hypoxia and the protective role of melatonin: I cauda epididymal spermatozoa. *Int J Morphol* (2009) 27:1275–84. doi: 10.4067/S0717-95022009000400049

52. Kurcer Z, Hekimoglu A, Aral F, Baba F, Sahna E. Effect of melatonin on epididymal sperm quality after testicular ischemia/reperfusion in rats. *Fertil Steril* (2010) 93(5):1545–9. doi: 10.1016/j.fertnstert.2009.01.146

53. Kurcer Z, Oguz E, Ozbilge H, Baba F, Aksoy N, Celik N. Effect of melatonin on testicular ischemia/reperfusion injury in rats: is this effect related to the proinflammatory cytokines? *Fertil Steril* (2008) 89(5 Suppl):1468–73. doi: 10.1016/j.fertnstert.2007.04.065

54. Sekmenli T, Gunduz M, Öztürk B, Karabağlı P, Ciftci I, Tekin G, et al. The effects of melatonin and colchicine on ischemia-reperfusion injury in experimental rat testicular torsion model. *J Pediatr Surg* (2017) 52(4):582–6. doi: 10.1016/j.jpedsurg.2016.11.035

55. Vargas A, Bustos-Obregón E, Hartley R. Effects of hypoxia on epididymal sperm parameters and protective role of ibuprofen and melatonin. *Biol Res* (2011) 44(2):161–7. doi: 10.4067/S0716-97602011000200008

56. Yildirim A, Akkus M, Nergiz Y, Baran OP. The effect of melatonin on ductus epididymis. unilateral testicular torsion in rats. *Saudi Med J* (2007) 28(2):288–9.

57. Yuan XC, Wang P, Li HW, Wu QB, Zhang XY, Li BW, et al. Effects of melatonin on spinal cord injury-induced oxidative damage in mice testis. *Andrologia* (2017) 49(7). doi: 10.1111/and.12692

58. Saalu LC, Togun VA, Oyewopo AO, Raji Y. Artificial cryptorchidism and the moderating effect of melatonin (N-acetyl. 5 methoxy tryptamin) in sprague-dawley rats. *J Appl Sci* (2006) 6:2889–94. doi: 10.3923/jas.2006.2889.2894

59. Semercioz A, Baltaci AK, Mogulkoc R, Avunduk MC. Effect of zinc and melatonin on oxidative stress and serum inhibin-b levels in a rat testicular torsion-detorsion model. *Biochem Genet* (2017) 55(5-6):395–409. doi: 10.1007/s10528-017-9826-5

60. Abo El Gheit RE, Soliman NA, Nagla SA, El-Sayed RM, Badawi GA, Emam MN, et al. Melatonin epigenetic potential on testicular functions and fertility profile in varicocele rat model is mediated by silent information regulator 1. *Br J Pharmacol* (2022) 179(13):3363–81. doi: 10.1111/bph.15804

61. Asghari A, Akbari G, Meghdadi A, Mortazavi P. Effects of melatonin and metformin co-administration on testicular ischemia/reperfusion injury in rats. *J Pediatr Urol* (2016) 12(6):410.e1–.e7. doi: 10.1016/j.jpurol.2016.06.017

62. Duru FI, Noronha CC, Akinwande AI, Okanlawon AO. Effects of torsion, detorsion and melatonin on testicular malondialdehyde level. *West Afr J Med* (2007) 26 (4):312–5. doi: 10.4314/wajm.v26i4.28333

63. Ekici S, Doğan Ekici AI, Öztürk G, Benli Aksungar F, Sinanoğlu O, Turan G, et al. Comparison of melatonin and ozone in the prevention of reperfusion injury following unilateral testicular torsion in rats. *Urology* (2012) 80(4):899–906. doi: 10.1016/j.urology.2012.06.049

64. Gul SS, Gurgul S, Uysal M, Erdemir F. The protective effects of pulsed magnetic field and melatonin on testis torsion and detorsion induced rats indicated by scintigraphy, positron emission Tomography/Computed tomography and histopathological methods. *Urol J* (2018) 15(6):387–96. doi: 10.22037/uj.v0i0.4404

65. Jeong SJ, Choi WS, Chung JS, Baek M, Hong SK, Choi H. Preventive effects of cyclosporine a combined with prednisolone and melatonin on contralateral testicular damage after ipsilateral torsion-detorsion in pubertal and adult rats. *J Urol* (2010) 184 (2):790–6. doi: 10.1016/j.juro.2010.03.109

66. Kanter M. Protective effects of melatonin on testicular torsion/detorsion-induced ischemia-reperfusion injury in rats. *Exp Mol Pathol* (2010) 89(3):314–20. doi: 10.1016/j.yexmp.2010.07.006

67. Koksal M, Oğuz E, Baba F, Eren MA, Ciftci H, Demir ME, et al. Effects of melatonin on testis histology, oxidative stress and spermatogenesis after experimental testis ischemia-reperfusion in rats. *Eur Rev Med Pharmacol Sci* (2012) 16(5):582–8.

68. Mahmudi SAA, Ghasemi Hamidabadi H, Moayeri A, Nazm Bojnordi M, Zahiri M, Madani Z, et al. Melatonin ameliorates testes against forced treadmill exercise training on spermatogenesis in rats. *Folia Med (Plovdiv).* (2022) 64(1):75–83. doi: 10.3897/folmed.64.e57544

69. Minaii B, Moayeri A, Shokri S, Habibi Roudkenar M, Golmohammadi T, Malek F, et al. Melatonin improve the sperm quality in forced swimming test induced oxidative stress in nandrolone treated wistar rats. *Acta Med Iran.* (2014) 52 (7):496–504.

70. Mirhoseini M, Rezanejad Gatabi Z, Saeedi M, Morteza-Semnani K, Talebpour Amiri F, Kelidari HR, et al. Protective effects of melatonin solid lipid nanoparticles on testis histology after testicular trauma in rats. *Res Pharm Sci* (2019) 14(3):201–8. doi: 10.4103/1735-5362.258486

71. Moayeri A, Mokhtari T, Hedayatpour A, Abbaszadeh HA, Mohammadpour S, Ramezanikhah H, et al. Impact of melatonin supplementation in the rat spermatogenesis subjected to forced swimming exercise. *Andrologia* (2018) 50(3). doi: 10.1111/and.12907

72. Olayaki LA, Alagbonsi IA, Abdulkadir HO, Idowu FO. Low dose of melatonin ameliorates cryptorchidism-induced spermatotoxicity in rats. *J Anatom Soc India* (2017) 66(1):67–71. doi: 10.1016/j.jasi.2017.05.010

73. Onur R, Semercioöz A, Orhan I, Yekeler H. The effects of melatonin and the antioxidant defence system on apoptosis regulator proteins (Bax and bcl-2) in experimentally induced varicocele. *Urol Res* (2004) 32(3):204–8. doi: 10.1007/s00240-004-0403-0

74. Ozturk A, Baltaci AK, Mogulkoc R, Ozturk B. The effect of prophylactic melatonin administration on reperfusion damage in experimental testis ischemia-reperfusion. *Neuro Endocrinol Lett* (2003) 24(3-4):170–2.

75. Parlaktas BS, Atilgan D, Ozyurt H, Gencten Y, Akbas A, Erdemir F, et al. The biochemical effects of ischemia-reperfusion injury in the ipsilateral and contralateral testes of rats and the protective role of melatonin. *Asian J Androl* (2014) 16(2):314–8. doi: 10.4103/1008-682X.122202

76. Sahna E, Türk G, Atessahin A, Yilmaz S, Olmez E. Remote organ injury induced by myocardial ischemia and reperfusion on reproductive organs, and protective effect of melatonin in male rats. *Fertil Steril* (2007) 88(1):188–92. doi: 10.1016/j.fertnstert.2006.11.068

77. Semercioz A, Onur R, Ogras S, Orhan I. Effects of melatonin on testicular tissue nitric oxide level and antioxidant enzyme activities in experimentally induced left varicocele. *Neuro Endocrinol Lett* (2003) 24(1-2):86–90.

78. Yurtçu M, Abasiyanik A, Avunduk MC, Muhtaroğlu S. Effects of melatonin on spermatogenesis and testicular ischemia-reperfusion injury after unilateral testicular torsion-detorsion. *J Pediatr Surg* (2008) 43(10):1873–8. doi: 10.1016/j.jpedsurg.2008.01.065

79. Yurtçu M, Abasiyanik A, Biçer S, Avunduk MC. Efficacy of antioxidant treatment in the prevention of testicular atrophy in experimental testicular torsion. *J Pediatr Surg* (2009) 44(9):1754–8. doi: 10.1016/j.jpedsurg.2008.11.043

80. Abasiyanik A, Dağdönderen L. Beneficial effects of melatonin compared with allopurinol in experimental testicular torsion. *J Pediatr Surg* (2004) 39(8):1238–41. doi: 10.1016/j.jpedsurg.2004.04.018

81. Gurbilek M, Vatansev H, Gültekin F, Dilsiz A, Vatansev C, Aköz M. Prevention of testicular damage by free radical scavengers after acute experimental torsion. *Biomed Res* (2000) 11:315–9.

82. Soma H, Mrinmoy S, Sananda D, Prasanta G, Sujay KB, Debasish B, et al. Melatonin ameliorates heat stress induced dysregulation of testicular function in wistar rat by restoring tissue health, hormone and antioxidant status and modulating heat shock protein expression. *Int J Life Sci Pharma Res* (2020) 10(5):31–43. doi: 10.22376/ijpbs/lpr.2020.10.5.L31-43

83. Guo Y, Sun J, Li T, Zhang Q, Bu S, Wang Q, et al. Melatonin ameliorates restraint stress-induced oxidative stress and apoptosis in testicular cells *via* NF-κB/iNOS and Nrf2/HO-1 signaling pathway. *Sci Rep* (2017) 7(1):9599. doi: 10.1038/s41598-017-09943-2

84. Qin DZ, Cai H, He C, Yang DH, Sun J, He WL, et al. Melatonin relieves heat-induced spermatocyte apoptosis in mouse testes by inhibition of ATF6 and PERK signaling pathways. *Zool Res* (2021) 42(4):514–24. doi: 10.24272/j.issn.2095-8137.2021.041

85. Zhang P, Zheng Y, Lv Y, Li F, Su L, Qin Y, et al. Melatonin protects the mouse testis against heat-induced damage. *Mol Hum Reprod* (2020) 26(2):65–79. doi: 10.1093/molehr/gaaa002

86. Reiter RJ, Tan D-X, Galano A. Melatonin reduces lipid peroxidation and membrane viscosity. *Front Media SA* (2014) 5:377. doi: 10.3389/fphys.2014.00377

87. Haghi-Aminjan H, Asghari MH, Farhood B, Rahimifard M, Hashemi Goradel N, Abdollahi M. The role of melatonin on chemotherapy-induced reproductive toxicity. *J Pharm Pharmacol* (2018) 70(3):291–306. doi: 10.1111/jphp.12855

88. Zi T, Liu Y, Zhang Y, Wang Z, Wang Z, Zhan S, et al. Protective effect of melatonin on alleviating early oxidative stress induced by DOX in mice spermatogenesis and sperm quality maintaining. *Reprod Biol Endocrinol* (2022) 20(1):1–11. doi: 10.1186/s12958-022-00977-4

89. Moradi M, Goodarzi N, Faramarzi A, Cheraghi H, Hashemian AH, Jalili C. Melatonin protects rats testes against bleomycin, etoposide, and cisplatin-induced toxicity *via* mitigating nitro-oxidative stress and apoptosis. *Biomed Pharmacother* (2021) 138:111481. doi: 10.1016/j.biopha.2021.111481

90. Izzo G, Francesco A, Ferrara D, Campitiello MR, Serino I, Minucci S, et al. Expression of melatonin (MT1, MT2) and melatonin-related receptors in the adult rat testes and during development. *Zygote* (2010) 18(3):257–64. doi: 10.1017/S0967199409990293

91. Gwayi N, Bernard R. The effects of melatonin on sperm motility *in vitro* in wistar rats. *Andrologia* (2002) 34(6):391–6. doi: 10.1046/j.1439-0272.2002.00522.x

92. Yang W-C, Tang K-Q, Fu C-Z, Riaz H, Zhang Q, Zan L-S. Melatonin regulates the development and function of bovine sertoli cells via its receptors MT1 and MT2. *Anim Reprod Sci* (2014) 147(1-2):10–6. doi: 10.1016/j.anireprosci.2014.03.017

93. Hassan MH, El–Taieb MA, Fares NN, Fayed HM, Toghan R, Ibrahim HM. Men with idiopathic oligoasthenoteratozoospermia exhibit lower serum and seminal plasma melatonin levels: Comparative effect of night–light exposure with fertile males. *Exp Ther Med* (2020) 20(1):235–42. doi: 10.3892/etm.2020.8678

94. Wang R-S, Yeh S, Tzeng C-R, Chang C. Androgen receptor roles in spermatogenesis and fertility: lessons from testicular cell-specific androgen receptor knockout mice. *Endocr Rev* (2009) 30(2):119–32. doi: 10.1210/er.2008-0025

95. Bahrami N, Goudarzi M, Hosseinzadeh A, Sabbagh S, Reiter RJ, Mehrzadi S. Evaluating the protective effects of melatonin on di (2-ethylhexyl) phthalate-induced testicular injury in adult mice. *Biomed Pharmacother* (2018) 108:515–23. doi: 10.1016/j.biopha.2018.09.044

96. Yang M, Guan S, Tao J, Zhu K, Lv D, Wang J, et al. Melatonin promotes male reproductive performance and increases testosterone synthesis in mammalian leydig cells. *Biol Reprod* (2021) 104(6):1322–36. doi: 10.1093/biolre/ioab046

97. Cipolla-Neto J, Amaral FG, Soares JMJr., Gallo CC, Furtado A, Cavaco JE, et al. The crosstalk between melatonin and sex steroid hormones. *Neuroendocrinology* (2022) 112(2):115–29. doi: 10.1159/000516148

98. Kumanov P, Nandipati K, Tomova A, Agarwal A. Inhibin b is a better marker of spermatogenesis than other hormones in the evaluation of male factor infertility. *Fertil steril* (2006) 86(2):332–8. doi: 10.1016/j.fertnstert.2006.01.022

99. Jankowska K, Suszczewicz N, Rabijewski M, Dudek P, Zgliczyński W, Maksym RB. Inhibin-b and FSH are good indicators of spermatogenesis but not the best indicators of fertility. *Life* (2022) 12(4):511. doi: 10.3390/life12040511

100. Lu XL, Liu JJ, Li JT, Yang QA, Zhang JM. Melatonin therapy adds extra benefit to varicecelectomy in terms of sperm parameters, hormonal profile and total antioxidant capacity: A placebo-controlled, double-blind trial. *Andrologia* (2018) 50(6):e13033. doi: 10.1111/and.13033

101. Ko EY, Sabanegh ESJr, Agarwal A. Male Infertility testing: reactive oxygen species and antioxidant capacity. *Fertil steril* (2014) 102(6):1518–27. doi: 10.1016/j.fertnstert.2014.10.020

102. Agarwal A, Sharma RK, Sharma R, Assidi M, Abuzenadah AM, Alshahrani S, et al. Characterizing semen parameters and their association with reactive oxygen species in infertile men. *Reprod Biol Endocrinol* (2014) 12(1):1–9. doi: 10.1186/1477-7827-12-33

103. Reiter RJ, Manchester LC, Tan D-X. Neurotoxins: free radical mechanisms and melatonin protection. *Curr neuropharmacol* (2010) 8(3):194–210. doi: 10.2174/157015910792246236

104. Morvaridzadeh M, Sadeghi E, Agah S, Nachvak SM, Fazelian S, Moradi F, et al. Effect of melatonin supplementation on oxidative stress parameters: a systematic review and meta-analysis. *Pharmacol Res* (2020) 161:105210. doi: 10.1016/j.phrs.2020.105210

105. Wardyn JD, Ponsford AH, Sanderson CM. Dissecting molecular cross-talk between Nrf2 and NF-κB response pathways. *Biochem Soc Trans* (2015) 43(4):621–6. doi: 10.1042/BST20150014

106. Alam F, Syed H, Amjad S, Baig M, Khan TA, Rehman R. Interplay between oxidative stress, SIRT1, reproductive and metabolic functions. *Curr Res Physiol* (2021) 4:119–24. doi: 10.1016/j.crphys.2021.03.002

107. Fan X, Liu Y, Yue M, Yue W, Ren G, Zhang J, et al. Effect of cryptorchidism on the histomorphometry, proliferation, apoptosis, and autophagy in boar testes. *Animals* (2021) 11(5):1379. doi: 10.3390/ani11051379

108. Barqawi A, Caruso A, Meacham RB. Experimental varicocele induces testicular germ cell apoptosis in the rat. *J urol* (2004) 171(1):501–3. doi: 10.1097/01.ju.0000088775.69010.61

109. Take G, Erdogan D, Helvacioglu F, Göktas G, Ozbey G, Uluoglu C, et al. Effect of melatonin and time of administration on irradiation-induced damage to rat testes. *Braz J Med Biol Res* (2009) 42:621–8. doi: 10.1590/S0100-879X2009000700006

110. Khan S, Adhikari JS, Rizvi MA, Chaudhury NK. Radioprotective potential of melatonin against 60Co γ-ray-induced testicular injury in male C57BL/6 mice. *J Biomed Sci* (2015) 22(1):1–15. doi: 10.1186/s12929-015-0156-9

111. Armandeh M, Bameri B, Haghi-Aminjan H, Foroumadi R, Ataei M, Hassani S, et al. A systematic review on the role of melatonin and its mechanisms on diabetes-related reproductive impairment in non-clinical studies. *Front Endocrinol* (2022) 13. doi: 10.3389/fendo.2022.1022989

112. Nazeri T, Hedayatpour A, Kazemzadeh S, Safari M, Safi S, Khanehzad M. Antioxidant effect of melatonin on proliferation, apoptosis, and oxidative stress variables in frozen-thawed neonatal mice spermatogonial stem cells. *Biopreserv Biobanking* (2022) 20(4):374–83. doi: 10.1089/bio.2021.0128

113. Chen S, Li Y, Fu S, Li Y, Wang C, Sun P, et al. Melatonin alleviates arginine vasopressin-induced cardiomyocyte apoptosis via increasing Mst1-Nrf2 pathway activity to reduce oxidative stress. *Biochem Pharmacol* (2022) 206:115265. doi: 10.1016/j.bcp.2022.115265

114. Najafi A, Adutwum E, Yari A, Salehi E, Mikaeili S, Dashtestani F, et al. Melatonin affects membrane integrity, intracellular reactive oxygen species, caspase3 activity and AKT phosphorylation in frozen thawed human sperm. *Cell Tissue Res* (2018) 372(1):149–59. doi: 10.1007/s00441-017-2743-4

115. Koppers AJ, Mitchell LA, Wang P, Lin M, Aitken RJ. Phosphoinositide 3-kinase signalling pathway involvement in a truncated apoptotic cascade associated with motility loss and oxidative DNA damage in human spermatozoa. *Biochem J* (2011) 436(3):687–98. doi: 10.1042/BJ20110114

116. Espino J, Ortiz Á, Bejarano I, Lozano GM, Monllor F, García JF, et al. Melatonin protects human spermatozoa from apoptosis *via* melatonin receptor–and extracellular signal–regulated kinase-mediated pathways. *Fertil steril* (2011) 95(7):2290–6. doi: 10.1016/j.fertnstert.2011.03.063

117. Xue R, Li S, Wei Z, Zhang Z, Cao Y. Melatonin attenuates di-(2-ethylhexyl) phthalate-induced apoptosis of human granulosa cells by inhibiting mitochondrial fission. *Reprod Toxicol* (2022) 113:18–29. doi: 10.1016/j.reprotox.2022.08.004

118. Feng J, Ma W-W, Li H-X, Pei X-Y, Deng S-L, Jia H, et al. Melatonin prevents cyclophosphamide-induced primordial follicle loss by inhibiting ovarian granulosa cell apoptosis and maintaining AMH expression. *Front Endocrinol* (2022) 13. doi: 10.3389/fendo.2022.895095

119. Sun J, Pan J, Liu Q, Cheng J, Tang Q, Ji Y, et al. Melatonin attenuates mitochondrial damage in aristolochic acid-induced acute kidney injury. *Biomol Ther* (2022) 31(1):97–107. doi: 10.4062/biomolther.2022.054

120. Sokolović D, Lazarević M, Milić D, Stanojković Z, Mitić K, Sokolović DT. Melatonin arrests excessive inflammatory response and apoptosis in lipopolysaccharide-damaged rat liver: A deeper insight into its mechanism of action. *Tissue Cell* (2022) 79:101904. doi: 10.1016/j.tice.2022.101904

121. Zhang Y, Cook A, Kim J, Baranov SV, Jiang J, Smith K, et al. Melatonin inhibits the caspase-1/cytochrome c/caspase-3 cell death pathway, inhibits MT1 receptor loss and delays disease progression in a mouse model of amyotrophic lateral sclerosis. *Neurobiol dis* (2013) 55:26–35. doi: 10.1016/j.nbd.2013.03.008

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 26 May 2023
DOI 10.3389/fendo.2023.1089315

OPEN ACCESS

EDITED BY
Dipak Kumar Sahoo,
Iowa State University, United States

REVIEWED BY
Isabel Castro-Piedras,
Texas Tech University Health Sciences Center, United States
Biswaranjan Paital,
Odisha University of Agriculture and Technology, India

*CORRESPONDENCE
Arian Karimi Rouzbahani
ariankarimi1998@gmail.com

RECEIVED 04 November 2022
ACCEPTED 09 May 2023
PUBLISHED 26 May 2023

CITATION
Farasati Far B, Broomand Lomer N, Gharedaghi H, Sahrai H, Mahmoudvand G and Karimi Rouzbahani A (2023) Is beta-carotene consumption associated with thyroid hormone levels?
Front. Endocrinol. 14:1089315.
doi: 10.3389/fendo.2023.1089315

Is beta-carotene consumption associated with thyroid hormone levels?

Bahareh Farasati Far[1], Nima Broomand Lomer[2], Hossein Gharedaghi[3], Hadi Sahrai[4], Golnaz Mahmoudvand[5,6] and Arian Karimi Rouzbahani[5,6]*

[1]Department of Chemistry, Iran University of Science and Technology, Tehran, Iran, [2]Faculty of Medicine, Guilan University of Medical Sciences, Guilan, Iran, [3]School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran, [4]Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran, [5]Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran, [6]USERN Office, Lorestan University of Medical Sciences, Khorramabad, Iran

The thyroid hormones play a pivotal role in various physiological processes, including growth, metabolism regulation, and reproduction. While non-modifiable factors are known to impact thyroid function, such as genetics and age, nutritional factors are also important. Diets rich in selenium and iodine are conventionally acknowledged to be beneficial for the production and release of thyroid hormones. Recent studies have suggested a potential link between beta-carotene, a precursor to vitamin A (retinol), and thyroid function. Beta-carotene is known for its antioxidant properties and has been shown to play a role in the prevention of various clinical conditions such as cancer and cardiovascular and neurological diseases. However, its impact on thyroid function is still unclear. Some studies have suggested a positive association between beta-carotene levels and thyroid function, while others have found no significant effect. Conversely, the hormone produced by the thyroid gland, thyroxine, enhances the conversion of beta-carotene to retinol. Furthermore, vitamin A derivatives are being explored as potential therapeutic options for thyroid malignancies. In this review, we highlight the mechanisms through which beta-carotene/retinol and thyroid hormones interact and review the findings of clinical studies examining the association between beta-carotene consumption and thyroid hormone levels. Our review underscores the need for further research to clarify the relationship between beta-carotene and thyroid function.

KEYWORDS

beta carotene, thyroid hormone levels, thyroid cancer, hormonal imbalance, vitamin A, retinol

1 Introduction

Thyroid hormones (THs) have various functions in almost all cell types. Thyroid malfunction is prevalent across the globe, and it is identified as a controllable and treatable disease. Taking into account the greater knowledge of thyroid disorders we do have today and the accessibility of effective diagnostic tests for assessing THs, it is vital to manage TH

secretion abnormalities cautiously (1). Subclinical hypothyroidism is when thyroid-stimulating hormone (TSH) levels are slightly elevated (between 4.6 and 8.0 mIU/ml) despite normal free T4 levels, while overt hypothyroidism is characterized by decreased free T4 levels. On the other hand, subclinical hyperthyroidism is marked by mildly suppressed TSH levels (usually still above 0.1 mIU/ml) and normal TH levels, while overt hyperthyroidism is characterized by increased TH levels (2). In most cases, hypothyroidism and hyperthyroidism are caused by pathological processes within the thyroid gland known as primary thyroid disease. However, in uncommon situations, hypothyroidism and hyperthyroidism are caused by dysfunction of the hypothalamus, pituitary, struma ovary, or responsive thyroid cancer metastases (3, 4). Autoimmunity is the most common cause of thyroid gland dysfunction in iodine-deficient populations, resulting in Graves' disease, Hashimoto's thyroiditis, and pregnancy hypothyroidism. Also, recent studies reported that oxidative stress (OS) is associated with hyperthyroidism and hypothyroidism (5). The mechanism of these two clinical conditions is different, and OS is produced by separate pathways: elevated reactive oxygen species (ROS) formation in hyperthyroidism and decreased antioxidant availability in hypothyroidism (6). In addition, THs can play an oxidative role in target cells (7).

Beta-carotene (β-carotene), known as a fat-soluble pigment, is found in red, orange, and yellow vegetables and fruits. When the body is deprived of vitamin A, beta-carotene is transformed into vitamin A. β-Carotene is an antioxidant and a substance that prevents activated oxygen molecules from damaging cells (8). Phytochemicals like β-carotene are crucial in fighting free radicals as they target various enzymes across multiple pathways (9). Also, the intake of other antioxidants such as vitamin C and vitamin E in rats showed that they can reduce oxidative stress (10, 11). Alongside β-carotene, selenium is also an antioxidant that is associated with TH levels. Rostami et al. reported that decreased selenium level in the serum is a trigger of oxidative stress, resulting in thyroid dysfunction (12). As a result, these findings can be helpful in the treatment of thyroid disorders and in decreasing harmful events caused by OS. However, there is a lack of both *in vivo* and *in vitro* studies to understand the association and mechanism between β-carotene and TH levels with OS events and other thyroid-related complications. This study was conducted in order to review the mechanisms through which beta-carotene/retinol and THs interact and further review the findings of clinical studies in this regard.

The aim of this review paper is to investigate the potential association between beta-carotene consumption and thyroid hormone levels. Beta-carotene is a precursor to vitamin A, which plays an important role in thyroid hormone synthesis and metabolism. There is growing interest in the potential health benefits of beta-carotene, particularly its ability to prevent chronic diseases such as cancer and cardiovascular disease. However, some studies have suggested that high levels of beta-carotene intake may interfere with thyroid function, leading to alterations in thyroid hormone levels. This review will examine the available evidence on the relationship between beta-carotene consumption and thyroid hormone levels, including both observational and interventional studies. We will also explore the potential mechanisms by which beta-carotene may affect thyroid function and identify areas for further research. Overall, this review aims to provide a comprehensive assessment of the current evidence on the association between beta-carotene consumption and thyroid hormone levels and to inform future research in this area.

Research on the relationship between beta-carotene consumption and thyroid hormone levels is an area of active investigation, and future studies are likely to build on the existing body of research. One possible direction for future research is to conduct clinical trials that examine the effects of beta-carotene supplementation on thyroid hormone levels in humans. This type of study would involve giving participants beta-carotene supplements and monitoring their thyroid hormone levels over time to determine whether there is a causal relationship between beta-carotene consumption and changes in thyroid function. Another potential avenue for future research is to explore the mechanisms by which beta-carotene affects thyroid hormone levels. For example, studies could investigate the role of beta-carotene in regulating the activity of enzymes that are involved in thyroid hormone synthesis or metabolism. In addition, future studies could examine the relationship between beta-carotene consumption and other markers of thyroid function beyond just thyroid hormone levels, such as TSH or thyroid antibodies. This could provide a more comprehensive understanding of the relationship between beta-carotene and thyroid health. Therefore, the field of research on beta-carotene consumption and thyroid hormone levels is still evolving, and future studies are likely to shed more light on this important topic.

2 Search strategy

The search strategy used a combination of relevant medical subject headings (MeSH terms) and keywords, including ("beta carotene" OR "beta-Carotene 15,15-Monooxygenase") AND ("Thyroid Hormones" OR "hypothyroidism" OR "hyperthyroidism" OR "T3 thyroid hormone" OR "T4 thyroid hormones" OR "mechanism of action" OR "thyroid" OR "vitamin A" OR "Retinoid" OR "Retinol" OR "thyroid stimulating hormone" OR "TSH"). The search strategy was designed to be comprehensive and was iteratively refined based on the initial search results. The search results were then screened based on the inclusion and exclusion criteria, and the relevant studies were selected for full-text review.

2.1 Inclusion criteria

To identify relevant studies for this review, a comprehensive search strategy was employed using electronic databases, including PubMed, MEDLINE, and Google Scholar. The search was limited to studies published between January 1990 and December 2022, and only English-language articles were included. The following inclusion criteria were used:

- Studies reporting on the relationship between beta-carotene consumption and thyroid hormone levels in humans
- Studies reporting on the effects of beta-carotene supplementation or dietary intake on thyroid function tests, including the levels of TSH, triiodothyronine (T3), and thyroxine (T4)
- Studies that reported beta-carotene intake through dietary sources or supplements
- Studies that included adult human subjects

2.2 Exclusion criteria

The following exclusion criteria were used to ensure the relevance and quality of the studies included in this review:

- Studies that did not report on the relationship between beta-carotene consumption and thyroid hormone levels
- Studies that did not include human subjects
- Studies that were not published in English or were published before January 1990 or after December 2022
- Studies that were not peer-reviewed or were of low quality, as assessed by the authors

3 Is beta-carotene level different among men and women?

Studies have reported sex differences in beta-carotene levels, with women generally having higher concentrations than men. The underlying mechanisms for these differences are not yet fully understood, but sex hormones such as estrogen and testosterone may play a role. For instance, estrogen can increase the expression of beta-carotene transporter proteins in the liver, leading to higher plasma concentrations of beta-carotene in women (13). Thyroid hormones are important regulators of metabolism, growth, and development in the body. The thyroid gland produces two main hormones: T4 and T3. Several studies have reported sex differences in thyroid hormone levels, with women having higher levels than men. This may be due to the influence of estrogen on thyroid hormone production and metabolism. Estrogen can enhance the conversion of T4 to T3, the more active form of thyroid hormone, leading to higher circulating levels of T3 in women. Additionally, estrogen can increase the number of thyroid hormone receptors in target tissues, further amplifying the hormonal effects (14). The differences in beta-carotene and thyroid hormone levels between men and women may have important implications for overall health and disease risk. For instance, low levels of beta-carotene have been linked to increased risk of cancer, cardiovascular disease, and other chronic conditions. On the other hand, high levels of thyroid hormone may increase the risk of osteoporosis, atrial fibrillation, and other health problems (15). Therefore, understanding the sex differences in these biomarkers can help identify individuals who may be at higher risk of certain diseases and inform targeted prevention and treatment strategies.

4 Beta-carotene and retinoid: the peripheral metabolism of thyroid hormones

Carotenoids are lipophilic antioxidants copiously present as colored pigments in vegetables and fruits. Carotenoids can be extracted from various parts of plants, including the green parts, flowers, fruits, seeds, roots, and tubers. These compounds are found in a range of vegetables like carrots, pumpkins, spinach, and tomatoes, as well as fruits such as watermelons and raspberries (Figure 1). Within plant cells, carotenoids are situated in the organelles of chloroplast known as thylakoid membranes.

There are hundreds of types of carotenoids in nature; however, almost 20 types, including beta-carotene, can be found in human tissues (16). Beta-carotene, a tetra-terpenoid containing two β-ionic rings, is one of the most common carotenoids in the human diet (17). It plays countless biological roles in the human body. The most vital function of beta-carotene is acting as a precursor of vitamin A (8). As beta-carotene cannot be produced in the human body, it must be supplied by consuming plant-based nutrition. In the intestine, beta-carotene is absorbed by enterocytes concurrently with consumed lipids. At the basolateral aspect of the enterocytes, beta-carotene gets cleaved by beta-carotene 15–15′-oxygenase, synthesizing retinal, which then converts to retinoic acid (RA) and retinol (17, 18). Vitamin A derivates subsequently influence physiological growth and development, as well as eyesight, regulation of the immune system, and reproduction (8, 17, 19). In addition to these effects, the endocrine system has a significant link with the retinoid system (20). Thyroid hormones, including T3 and T4, are vital hormones in the metabolism of different organs. These hormones mainly act through the cytosolic functions of T3, which adjust gene expression by attaching to TH receptors (21). RA does not participate in thyroid organogenesis; nevertheless, it plays a key role in preserving an improved thyroid cell phenotype (22). In a flatfish model, it was discovered that dietary vitamin A content significantly affected thyroid follicle development, increasing the

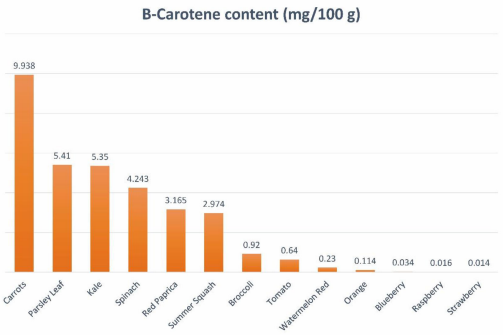


FIGURE 1
Quantitative sources of β-carotene.

number of follicles as well as elevating the colloid content of THs during the pre-metamorphosis stage and reducing THs, particularly T3, in cortical vesicles during the pro-metamorphosis stage (23). Vitamin A contributes to the synthesis of T4 as well as the establishment of an intracellular receptor for T3 (24). It has been observed that vitamin A deficiency damages the formation of thyroglobulin, which is a protein precursor of THs (25, 26). Individuals with elevated serum levels of TSH and decreased or normal free T4 have shown high levels of serum retinol and beta-carotene (27). Patients undergoing RA therapy are supposed to be at an increased risk of hypothyroidism (28). Some may suppose that, as the conversion of beta-carotene to retinol is reduced in patients with hypothyroidism, there must be a negative association between their serum concentrations in these patients. Studies have shown that as retinol synthesized from retinyl esters stacks in the serum, both beta-carotene and retinol levels rise in hypothyroidism (29). Furthermore, certain links have been reported between vitamin A and iodine metabolism. Vitamin A and iodine co-deficiency in children elevates TSH levels and the chance of goiter (30). The use of RA can also decrease the risk of hypothyroidism in patients undergoing radioactive iodine therapy for functional nodules (31, 32). All in all, RA has been linked to thyroid function at different levels of the hypothalamus–pituitary–thyroid axis. Table 1 represents an overview of the potential therapeutic benefits of vitamin A derivatives in different thyroid disorders.

5 Molecular mechanism of vitamin A metabolization and relation to thyroid hormones

As the understanding of the link between vitamin A and the thyroid grows, many investigators are exploring the molecular structures by which β-carotene and RA affect thyroid performance. RA exerts its effects through nuclear receptors belonging to the steroid family, known as RA receptors (RAR). These receptors, together with the retinoid X receptor (RXR), form heterodimers to bind DNA on RA response element sequences situated in the promoter area to promote or suppress gene expression (20). These interactions may trigger biological pathways involving different tissues and organs (39). The endocrine system has an inseparable relationship with the retinoid system as the RXR provides heterodimers for certain hormone nuclear receptors, including thyroid receptors (20).

A crosstalk between THs and RA signaling has been observed in previous studies. In a preclinical study of mouse models, it was observed that iodothyronine deiodinase type 2 (DIO2) and iodothyronine deiodinase type 3 (DIO3) switching is reduced in fetuses with neural tube defects born from mothers with hyperthyroidism. It should be noted that the enzyme DIO3 degrades T3 to diiodo-l-thyronine and converts T4 to reverse T3, while DIO2 converts T4 to T3. Researchers explained the reduction in DIO3 expression by elevated levels of inhibitory histone in the DIO3 promoter area, indicating that overactive RA signaling can ectopically de-repress TH signaling (40). Fernández et al. evaluated the influence of vitamin A on flatfish development in terms of TH signaling. Gene expression analysis revealed that the mRNA levels of retinoid receptors, retinol-binding protein, and TH receptors including TRαa, TRαb, and TRβ along with TSHβ were upregulated in larvae fed with rotifers with 50 times above normal vitamin A content compared with the control group (23). Froöhlich et al. found that retinol promoted the binding of TSH to thyroid cells but did not influence the expression of the sodium–iodide symporter mRNA and protein (41). Another group of researchers investigated the effects of RA on thyroid function in rats treated with all different doses of RA. The thyroid iodine content diminished in rats treated with all-trans-retinoic-acid (ATRA) for 14 days and rose in those treated with 13-cis-RA. In the ATRA group, sodium–iodide symporter function and thioperoxide activity remained unmodified; however, dual oxidase activity remarkably decreased (42).

RA also regulates the effects of THs on target tissues. It has been reported that ATRA or RAR enhances the uptake of THs through transcriptional upregulation of monocarboxylate transporter in the extraembryonic endoderm development period (43). Furthermore, RAR and thyroid receptors share some cofactors such as cytoplasmic adaptor for RAR and thyroid receptor (CART1). It has been suggested that the C-terminal CoRNR box of CART1 is in charge of the interaction with the nuclear receptor co-repressor 1 binding region of RAR and thyroid receptor (44).

In conclusion, an increasing number of evidence demonstrates the regulation of thyroid hormones by vitamin A and its derivatives from the gene expression level to their effect on target tissues.

TABLE 1 Overview of the potential therapeutic benefits of vitamin A derivatives in different thyroid disorders.

Disorder	Therapeutic effects	Ref
Hypothyroidism	Administration of vitamin A increases free T4 concentrations in patients with hypothyroidism.	(33)
Subclinical hypothyroidism	Retinyl palmitate supplementation is beneficial for reducing the risk of subclinical hypothyroidism in women of reproductive age.	(34)
Hyperthyroidism	High doses of vitamin A are effective in decreasing symptoms and metabolic rates in patients suffering from hyperthyroidism.	(35)
Goiter	Vitamin A supplementation in combination with iodized salt reduces the thyroid volume and serum TSH levels significantly.	(36)
Thyroid eye disease	β–Carotene reduces the H_2O_2–induced proliferation in eye involvement secondary to thyroid diseases.	(37)
Thyroid cancer	ATRA inhibits the malignant properties of thyroid cancer cells and stimulates apoptosis.	(38)

T4, thyroxine; TSH, thyroid-stimulating hormone; ATRA, all-trans retinoic acid.

6 Beta-carotene level in hypothyroidism: past history and molecular mechanism

Hypothyroidism is a prevalent disorder that is more common in women, the elderly, and certain ethnic groups. Clinical hypothyroidism is characterized by high levels of TSH and low FT4 levels. In contrast, subclinical hypothyroidism is characterized by normal FT4 levels and high serum TSH levels. For instance, the hypothalamic, pituitary, and thyroid axis must be unimpaired. There must be no associated disease, and this pattern must be persistent for a minimum of 4 weeks. The subjective nature of the symptoms of hypothyroidism varies with the level of biochemical thyroid hormones. Common symptoms include weariness, cold intolerance, dry skin, constipation, voice changes, and aches and pains in the muscles. The sensitivity and specificity of these symptoms and the scoring system to identify hypothyroidism are inadequate (45). Moreover, patients with overt hypothyroidism express more and more significant symptoms. Hypothyroidism is mainly diagnosed with biochemical changes rather than clinical symptoms (46). Indeed, in adults, the upper limit of the TSH baseline range typically rises with age. Additionally, patients have their TSH reference range, which effectively spans just 25% of the general reference range (47). Currently, the monotherapy of L-thyroxine is the first-line treatment of hypothyroidism to normalize the T3 and T4 levels; also, it is approved that patients with higher TSH levels (≥10 mIU/L) should receive medication. The initial dose of L-thyroxine is 50 mg daily; later, in non-pregnant individuals, experts suggest that the dose of L-thyroxine be adjusted by maintaining the TSH level within the standard reference range (48). Furthermore, recent clinical trials suggest combination therapy of L-thyroxine and LT3, which had better results in preventing disease progression (49, 50).

Several factors cause hypothyroidism. The most prevalent cause of hypothyroidism is iodine deficiency, one of the crucial elements of thyroid hormones (51). Recently, it was believed that the high profile of β-carotene is also related to hypothyroidism. There is evidence that thyroid hormones are associated with retinol and β-carotene metabolism (52). In a recent trial of 101 patients, Kiuchi et al. reported that high levels of β-carotene were associated with a lower free T4 and a high concentration of TSH in plasma; the prevalence of high serum β-carotene was 8% in patients with primary hypothyroidism and 6.5% in patients with subclinical hypothyroidism (27). Also, in another study, Goswami et al., in agreement with a previous study, reported that in women with hypothyroidism, higher levels of β-carotene and retinol were observed; however, the mentioned study did not include men (53). As a result, it is believed that the high β-carotene level is correlated with a lower level of T4; however, in terms of retinol, there are controversial reports. Aktuna et al. reported that in hypothyroidism, β-carotene levels were substantially greater than in hyperthyroidism. In addition, serum retinol concentrations did not differ between hypothyroidism, euthyroidism, and hyperthyroidism, nor was a substantial rise found in hypothyroidism (52). T3 increases CMO1 (BCMO1) mRNA expression and enzyme performance in Caco-2 BBe cells in the human intestine, resulting in high serum β-carotene profile in patients with hypothyroidism. Furthermore, the conversion of β-carotene to retinol is inhibited in patients with hypothyroidism, and a negative relationship persists between β-carotene and retinol (54). In adults, RAR, RXR heterodimer, intestine-specific homeodomain transcription factor, and intestinal scavenger class B type 1 regulate β-carotene intake and intestinal BCMO1 activity. With sufficient vitamin A, intestinal transcription factor (ISX) inhibits both BCMO1 activity and intestine β-carotene intake *via* SRB1. ISX regulation, which suppresses BCMO1 and SRB1, is decreased in vitamin A deficiency (VAD) due to decreased retinoic acid profile, resulting in normal regulation of BCMO1 and SRB1. One possible explanation for the relationship between β-carotene and THs is that β-carotene is absorbed by cells in the intestine, which may lead to lowering the levels of THs in individuals with β-carotenemia, and this is supported by current evidence. In conclusion, in order to understand the exact relationship of retinol and β-carotene in thyroid disorders, further *in vivo* and *in vitro* studies are required (55). Figure 2 shows the proposed molecular mechanism of action.

7 Beta-carotene level in hyperthyroidism and its possible mechanism of action

The clinical condition known as hyperthyroidism is carried on by the blood rising level of thyroid hormones. In the United States, the overall prevalence of hyperthyroidism is 1.2% compared with 0.5% in overt hyperthyroidism and 0.7% in subclinical hyperthyroidism (56). The most prevalent cause of hyperthyroidism is Graves' disease. Graves' disease is an autoimmune condition in which antibodies activate TSH receptors and increase TH secretion (51). Hyperthyroidism can cause a variety of symptoms such as heat intolerance, sweating, trembling, widened eyes, drooping eyelids, weight loss, muscle weakness, and psychiatric conditions (57). Several laboratory tests are used in the diagnosis of hyperthyroidism. Low TSH and elevated levels of free T4 are usually characterized as laboratory findings of hyperthyroidism. Also, we have to mention that free T3 tests are not reliable (58). Furthermore, the radioactive iodine uptake test quantifies the amount of iodine that is absorbed by the thyroid gland. An elevated level of iodine uptake is commonly observed in individuals with Graves' disease, as opposed to those with a toxic multinodular goiter or an adenoma (59). The treatment options for hyperthyroidism range from conservative therapy to total thyroidectomy. Despite the originating cause of hyperthyroidism, beta-blockers suppress the adrenergic symptoms. Theoretically, propranolol also inhibits 5-monodeiodinase, preventing the peripheral conversion of T4 to T3. The management pathway for hyperthyroidism produced by an excess of thyroid hormones is determined by the patient's age, symptoms, comorbidities, and individual preferences (60). As previously mentioned, the potential link between hyperthyroidism and elevated β-carotene levels remains unclear. Additional preclinical and clinical studies are

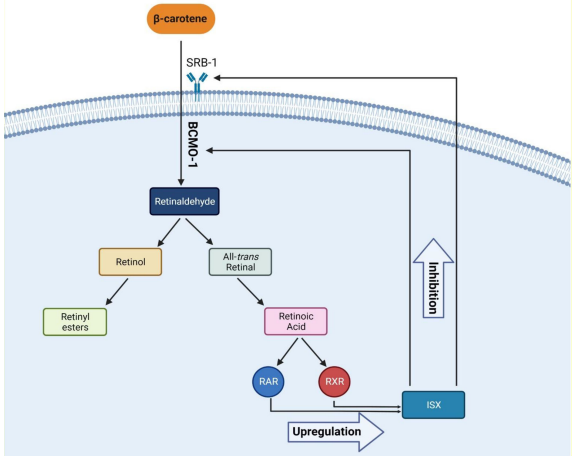


FIGURE 2
The proposed molecular mechanism of β-carotene metabolism and its effects. Abbreviations: RAR, retinoic acid receptor; RXR, retinoid X receptor; ISX, intestinal transcription Factor; SRB-1, scavenger receptor class B type 1; BCMO-1, beta-carotene oxygenase 1.

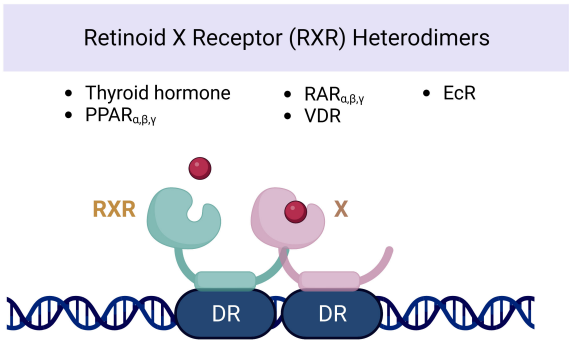


FIGURE 3
Nuclear receptor of retinoid X heterodimers and its association with thyroid hormonal secretion.

needed to fully understand the link between hyperthyroidism and β-carotene. Although numerous biochemical factors accompany hyperthyroidism, an increase in β-carotene or a decrease in vitamin A levels is not among them. Further investigation is required to elucidate the exact correlation between elevated thyroid hormone levels and the presence of β-carotene and retinol.

8 Retinoid and the inhibition of TSH secretion

Several observational studies have reported a correlation between VAD and thyroid dysfunction or enlargement of the thyroid gland (goiter) (61, 62). Figure 3 represents the schematic nuclear receptor of retinoid X heterodimers and its association with thyroid hormonal secretion. Studies in children with severe goiter have shown lower serum retinol and retinol-binding protein levels compared with children with mild or no goiter (63). Various impacts on the pituitary–thyroid axis have been observed in relation to VAD. The level of vitamin A plays a role in regulating both thyroid metabolism (64) and peripheral thyroid hormone metabolism (65–67). An *in vitro* preclinical study on rats demonstrated that vitamin A influences the secretion of thyrotropin or TSH by the pituitary gland. Moreover, VAD leads to enlargement of the thyroid gland (68) and reduced thyroid iodine uptake (69). VAD might also affect thyroid metabolism *via* a central mechanism. In the central axis, the thyroid hormone receptor is found in two isoforms: α and β (70, 71). The effect of RA on the expression of the pituitary TSHβ gene was investigated through its interaction with the RXR receptor. It was found that RA inhibits gene expression by binding to half-sites located on the promoter DNA. However, no significant effect was observed on the hypothalamic TRH level. Such findings suggest a potential mechanism for regulating thyroid hormone synthesis and secretion (72). Additionally, the authors discovered that retinoids increased thyroid hormone metabolic clearance *via* mechanisms mediated by deiodinase and non-deiodinase enzymes in some organs such as the liver and pituitary gland. The administration of VAD in rats increased the levels of plasma T3, T4, and free T4 index, as well as pituitary TSHβ mRNA. Despite the presence of high serum TT4, the expression of elevated TSH mRNA indicates that the impact of VAD has caused a general insensitivity in the pituitary thyrotrope's response to thyroid hormone. This suggests that further investigation into the mechanisms behind this effect may be warranted. In patients with cutaneous T-cell lymphoma receiving bexarotene therapy (synthetic retinoid), blood TSH levels and free T4 have been found to decline (70). In one report, 19 of 27 patients with bexarotene therapy experienced symptoms of hypothyroidism (fatigue, constipation). High TSH suppression was observed in patients receiving high doses of bexarotene (>300 mg/m^2 daily) (71). In a double-blind, randomized study of six healthy subjects treated with a single dose of bexarotene (400 mg/m^2) or placebo, plasma TSH levels decreased as early as 12 h after therapy and reached a nadir at 24 h. Free T4 and free T3 levels were also significantly lower than placebo over 48 h (73).

9 Retinoid influences the glycoprotein α-subunit and TRH gene expressions

Pituitary glycoprotein hormones are formed by two different subunits, the alpha (α) and beta (β) subunits. Among FSH, LH, and TSH hormones, the alpha subunit is common, but the beta subunit is unique for each of the three hormones (74). Retinoids are a group of vitamin A derivatives that are part of the vision system (11-cis-retinal), and they regulate the genes involved in cell death: how they differentiate and proliferate. Vitamin A is transformed by enzymes to all-trans-retinoic acid; after that, it is converted to isotretinoin and 9-cis-retinoic acid in the microsomes of the liver (70). Two groups of nuclear receptors mediate the activity of all-trans- and 9-cis-retinoic acid: one is called the RXRs and the other one is RARs (75, 76). Both 9-cis- and all-trans-retinoic acids can activate RARs, but on the other hand, 9-cis-retinoic acid, unsaturated fatty acids,

and many natural or synthetic ligands named rexinoids can activate RXRs (77). LGD1069 called bexarotene and LG100268 are two of the highly RXR-selective synthetic rexinoids (78). Janssen et al. in their preclinical study among murine TαT1 thyrotrope cells found that a retinoid X receptor antagonist named LG101208 makes a 71%–81% increase in the levels of TSHβ mRNA *in vitro* at 24 and 48 h and a 47% to 53% increase in the levels of D2 and glycoprotein α-subunit mRNA, and the RXR agonist LG100268 decreases the levels of the glycoprotein α-subunit, TSHβ, and D2 mRNA (79).

Hypothalamus TRH controls TH production. The TRH gene promoter site is made up of three isolated sites that altogether suppress the promoter. Two of the three sites only bind to TH receptor monomers, while the last one can bind to monomers or homodimers of the TH receptor and even to heterodimers of TR/RXR (80). The *in vivo* usage of LG100268 among mice in a preclinical study decreased serum TSH and T4 levels but had no effects on hypothalamic TRH mRNA levels (70). Accordingly, after RXR antagonist usage, similar hypothalamic preproTRH mRNA levels were observed in murines compared with the controls, despite raised T4 levels (79). Altogether, bexarotene is demonstrated to cause central hypothyroidism by affecting the TSHβ gene regulatory regions and concurrent influence on the α-subunit and TRH synthesis (81). Sharma et al. in an experiment in TaT1 cells and after 48 h of treatment with LG100268 (RXR selective) and TTNPB (RAR selective) found that treatment with LG100268 significantly reduced both TSH subunits and D2 mRNA levels, whereas TTNPB showed no influence (70). In summary, retinoid agonists decrease glycoprotein α-subunit and TRH gene expressions, and retinoid antagonists increase them.

10 Retinoid in thyroid eye disease

Thyroid eye disease (TED) is an autoimmune infiltrative disease known as the most common cause of orbitopathy or dysthyroid ophthalmopathy worldwide (82, 83), found in up to 50% of patients with Graves' disease (GD) (84). Graves' orbitopathy (GO) pathogenesis is uncertain and the underlying cause remains undetected (85). The clinical features of GO consist of proptosis, eyelid edema, diplopia, corneal abrasion, and optic neuropathy (86, 87). GO standard treatment is based on corticosteroids and radiotherapy (RT) to reduce the activity and duration of the disease. Oxidative stress plays a significant role in the worsening of TED leading to the investigation of antioxidants such as selenium, which have the potential to limit the progression of TED (88). Increased oxidative stress is a defining feature of hyperthyroidism (89). It appears that increases in many types of oxidative stress are involved in GO progression (89, 90). Antioxidants have been studied to find out how they counteract the effects of oxidative stress in TED (88, 90–93). This imbalance of antioxidants in TED patients is due to the growth of orbital fibroblasts, synthesis of autoantibodies, degradation of preadipocytes to adipocytes, and secretion of endogenous cytokines (TNFα, IL1-β, and IFNγ), which consequently leads to fibro-adipose tissue development and inflammation of the extraocular muscle. A few studies have tried to find out the role of antioxidants in autoimmune hyperthyroidism and thyroiditis in TED treatment, with conflicting results regarding the effects of different types of antioxidants. Rotondo Dottore et al. reported that retinol, beta-carotene, and vitamin E significantly reduced H_2O_2-induced secretion of glutathione disulfide and IL-1 in GD, but not in control fibroblasts. Beta-carotene increased orbital fibroblast growth in GD and retinol decreased IFN-γ in GD and controlled fibroblasts (94). In a systematic review about the effect of antioxidants in TED, results showed that β-carotene, retinol, N-acetyl-l-cysteine (NAC), vitamin C, melatonin, resveratrol, vitamin E, and quercetin may have some efficacy in the management of TED. Although various antioxidants have shown potential for managing TED, there is insufficient evidence to support the implementation of any specific antioxidant or combination thereof in routine clinical practice. It appears that more clinical studies are needed to confirm the effects of beta-carotene on GD (95).

11 Beta-carotene thyroid cancer association and its possible mechanism of action

RAs modulate cell division and development of various cell types by binding to a RAR and also RXR (96). Each receptor has three subtypes, namely, α, β, γ, and different analogs of RA have been utilized for the treatment and chemoprophylaxis of neoplasms such as acute promyelocytic leukemia (97) and skin carcinoma (98, 99). Recently, a reduction of RAR-β expression has been discovered in several malignancies, such as non-small cell lung carcinoma, squamous cell carcinoma of the head and neck, and breast and cervical cancers. Some studies show that downregulation of RAR-β and RXR-β correlates with end-stage lung cancer (100, 101). Rochaix et al. (102) reported that reduced RAR-β protein expression was found in all cases of papillary thyroid carcinoma (PTC) and in 50% of cases of follicular carcinoma. Carotenoid concentration is an indicator of PTCs, and underexpression of RA receptors also appears to be related to PTC deterioration and stage, likely providing an additional diagnostic indicator of PTC. Differentiated thyroid carcinomas (DTCs) have a mild proliferation that can be completely treated with the combination therapy of surgery and radioiodine therapy. The efficacy of therapeutic methods is reduced in cases of dedifferentiated thyroid carcinomas. *In vitro* studies have demonstrated the potential of RA therapy to restore thyroid cancer cells to their original specialized state, as evidenced by the increased transcription of thyroid-specific proteins (103–106) and elevated cellular radioiodine uptake (107). RA also plays a role in opposite growth pathways, such as limiting cell division and stimulating cell death. Simon et al. (108) reported an investigation of the efficiency of RA treatment for end-stage thyroid carcinomas (13-cis-retinoic acid: 1.5 mg/kg/day for 5 weeks). A positive effect was observed in 20% of the patients, in whom tumor regression or stabilization correlated with decreased Tg plasma level or increased iodine uptake.

Malignancy size showed no alteration or regression in 56% of the patients.

12 The relation between vitamin A and thyroid function in pregnant and obese individuals

Thyroid physiologic changes during pregnancy include a moderate increase in gland size and vascularization. Thyroid stimulation occurs since the first trimester, caused by beta-human chorionic gonadotropin (β-HCG), due to structural similarity with TSH (109). β-HCG has a thyrotropic activity that causes a decrease in serum TSH in the first trimester (110). There is estrogen stimulation and the level of circulating thyroid-binding globulin (TBG) in the blood increases (111). In early pregnancy, total thyroxine (TT4) and triiodothyronine (TT3) are increased and peak in the second trimester (112). Generally, pregnant women have lower free-hormone concentrations compared with non-pregnant women (113, 114). During pregnancy, thyroglobulin often rises, indicating increased thyroid activity (115). The fetal THs are synthesized after 12 weeks of gestation; before this time, maternal THs are responsible for the physiological development of the fetal brain (116, 117). Vitamin A is essential for pregnant women to maintain night vision and for fetal ocular health, fetal skeleton development, and immune system maintenance (118–121). The most important adverse effects caused by additional vitamin A intake, especially in the early stages of pregnancy, are spontaneous abortion and congenital anomalies of the central nervous and cardiovascular systems (122, 123). Teratogenicity risk is increased when daily usage of vitamin A exceeds 10,000 IU. Malformations (e.g., urinary tract malformations) are reported in children whom their mothers use high doses of vitamin A (>25,000 IU/day) (124, 125).

The prevalence of obesity is increasing worldwide with lifestyle and environmental alterations (126). Disorders of glucose and lipid and vitamin deficiency are common in obese individuals (127). Obese individuals are vitamin-deficient, particularly the fat-soluble vitamins like vitamin A (VA) (127). VA is significantly lower in individuals with metabolic syndrome (128, 129). Obesity and TH dysfunction are significantly linked (130, 131). Iodine is required for the metabolism of THs as well as micronutrients such as VA (25). Thyroid metabolism, TH synthesis, TH peripheral function, and TSH secretion are all regulated by VA (68, 132). The synthesis of thyroglobulin, T4 and T3 formation, and thyroid iodine uptake are affected by VAD (68, 132), while VA supplementation reduces TSH, mean thyroglobulin, and thyroid size (133). Bingwei Ma et al. in a study found that subclinically hypothyroid (SH) obese had lower VA levels than euthyroid obese and also VA-deficient obese had lower FT4 and higher TSH than normal obese (134). Farhangi et al. in an interventional study found that VA supplementation in both obese and non-obese women significantly decreases TSH and increases serum T3 (34). This might be due to decreased responsiveness of tissue to THs or increased hepatic conversion of T4 to T3 by VA supplementation as reported by Morley et al. (135). In summary, obesity is associated with lower vitamin A and elevated TSH levels, and vitamin A supplementation might reduce the risk of hypothyroidism.

13 β-carotene conversion products and their effects on thyroid hormone level

All the fruits that are colored and leafy vegetables that are green have pigments called carotenoids (136). Carotenoids are various, and among them, β-carotene has provitamin A activity and is assumed to be of great nutritional value. Up to 80% of our daily vitamin A originates from provitamin A carotenoids which can be found in fruits and vegetables (137, 138). Animals cannot synthesize vitamin A. It is supplied as animal food resources or as provitamin A carotenoids (139). Vitamin A, which is called retinol, and RA as its active metabolite play key roles in the growth and differentiation of cells and vertebral development (139). Vitamin A deficiency may lead to xerophthalmia, immune system impairment, blindness, and a higher risk of death (137). β-Carotene supplementation can fight against vitamin A deficiency complications such as reducing night blindness incidence (140–143). Absorbed β-carotene is metabolized throughout centric and eccentric pathways. The centric pathway is carried out by β-carotene 15,15'-monooxygenase enzyme (BCO1) which produces retinal, and in the eccentric pathway, apo-10'-carotenal is produced by β-carotene 9',10'-dioxygenase enzyme (BCO2). Retinal dehydrogenase enzymes metabolize retinal to retinol, and after esterification to retinyl esters, it is packed with non-converted β-carotene into chylomicrons (144, 145). Toxicity is not observed in individuals consuming large amounts of β-carotene because of the reduction in vitamin A production efficiency at increasing doses (137, 146, 147). Vitamin A alterations on thyroid function include alteration in the TH-binding capacity of the serum proteins, decrease in total T4 and T3 levels, increase in thyroid radioactive iodine uptake, and alteration in T4 to T3 conversion (135). McCarrison in a preclinical study found that a deficiency of vitamin A leads to rat thyroid hypertrophy (148). Drill found that vitamin A deficiency leads to hypertrophy of the thyroid gland and excessive vitamin A decreases the amount of thyroid colloid (149). Morley et al. found an integral thyroxine and vitamin A relationship. An increase in vitamin A leads to TH reduction, and vitamin A deficiency leads to an increase in THs (150). Di Bella et al., in a preclinical study among rats, demonstrated that vitamin A affects the thyroid gland by producing a dwindled gland, higher radioiodine uptake, and enhanced hepatic conversion of T4 to T3. Thyroid gland overactivity may be explained by vitamin A-based stimulation of cathepsin activity which controls proteolysis (151). In summary, β-carotene in the human body converts to vitamin A which if decreased leads to thyroid hypertrophy and TH increase. Higher vitamin A intake may increase radioiodine uptake and raise the hepatic conversion of T4 to T3. In addition, the β-carotene-metabolizing pathways are shown in Figure 4.

14 The effect of hypo/hyperthyroidism and their association with antioxidant status

Oxygen-free radicals have an effect on the mechanism of tissue damage of multiple pathologic pathways (152), including hypothyroidism and hyperthyroidism (153–155). The accessible data related to oxidant stress and antioxidant role in hypothyroidism are rare and inconsistent. Hypothyroidism is a decrease in free radical production as a result of the metabolic suppression brought about by the reduction in serum thyroid level (154, 156). However, other lines of evidence reported that hypothyroidism increased oxidative stress (157), and hyperthyroidism is associated with a metabolic increase and, therefore, an increased amount of oxidative stress and peroxides and even an increase (158–160) or a decrease (161) in antioxidant enzymes. Studies showed that hyperthyroidism treatment reduced oxidative stress (162). A study examined the effects of hyperthyroidism on the antioxidant defense system, specifically serum levels of thiobarbituric acid-reactive substance (TBARS), vitamin E, and coenzyme Q10. The results show that hyperthyroidism leads to increased TBARS and reduced antioxidants. However, these values were normalized in euthyroid patients. In addition, studies take into account the possible negative effects of hyperthyroidism on important antioxidant enzymes such as Mn or Cu, Zn, superoxide dismutase, catalase, and glutathione peroxidase (163). In the systemic investigation, hyperthyroidism had a role in the decline of serum alpha-tocopherol (162, 164) and CoQ10 in humans (162, 165). Baskow et al. found in hypothyroid patients (166) a high malondialdehyde (MDA) level, an oxidative stress agent that is made by lipid peroxidation, as well as NO, and a low activity of paraoxonase-1 (PON-1), a liver enzyme made with an antioxidant agent. On the other hand, levothyroxine therapy reduced MDA levels and increased PON-1 activity, although several similar studies had different conclusions (166). They claimed that in hypothyroidism the prooxidant environment could affect the development of atherosclerosis. MDA rising levels also represent mild thyroid failure (167). Increased OS has a role in the reduction of antioxidants and also leads to hyperlipidemia. Furthermore, a considerable association exists between MDA and LDL-cholesterol, serum cholesterol, and triglyceride levels. The antioxidant level was not significantly different in clinical hypothyroidism, mild thyroid failure, and controls. OS can also damage the thyroid itself, which appears in iodine-excess conditions. This matter was investigated both *in vitro* and in animals (168, 169). Iodide induced action on hydrogen peroxide production in the thyroid and damaged thyrocytes at high accumulation (170). Subudhi and colleagues discovered that when hypothyroid rats were given vitamin E and curcumin supplements, the mitochondrial oxidative stress levels of their livers were improved and hyperlipidemia was reduced (171). Table 2 is a summary of reports on β-carotene and vitamin A levels in association with thyroid hormones.

15 Recent clinical studies

Due to the importance of the thyroid gland in maintaining normal metabolism in the human body, many researchers have tried to evaluate the crosstalk between vitamin A derivatives and THs in human subjects and compare the results of animal studies at a clinical level. In developing countries, because of the prevalence of iodine and vitamin A co-deficiency, the risk of thyroid disorders, particularly in children, has been of interest. Zimmermann et al., in a study on 404 children from regions with mild to moderate iodine and vitamin A deficiency, showed that vitamin A supplementation caused a significant decrease in mean thyroglobulin, median TSH, and thyroid volume. They proposed that in the presence of vitamin A, the thyroid gland is less triggered by excess TSH and the chance of goiter is reduced (133). In a similar study of 138 pediatric patients

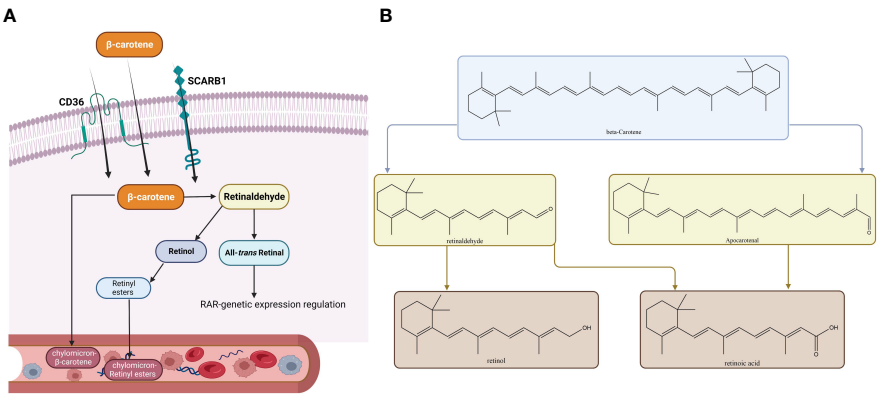


FIGURE 4
Metabolic pathways of β-carotene. The membrane proteins SCARB1 and CD36 can mediate absorption in the intestinal epithelium, or absorption can occur passively by diffusion. When inside the cytoplasm of an enterocyte, there are two ways a substance can be metabolized. **(A)** The metabolic route most frequently taken, which culminates in the release of retinyl esters (RE) or β-carotene into the bloodstream in tandem with chylomicrons. **(B)** Both the conventional metabolic route and the alternate cleavage-generating apo-carotenal molecule are depicted. Similar metabolic by-products are produced by both substances (all-trans-RA, ATRA). Abbreviations: RAL, retinal; ROH, retinol; B1ChM-β, carotene–chylomicron–β-carotene; CD36, cluster of differentiation 36; SCARB1, scavenger receptor class; ChM-RE, chylomicron-RE.

TABLE 2 Reports of β-carotene and vitamin A levels in association with thyroid hormones.

Author	Year	Status	Outcome	Ref
Sachiko Kiuchi et al.	2018	β-Carotene and hypothyroidism	The prevalence of hyper β-carotenemia in patients with primary hypothyroidism is 8%, and in patients with subclinical hypothyroidism, the prevalence is 6.5%.	(27)
Goswami UC et al.	1999	β-Carotene, hypothyroidism, and hyperthyroidism	β-Carotene and retinol levels were elevated in hypothyroidism but were decreased in hyperthyroidism.	(53)
Steven I. Sherman et al.	1999	Retinoid and thyroid hormones in a patient with cutaneous T-cell lymphoma	Retinoid treatment of the patient with cutaneous T-cell lymphoma decreases the level of thyroid hormones.	(71)
A. R. Angioni et al.	2005	Retinoid and TSH	Chronic retinoid intake reduces the level of TSH in serum.	(172)
Wendy M. Golden et al.	2007	Retinoid and TSH	Retinoid administration can reduce TSH concertation in healthy adults.	(73)
Mervat M. El-Eshmawy et al.	2016	Vitamin A and thyroid hormones in patients with hepatitis C virus	Vitamin A deficiency is associated with hyperthyroidism condition.	(173)
Mahdieh Abbasalizad Farhangi et al.	2012	Vitamin A and TSH in premenopausal women	Vitamin A treatment may decrease the likelihood of subclinical hypothyroidism in premenopausal women since serum TSH levels were considerably reduced in vitamin A-treated participants.	(34)
G. Ceresini et al.	2002	Vitamin A and TSH	In healthy individuals, treatment of vitamin A appears to have little effect on TSH production; the findings also imply that the interaction between T3 and vitamin A in the regulation of TSH secretion is unlikely.	(174)
RK Miller et al.	1998	Safe dosage of vitamin A and β-carotene in pregnant women	It is believed that a daily dose of 10,000 IU/day is safe in pregnant women, and intake of β-carotene is not teratogenic at any dose.	(122)

with goiter, vitamin A supplementation in combination with iodized salt reduced thyroid volume and serum TSH levels more significantly than iodized salt alone (36). Similar studies have also been conducted on adult populations. The studies mainly focused on assessing the impact of vitamin A supplementation on the thyroid function of patients with thyroid disorders. However, some researchers evaluated the baseline status of vitamin A in patients with such diseases. For instance, Ibrahim et al. studied the nutritional condition of female patients with thyroid disorders. They found that in women with hyperthyroidism, the average vitamin A intake was considerably higher than in those with hypothyroidism (175). Farhangi et al. in a randomized controlled trial assessed the effects of vitamin A supplementation on thyroid status in obese women. The obese women received placebo or vitamin and non-obese subjects received vitamin A. They found that vitamin A supplementation led to a noticeable decrease in TSH levels in both obese and non-obese subjects. Furthermore, serum T3 levels rose in both treated groups, while serum T4 levels dropped in the three groups after the trial. Their findings confirmed a marked decrease in serum retinol-binding protein in the obese group treated with vitamin A, but no meaningful change was detected in serum transthyretin (34). Haugen reported that a high dose of vitamin A was effective in reducing symptoms and metabolic rate in individuals suffering from hyperthyroidism (35). On the other hand, recent studies have shown the benefits of vitamin A supplementation in individuals with hypothyroidism. As an illustration, Rabbani et al. designed a randomized controlled trial where 86 hypothyroid patients either were treated with supplementation including zinc gluconate, magnesium oxide, and vitamin A or received placebo. They demonstrated that in the intervention group, serum free T4 concentrations elevated considerably, while after the intervention, the differences in serum total T4, TSH, and free T3 were not significant (33). As might be noticed, the results of previous studies in terms of the effects of vitamin A on THs have been controversial. These discrepancies might be justified by the presence of complex feedback between the hypothalamus–pituitary–thyroid axis (176). In T4, as most of this hormone is bound to transporter proteins, the alterations in its serum levels may be masked. Regarding TSH, T3 is the main regulator of TSH release from the pituitary gland negatively influencing TSH synthesis. Thus, a lack of alteration in serum FT3 can lead to no change in TSH levels (33). Overall, despite differences between the findings of previous studies on this topic, there is a strong body of evidence on the potential of vitamin A supplementation in the treatment of thyroid disorders in human subjects.

16 Discussion and future perspectives

TH receptors are nuclear receptors that require heterodimerization with RXR. In fact, RXR regulates the function of thyroid receptors with the outcome being determined by the ligands of the two receptors (177). Various studies have confirmed the influential role of vitamin A on THs from gene expression to impact on the target cells; a reciprocal effect has also been discovered suggesting the effects of THs on vitamin A metabolism. Hence, vitamin A and thyroid functions are inseparably linked; however, some underlying mechanisms are still unclear (19, 178, 179). In the case of these mutual

interactions, a major factor that should be noticed is the accessibility of vitamin A, which is dependent on the equilibrium between the enzymes which are responsible for the production or inactivation of the RA RAR or could be limiting in rigorous nutritional deficiencies (180). This matter becomes more complex knowing the severe impact of vitamin A and iodine co-deficiency on thyroid function in individuals from regions with nutritional crises. Indeed, providing sufficient intake of these micronutrients is a major health issue that needs to be tackled in developing countries (22). Even with providing adequate nutritional intake, there are still complexities in the case of RA–thyroid interactions. RA synthesis and deactivation are regulated *via* complex pathways. Knowledge of the mechanisms can facilitate understanding of the links between RA and endocrine hormones including THs (181, 182). At the clinical levels, the results of studies assessing the impact of vitamin A supplementation on human subjects with thyroid disorders have been controversial yet mostly promising. Recently, the use of vitamin A derivatives as a potential therapeutic option in thyroid cancer has gained the interest of researchers like never before (38, 183, 184). At this moment, we might have effective techniques to tackle some problems in this area. With recent improvements in genome engineering, we will be able to assess the effects of small modifications to RARs/RXRs encoding genes on thyroid hormonal pathways. On larger scales, these types of studies might help discover new pathways in the regulation of the hypothalamus–pituitary axis affecting other endocrine organs. With advances in molecular pharmaceutics and nanoformulation, synergistic drug combinations and polymer micelles might be offered to improve the efficacy of vitamin A derivatives and reduce their adverse effects. Novel machine learning techniques can help analyze large data sets produced by genomic studies and facilitate the understanding of the dynamic associations between RA-related genes and subsequent hormonal processes (185). The proposed mechanism of the role of beta-carotene in affecting thyroid hormones is shown in Figure 5.

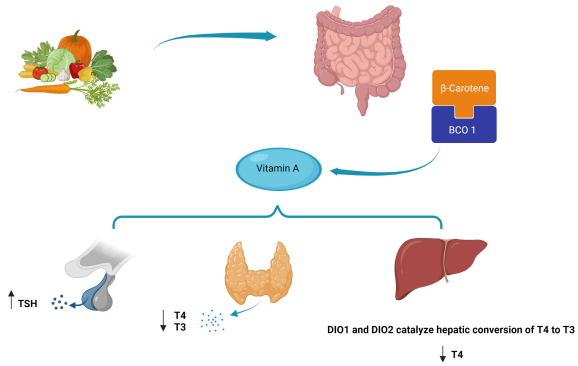


FIGURE 5
Proposed mechanism of beta-carotene's role in affecting thyroid hormones.

17 Conclusion

In recent years, significant progress has been made in advancing our comprehension of retinoic acid and thyroid interactions at both the molecular and clinical levels. Although the positive effects of vitamin A on patients with thyroid disorders and malignancies are widely accepted, the underlying mechanisms still require further elucidation. Additionally, the efficacy of vitamin A supplementation in thyroid disorders can be improved by conducting large-scale studies utilizing novel pharmacological techniques. These studies will allow for a more comprehensive understanding of the intricate interactions between retinoic acid and thyroid hormones, paving the way for the development of more effective therapeutic strategies. Thus, continued research in this area is crucial for improving patient outcomes and advancing our knowledge of the complex relationship between retinoic acid and thyroid function.

Author contributions

Data collection: BF, HG and NL. Figures: BF and HS. Review and editing: BF, HG, GM, HS, NL and AR. All authors contributed to the article and approved the submitted version.

Acknowledgments

We are thankful to Biorender.com for helping us draw the figures.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Rice SP, Boregowda K, Williams MT, Morris GC, Okosieme OE. A welsh-sparing dysphasia. *Lancet* (2013) 382(9904):1608. doi: 10.1016/S0140-6736(13)61837-1

2. Sheehan MT. Biochemical testing of the thyroid: TSH is the best and, oftentimes, only test needed - a review for primary care. *Clin Med Res* (2016) 14(2):83–92. doi: 10.3121/cmr.2016.1309

3. Persani L. Central hypothyroidism: pathogenic, diagnostic, and therapeutic challenges. *J Clin Endocrinol Metab* (2012) 97(9):3068–78. doi: 10.1210/jc.2012-1616

4. Sahoo DK, Roy A. Compromised rat testicular antioxidant defence system by hypothyroidism before puberty. *Int J Endocrinol* (2012) 2012:637825. doi: 10.1155/2012/637825

5. Chainy GBN, Sahoo DK. Hormones and oxidative stress: an overview. *Free Radical Res* (2020) 54(1):1–26. doi: 10.1080/10715762.2019.1702656

6. Sahoo DK, Chainy GBN. Chapter eight - hormone-linked redox status and its modulation by antioxidants. In: Litwack G, editor. *Vitamins and hormones*, vol. 121. Academic Press, Cambridge, MA (2023). p. 197–246. doi: 10.1016/bs.vh.2022.10.007

7. Dobrzyńska MM, Baumgartner A, Anderson D. Antioxidants modulate thyroid hormone-and noradrenaline-induced DNA damage in human sperm. *Mutagenesis* (2004) 19(4):325–30. doi: 10.1093/mutage/geh037

8. Bogacz-Radomska L, Harasym J. β-Carotene–properties and production methods. *Food Qual Safety* (2018) 2(2):69–74. doi: 10.1093/fqsafe/fyy004

9. Chhabria S, Mathur S, Vadakan S, Sahoo DK, Mishra P, Paital B. A review on phytochemical and pharmacological facets of tropical ethnomedicinal plants as reformed DPP-IV inhibitors to regulate incretin activity. *Front Endocrinol (Lausanne)* (2022) 13:1027237. doi: 10.3389/fendo.2022.1027237

10. Peepre K, Deshpandey U, Choudhary P. Role of antioxidants on thyroid hormones in wister rats. *Int J Sci Res* (2014) 3(1):34–8.

11. Chattopadhyay S, Sahoo DK, Subudhi U, Chainy GBN. Differential expression profiles of antioxidant enzymes and glutathione redox status in hyperthyroid rats: a temporal analysis. *Comp Biochem Physiol Part C: Toxicol Pharmacol* (2007) 146 (3):383–91. doi: 10.1016/j.cbpc.2007.04.010

12. Rostami R, Nourooz-Zadeh S, Mohammadi A, Khalkhali HR, Ferns G, Nourooz-Zadeh J. Serum selenium status and its interrelationship with serum biomarkers of thyroid function and antioxidant defense in hashimoto's thyroiditis. *Antioxidants* (2020) 9(11):1070. doi: 10.3390/antiox9111070

13. Shibata A, Sasaki R, Ito Y, Hamajima N, Suzuki S, Ohtani M, et al. Serum concentration of beta-carotene and intake frequency of green-yellow vegetables among healthy inhabitants of Japan. *Int J Cancer* (1989) 44(1):48–52. doi: 10.1002/ijc.2910440109

14. Baksi S, Pradhan A. Thyroid hormone: sex-dependent role in nervous system regulation and disease. *Biol Sex Differ* (2021) 12(1):1–13. doi: 10.1186/s13293-021-00367-2

15. Delitala AP, Scuteri A, Doria C. Thyroid hormone diseases and osteoporosis. *J Clin Med* (2020) 9(4):1034. doi: 10.3390/jcm9041034

16. Johra FT, Bepari AK, Bristy AT, Reza HM. A mechanistic review of β-carotene, lutein, and zeaxanthin in eye health and disease. *Antioxidants* (2020) 9(11):1046. doi: 10.3390/antiox9111046

17. Bohn T, Desmarchelier C, El SN, Keijer J, van Schothorst E, Rühl R, et al. β-carotene in the human body: metabolic bioactivation pathways–from digestion to tissue distribution and excretion. *Proc Nutr Society* (2019) 78(1):68–87. doi: 10.1017/S0029665118002641

18. Szabo K, Teleky BE, Ranga F, Simon E, Pop OL, Babalau-Fuss V, et al. Bioaccessibility of microencapsulated carotenoids, recovered from tomato processing industrial by-products, using *in vitro* digestion model. *Lwt* (2021) 152:112285. doi: 10.1016/j.lwt.2021.112285

19. Saleh SR, Zaki R, Hassan R, El-Kersh MA, El-Sayed MM, Abd Elmoneam AA. The impact of vitamin a supplementation on thyroid function and insulin sensitivity: implication of deiodinases and phosphoenolpyruvate carboxykinase in male wistar rats. *Eur J Nutr* (2022) 61(8):4091–105. doi: 10.1007/s00394-022-02945-5

20. Marie A, Darricau M, Touyarot K, Parr-Brownlie LC, Bosch-Bouju C. Role and mechanism of vitamin a metabolism in the pathophysiology of parkinson's disease. *J Parkinson's Dis* (2021) 11(3):949–70. doi: 10.3233/JPD-212671

21. Lamparelli EP, Ciardulli MC, Scala P, Scognamiglio M, Charlier B, Di Pietro P, et al. Lipid nano-vesicles for thyroid hormone encapsulation: a comparison between different fabrication technologies, drug loading, and an *in vitro* delivery to human tendon stem/progenitor cells in 2D and 3D culture. *Int J Pharmaceut* (2022) 624:122007. doi: 10.1016/j.ijpharm.2022.122007

22. Brossaud J, Pallet V, Corcuff J-B. Vitamin a, endocrine tissues and hormones: interplay and interactions. *Endocrine Connections* (2017) 6(7):R121–R30. doi: 10.1530/EC-17-0101

23. Fernández I, Ortiz-Delgado JB, Darias MJ, Hontoria F, Andree KB, Manchado M, et al. Vitamin a affects flatfish development in a thyroid hormone signaling and metamorphic stage dependent manner. *Front Physiol* (2017) 8:458. doi: 10.3389/fphys.2017.00458

24. Temple LM, Saigal P. Hypothyroidism. In: Rakel D, Minichiello V. (eds) *Integrative Medicine*, *5th* Edition, Elsevier (2023). p.322–333.

25. O'Kane SM, Mulhern MS, Pourshahidi LK, Strain J, Yeates AJ. Micronutrients, iodine status and concentrations of thyroid hormones: a systematic review. *Nutr Rev* (2018) 76(6):418–31. doi: 10.1093/nutrit/nuy008

26. Coscia F, Taler-Verčič A, Chang VT, Sinn L, O'Reilly FJ, Izoré T, et al. The structure of human thyroglobulin. *Nature* (2020) 578(7796):627–30. doi: 10.1038/s41586-020-1995-4

27. Kiuchi S, Ihara H, Koyasu M, Tani A, Kakinoki T, Shino Y, et al. Relation between serum levels of thyroid hormone and serum β-carotene concentrations in patients with thyroid disorders. *Int J Anal Bio-Sci* (2018) 6(1):1–9.

28. Marino A, Albanese I, Larose S, Fantus IG. Combined central hypothyroidism and adrenal insufficiency associated with retinoic acid therapy for cutaneous T-cell lymphoma. *AACE Clin Case Rep* (2022) 8(6):251–4. doi: 10.1016/j.aace.2022.08.004

29. Sahoo DK, Roy A, Bhanja S, Chainy GBN. Hypothyroidism impairs antioxidant defence system and testicular physiology during development and maturation. *Gen Comp Endocrinol* (2008) 156(1):63–70. doi: 10.1016/j.ygcen.2007.11.007

30. Zimmermann MB. Iodine and the iodine deficiency disorders. In: *Present knowledge in nutrition*. Academic Press, Cambridge, MA (2020). p. 429–41. doi: 10.1210/er.2009-0011

31. Lim JPL, Mac Kevin EB, Nellas RB. The effect of ligand affinity to the contact dynamics of the ligand binding domain of thyroid hormone receptor-retinoid X receptor. *J Mol Graphics Modelling* (2021) 104:107829. doi: 10.1016/j.jmgm.2020.107829

32. Sahoo DK, Roy A, Chainy GBN. Protective effects of vitamin e and curcumin on l-thyroxine-induced rat testicular oxidative stress. *Chemico-Biol Interactions* (2008) 176 (2):121–8. doi: 10.1016/j.cbi.2008.07.009

33. Rabbani E, Golgiri F, Janani L, Moradi N, Fallah S, Abiri B, et al. Randomized study of the effects of zinc, vitamin a, and magnesium co-supplementation on thyroid function, oxidative stress, and hs-CRP in patients with hypothyroidism. *Biol Trace Element Res* (2021) 199(11):4074–83. doi: 10.1007/s12011-020-02548-3

34. Farhangi MA, Keshavarz SA, Eshraghian M, Ostadrahimi A, Saboor-Yaraghi AA. The effect of vitamin a supplementation on thyroid function in premenopausal women. *J Am Coll Nutr* (2012) 31(4):268–74. doi: 10.1080/07315724.2012.10720431

35. Haugen BR. The effect of vitamin a, retinoids and retinoid receptors on the hypothalamic-pituitary-thyroid axis. In: Beck-Peccoz P. editor. *Syndromes of hormone resistance on the hypothalamic-Pituitary-Thyroid axis*. Endocrine Updates. Springer, Boston, MA (2004). p. 149–63. doi: 10.1007/978-1-4020-7852-1_10

36. Zimmermann MB, Wegmöller R, Zeder C, Chaouki N, Torresani T. The effects of vitamin a deficiency and vitamin a supplementation on thyroid function in goitrous children. *J Clin Endocrinol Metab* (2004) 89(11):5441–7. doi: 10.1210/jc.2004-0862

37. Rotondo Dottore G, Ionni I, Menconi F, Casini G, Sellari-Franceschini S, Nardi M, et al. Antioxidant effects of β-carotene, but not of retinol and vitamin e, in orbital fibroblasts from patients with graves' orbitopathy (GO). *J Endocrinol Invest* (2018) 41 (7):815–20. doi: 10.1007/s40618-017-0809-5

38. Mei D, Lv B, Chen B, Xiao S, Jiang J, Xie Y, et al. All-trans retinoic acid suppresses malignant characteristics of CD133-positive thyroid cancer stem cells and induces apoptosis. *PloS One* (2017) 12(8):e0182835. doi: 10.1371/journal.pone.0182835

39. Brtko J, Dvorak Z. Natural and synthetic retinoid X receptor ligands and their role in selected nuclear receptor action. *Biochimie* (2020) 179:157–68. doi: 10.1016/j.biochi.2020.09.027

40. Li H, Bai B, Zhang Q, Bao Y, Guo J, Chen S, et al. Ectopic cross-talk between thyroid and retinoic acid signaling: a possible etiology for spinal neural tube defects. *Gene* (2015) 573(2):254–60. doi: 10.1016/j.gene.2015.07.048

41. Froöhlich E, Witke A, Czarnocka B, Wahl R. Retinol has specific effects on binding of thyrotrophin to cultured porcine thyrocytes. *J Endocrinol* (2004) 183 (3):617–26. doi: 10.1677/joe.1.05693

42. Ferreira ACF, de Carvalho DP. Retinoic acid modulation of thyroid dual oxidase activity in rats and its impact on thyroid iodine organification. *J Endocrinol* (2010) 205 (3):271–7. doi: 10.1677/JOE-09-0421

43. Kogai T, Liu Y-Y, Richter LL, Mody K, Kagechika H, Brent GA. Retinoic acid induces expression of the thyroid hormone transporter, monocarboxylate transporter 8 (Mct8). *J Biol Chem* (2010) 285(35):27279–88. doi: 10.1074/jbc.M110.123158

44. Park U-H, Kim E-J, Um S-J. A novel cytoplasmic adaptor for retinoic acid receptor (RAR) and thyroid receptor functions as a derepressor of RAR in the absence of retinoic acid. *J Biol Chem* (2010) 285(44):34269–78. doi: 10.1074/jbc.M110.143008

45. Kostoglou-Athanassiou I, Ntalles K. Hypothyroidism-new aspects of an old disease. *Hippokratia* (2010) 14(2):82.

46. Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado thyroid disease prevalence study. *Arch Internal Med* (2000) 160(4):526–34. doi: 10.1001/archinte.160.4.526

47. Andersen S, Pedersen KM, Bruun NH, Laurberg P. Narrow individual variations in serum T4 and T3 in normal subjects: a clue to the understanding of subclinical thyroid disease. *J Clin Endocrinol Metab* (2002) 87(3):1068–72. doi: 10.1210/jcem.87.3.8165

48. McAninch EA, Bianco AC. The history and future of treatment of hypothyroidism. *Ann Internal Med* (2016) 164(1):50–6. doi: 10.7326/M15-1799

49. Perros P. European Thyroid association guidelines on l-T4+ l-T3 combination for hypothyroidism: a weary step in the right direction. *Eur Thyroid J* (2012) 1(2):51. doi: 10.1159/000338637

50. Wiersinga WM, Duntas L, Fadeyev V, Nygaard B, Vanderpump MP. ETA Guidelines: the use of l-T4+ l-T3 in the treatment of hypothyroidism. *Eur Thyroid J* (2012) 1(2):55–71. doi: 10.1159/000339444

51. Vanderpump MP. The epidemiology of thyroid disease. *Br Med Bull* (2011) 99 (1):39–51. doi: 10.1093/bmb/ldr030

52. Aktuna D, Buchinger W, Langsteger W, Meister E, Sternad H, Lorenz O, et al. Beta-carotene, vitamin a and carrier proteins in thyroid diseases. *Acta Med Austriaca* (1993) 20(1-2):17–20.

53. Goswami U, Choudhury S. The status of retinoids in women suffering from hyper-and hypothyroidism: interrelationship between vitamin a, beta-carotene and

thyroid hormones. *Int J Vitamin Nutr Res Internationale Z Fur Vitamin-und Ernahrungsforschung J Int Vitaminol Nutr* (1999) 69(2):132–5. doi: 10.1024/0300-9831.69.2.132

54. Yamaguchi N, Suruga K. Triiodothyronine stimulates CMO1 gene expression in human intestinal caco-2 BBe cells. *Life Sci* (2008) 82(13-14):789–96. doi: 10.1016/j.lfs.2008.01.010

55. Lietz G, Oxley A, Bosch-Saadatmandi C. Consequences of common genetic variations on beta-carotene cleavage for vitamin a supply. *Carotenoids Vitamin A Trans Med* (2013), p. 383–396. doi: 10.1201/b14569-26

56. Kravets I. Hyperthyroidism: diagnosis and treatment. *Am Family Physician* (2016) 93(5):363–70.

57. Silva JE, Bianco SD. Thyroid–adrenergic interactions: physiological and clinical implications. *Thyroid* (2008) 18(2):157–65. doi: 10.1089/thy.2007.0252

58. Association AT, Bahn RS, Burch HB, Cooper DS, Garber JR, Greenlee MC, Klein I, et al. Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American thyroid association and American association of clinical endocrinologists. *Thyroid* (2011) 21(6):593–646. doi: 10.1089/thy.2010.0417

59. Cappelli C, Pirola I, De Martino E, Agosti B, Delbarba A, Castellano M, et al. The role of imaging in graves' disease: a cost-effectiveness analysis. *Eur J Radiol* (2008) 65(1):99–103. doi: 10.1016/j.ejrad.2007.03.015

60. Abraham P, Avenell A, McGeoch SC, Clark LF, Bevan JS. Antithyroid drug regimen for treating graves' hyperthyroidism. *Cochrane Database Systematic Rev* (2010) 1:CD003420. doi: 10.1002/14651858.CD003420.pub4

61. Ingenbleek Y, Luypaert B, De Nayer PH. Nutritional status and endemic goitre. *Lancet* (1980) 315(8165):388–92. doi: 10.1016/S0140-6736(80)90943-5

62. Sahoo DK, Jena S, Chainy GBN. Chapter 2.7 - thyroid dysfunction and testicular redox status: an intriguing association. In: Henkel R, Samanta L, Agarwal A, editors. *Oxidants, antioxidants and impact of the oxidative status in Male reproduction*. Academic Press, Cambridge, MA (2019). p. 149–70. doi: 10.1016/C2016-0-03860-3

63. Wolde-Gebriel Z, West CE, Gebru H, Tadesse A, Fisseha T, Gabre P, et al. Interrelationship between vitamin a, iodine and iron status in school children in shoa region. central Ethiopia. *Br J Nutr* (1993) 70:593–607. doi: 10.1079/BJN19930151

64. Ingenbleek Y. Vitamin a-deficiency impairs the normal mannosylation, conformation and iodination of thyroglobulin: a new etiological approach to endemic goitre. In: *Nutritional adequacy, nutrient availability and needs*. Springer, Basel AG (1983). p. 264–97. doi: 10.1007/978-3-0348-6540-1_15

65. Higueret P, Garcin H. Triiodothyronine and vitamin a-deficiency in the rat. *J Physiol* (1984) 79(5):373–7.

66. Higueret P, Pailler I, Garcin H. Vitamin a deficiency and tri-iodothyronine action at the cellular level in the rat. *J Endocrinol* (1989) 121(1):75–9. doi: 10.1677/joe.0.1210075

67. Robbins J, Braveman LE. Thyroid hormone transport proteins and the physiology of hormone binding. In: Braverman LE, Utiger RD (eds) *Werner and Ingbar's the Thyroid: A Fundamental and Clinical Text*. Lippincott, Philadelphia, PA (1991). p. 111–125.

68. Oba K, Kimura S. Effects of vitamin a deficiency on thyroid function and serum thyroxine levels in the rat. *J Nutr Sci Vitaminol* (1980) 26(4):327–34. doi: 10.3177/jnsv.26.327

69. Strum JM. Alterations within the rat thyroid gland during vitamin a deficiency. *Am J Anatomy* (1979) 156(2):169–81. doi: 10.1002/aja.1001560202

70. Sharma V, Hays WR, Wood WM, Pugazhenthi U, St. Germain DL, Bianco AC, et al. Effects of rexinoids on thyrotrope function and the hypothalamic-pituitary-thyroid axis. *Endocrinology* (2006) 147(3):1438–51. doi: 10.1210/en.2005-0706

71. Sherman SI, Gopal J, Haugen BR, Chiu AC, Whaley K, Nowlakha P, et al. Central hypothyroidism associated with retinoid X receptor–selective ligands. *New Engl J Med* (1999) 340(14):1075–9. doi: 10.1056/NEJM199904083401404

72. Kelestimur F, Jonsson P, Molvalilar S, Gomez JM, Auernhammer CJ, Colak R, et al. Sheehan's syndrome: baseline characteristics and effect of 2 years of growth hormone replacement therapy in 91 patients in KIMS–pfizer international metabolic database. *Eur J Endocrinol* (2005) 152(4):581–7. doi: 10.1530/eje.1.01881

73. Golden WM, Weber KB, Hernandez TL, Sherman SI, Woodmansee WW, Haugen BR. Single-dose rexinoid rapidly and specifically suppresses serum thyrotropin in normal subjects. *J Clin Endocrinol Metab* (2007) 92(1):124–30. doi: 10.1210/jc.2006-0696

74. Lindner J, Rivier JE, Vale WW, Pavlou SN. Regulation of pituitary glycoprotein alpha-subunit secretion after administration of a luteinizing hormone-releasing hormone antagonist in normal men. *J Clin Endocrinol Metab* (1990) 70(4):1219–24. doi: 10.1210/jcem-70-4-1219

75. Chambon P. A decade of molecular biology of retinoic acid receptors. *FASEB J* (1996) 10(9):940–54. doi: 10.1096/fasebj.10.9.8801176

76. Lefebvre P, Benomar Y, Staels B. Retinoid X receptors: common heterodimerization partners with distinct functions. *Trends Endocrinol Metab* (2010) 21(11):676–83. doi: 10.1016/j.tem.2010.06.009

77. Lengqvist J, Mata De Urquiza A, Bergman AC, Willson TM, Sjövall J, Perlmann T, et al. Polyunsaturated fatty acids including docosahexaenoic and arachidonic acid bind to the retinoid X receptor alpha ligand-binding domain. *Mol Cell Proteomics* (2004) 3(7):692–703. doi: 10.1074/mcp.M400003-MCP200

78. Liu S, Ogilvie KM, Klausing K, Lawson MA, Jolley D, Li D, et al. Mechanism of selective retinoid X receptor agonist-induced hypothyroidism in the rat. *Endocrinology* (2002) 143(8):2880–5. doi: 10.1210/endo.143.8.8930

79. Janssen JS, Sharma V, Pugazhenthi U, Sladek C, Wood WM, Haugen BR. A rexinoid antagonist increases the hypothalamic-pituitary-thyroid set point in mice and thyrotrope cells. *Mol Cell Endocrinol* (2011) 339(1-2):1–6. doi: 10.1016/j.mce.2011.03.014

80. Hollenberg AN, Monden T, Flynn TR, Boers ME, Cohen O, Wondisford FE. The human thyrotropin-releasing hormone gene is regulated by thyroid hormone through two distinct classes of negative thyroid hormone response elements. *Mol Endocrinol* (1995) 9(5):540–50. doi: 10.1210/mend.9.5.7565802

81. Lalloyer F, Pedersen TA, Gross B, Lestavel S, Yous S, Vallez E, et al. Rexinoid bexarotene modulates triglyceride but not cholesterol metabolism *via* gene-specific permissivity of the RXR/LXR heterodimer in the liver. *Arterioscler Thromb Vasc Biol* (2009) 29(10):1488–95. doi: 10.1161/ATVBAHA.109.189506

82. Kashkouli MB, Pakdel F, Kiavash V, Heidari I, Heirati A, Jam S. Hyperthyroid vs hypothyroid eye disease: the same severity and activity. *Eye* (2011) 25(11):1442–6. doi: 10.1038/eye.2011.186

83. Kashkouli MB, Jam S, Sabzvari D, Ketabi N, Azarinia S, SeyedAlinaghi S, et al. Thyroid-associated ophthalmopathy in Iranian patients. *Acta Med Iranica* (2011) 49 (9):612–8.

84. Bahn RS. Graves' ophthalmopathy. *New Engl J Med* (2010) 362(8):726–38. doi: 10.1056/NEJMra0905750

85. Marcocci C, Bartalena L, Bogazzi F, Panicucci M, Pinchera A. Studies on the occurrence of ophthalmopathy in graves' disease. *Eur J Endocrinol* (1989) 120(4):473–8. doi: 10.1530/acta.0.1200473

86. Kashkouli MB, Heidari I, Pakdel F, Jam S, Honarbakhsh Y, Mirarmandehi B. Change in quality of life after medical and surgical treatment of graves' ophthalmopathy. *Middle East Afr J Ophthalmol* (2011) 18(1):42–7. doi: 10.4103/0974-9233.75884

87. Brix TH, Kyvik KO, Christensen K, Hegedös L. Evidence for a major role of heredity in graves' disease: a population-based study of two Danish twin cohorts. *J Clin Endocrinol Metab* (2001) 86(2):930–4. doi: 10.1210/jcem.86.2.7242

88. Strianese D. Update on graves disease: advances in treatment of mild, moderate and severe thyroid eye disease. *Curr Opin Ophthalmol* (2017) 28(5):505–13. doi: 10.1097/ICU.0000000000000402

89. Halliwell B. Cellular responses to oxidative stress: adaptation, damage, repair, senescence and death. In: Halliwell B, Gutteridge JMC., (eds) *Free Radicals in Biology and Medicine*, *4th* Edition, Oxford University Press, New York (2007). p.187–267.

90. Marcocci C, Leo M, Altea MA. Oxidative stress in graves' disease. *Eur Thyroid J* (2012) 1(2):80–7. doi: 10.1159/000337976

91. Kashkouli MB, Aghamirsalim M, Karimi N, Shahrzad S. Autoimmune hyperthyroidism and thyroid eye disease: what is the role of pro-oxidants and antioxidants? *Expert Rev Ophthalmol* (2015) 10(2):135–43. doi: 10.1586/17469899.2015.1012499

92. Winther KH, Bonnema SJ, Hegedüs L. Is selenium supplementation in autoimmune thyroid diseases justified? *Curr Opin Endocrinol Diabetes Obes* (2017) 24(5):348–55. doi: 10.1097/MED.0000000000000356

93. Lanzolla G, Marcocci C, Marinò M. Antioxidant therapy in graves' orbitopathy. *Front Endocrinol* (2020) 11:608733. doi: 10.3389/fendo.2020.608733

94. Lisi S, Botta R, Lemmi M, Sellari-Franceschini S, Altea MA, Sisti E, et al. Quercetin decreases proliferation of orbital fibroblasts and their release of hyaluronic acid. *J Endocrinol Invest* (2011) 34(7):521–7. doi: 10.3275/7321

95. Akbarian S, Chaibakhsh S, Kashkouli MB, Karimi N, Abdolalizadeh P, Ghahvehchian H. A systematic review on the role of antioxidants in thyroid eye disease. *J Curr Ophthalmol* (2022) 34(1):16–24. doi: 10.4103/joco.joco_266_21

96. Coelho SM, Vaisman M, Carvalho DP. Tumour re-differentiation effect of retinoic acid: a novel therapeutic approach for advanced thyroid cancer. *Curr Pharm Design* (2005) 11(19):2525–31. doi: 10.2174/1381612054367490

97. Castaigne S, Chomienne C, Daniel MT, Ballerini P, Berger R, Fenaux P, et al. All-trans retinoic acid as a differentiation therapy for acute promyelocytic leukemia. *I Clin Results* (1990) 76(9):1704–9. doi: 10.1182/blood.V76.9.1704.1704

98. Lippman SM, Meyskens FLJr. Treatment of advanced squamous cell carcinoma of the skin with isotretinoin. *Ann Internal Med* (1987) 107(4):499–501. doi: 10.7326/0003-4819-107-4-499

99. Saade M, Debahy NE-S, Houjeily S. Clinical remission of xeroderma pigmentosum-associated squamous cell carcinoma with isotretinoin and chemotherapy: case report. *J Chemother* (1999) 11(4):313–7. doi: 10.1179/joc.1999.11.4.313

100. Khuri FR, Lotan R, Kemp BL, Lippman SM, Wu H, Feng L, et al. Retinoic acid receptor-beta as a prognostic indicator in stage I non–small-cell lung cancer. *J Clin Oncol* (2000) 18(15):2798–804. doi: 10.1200/JCO.2000.18.15.2798

101. Brabender J, Danenberg KD, Metzger R, Schneider PM, Lord RV, Groshen S, et al. The role of retinoid X receptor messenger RNA expression in curatively resected non-small cell lung cancer. *Clin Cancer Res* (2002) 8(2):438–43.

102. Rochaix P, Monteil-Onteniente S, Rochette-Egly C, Caratero C, Voigt JJ, Jozan S. Reduced expression of retinoic acid receptor beta protein (RARβ) in human papillary

thyroid carcinoma: immunohistochemical and Western blot study. *Histopathology* (1998) 33(4):337–43. doi: 10.1046/j.1365-2559.1998.00486.x

103. Kurebayashi J, Tanaka K, Otsuki T, Moriya T, Kunisue H, Uno M, et al. All-trans-retinoic acid modulates expression levels of thyroglobulin and cytokines in a new human poorly differentiated papillary thyroid carcinoma cell line, KTC-1. *J Clin Endocrinol Metab* (2000) 85(8):2889–96. doi: 10.1210/jcem.85.8.6732

104. Schmutzler C, Winzer R, Meissner-Weigl J, Köhrle J. Retinoic acid increases sodium/iodide symporter mRNA levels in human thyroid cancer cell lines and suppresses expression of functional symporter in nontransformed FRTL-5 rat thyroid cells. *Biochem Biophys Res Commun* (1997) 240(3):832–8. doi: 10.1006/bbrc.1997.7715

105. Schreck R, Schnieders F, Schmutzler C, Köhrle J. Retinoids stimulate type I iodothyronine 5'-deiodinase activity in human follicular thyroid carcinoma cell lines. *J Clin Endocrinol Metab* (1994) 79(3):791–8. doi: 10.1210/jcem.79.3.8077363

106. Schmutzler C, Brtko J, Bienert K, Köhrle J. Effects of retinoids and role of retinoic acid receptors in human thyroid carcinomas and cell lines derived therefrom. *Exp Clin Endocrinol Diabetes* (1996) 104(S 04):16–9. doi: 10.1055/s-0029-1211693

107. Van Herle AJ, Agatep ML, Padua Iii DN, Totanes TL, Canlapan DV, Herle HV, et al. Effects of 13 cis-retinoic acid on growth and differentiation of human follicular carcinoma cells (UCLA RO 82 wl) in vitro. *J Clin Endocrinol Metab* (1990) 71(3):755–63. doi: 10.1210/jcem-71-3-755

108. Simon D, Körber C, Krausch M, Segering J, Groth P, Görges R, et al. Clinical impact of retinoids in redifferentiation therapy of advanced thyroid cancer: final results of a pilot study. *Eur J Nucl Med Mol Imaging* (2002) 29(6):775–82. doi: 10.1007/s00259-001-0737-6

109. Soldin OP, Tractenberg RE, Hollowell JG, Jonklaas J, Janicic N, Soldin SJ. Trimester-specific changes in maternal thyroid hormone, thyrotropin, and thyroglobulin concentrations during gestation: trends and associations across trimesters in iodine sufficiency. *Thyroid* (2004) 14(12):1084–90. doi: 10.1089/thy.2004.14.1084

110. Negro R. Lazarus J, Pirags V, Butz S, editors. *The thyroid and reproduction*. New York: Georg Thieme Verlag (2009). p. 84–95.

111. Skjöldebrand L, Brundin J, Carlström A, Pettersson T. Thyroid associated components in serum during normal pregnancy. *Acta Endocrinol (Copenh)* (1982) 100 (4):504–11. doi: 10.1530/acta.0.1000504

112. Kurtz A, Dwyer K, Ekins R. Serum free thyroxine in pregnancy. *Br Med J* (1979) 2(6189):550–1. doi: 10.1136/bmj.2.6189.550-c

113. Boss AM, Kingstone D. Further observations on serum free thyroxine concentrations during pregnancy. *Br Med J (Clin Res Ed)* (1981) 283(6291):584. doi: 10.1136/bmj.283.6291.584

114. Hopton MR, Ashwell K, Scott IV, Harrop JS. Serum free thyroxine concentration and free thyroid hormone indices in normal pregnancy. *Clin Endocrinol (Oxf)* (1983) 18(4):431–7. doi: 10.1111/j.1365-2265.1983.tb00589.x

115. Glinoer D. The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology. *Endocr Rev* (1997) 18(3):404–33. doi: 10.1210/edrv.18.3.0300

116. de Escobar GM, Obregón MJ, del Rey FE. Maternal thyroid hormones early in pregnancy and fetal brain development. *Best Pract Res Clin Endocrinol Metab* (2004) 18 (2):225–48. doi: 10.1016/j.beem.2004.03.012

117. Kilby MD. Thyroid hormones and fetal brain development. *Clin Endocrinol (Oxf)* (2003) 59(3):280–1. doi: 10.1046/j.1365-2265.2003.01804.x

118. Downie D, Antipatis C, Delday MI, Maltin CA, Sneddon AA. Moderate maternal vitamin a deficiency alters myogenic regulatory protein expression and perinatal organ growth in the rat. *Am J Physiol Regul Integr Comp Physiol* (2005) 288(1):R73–9. doi: 10.1152/ajpregu.00186.2004

119. El-Khashab EK, Hamdy AM, Maher KM, Fouad MA, Abbas GZ. Effect of maternal vitamin a deficiency during pregnancy on neonatal kidney size. *J Perinat Med* (2013) 41(2):199–203. doi: 10.1515/jpm-2012-0026

120. Sommer A, Vyas KS. A global clinical view on vitamin a and carotenoids. *Am J Clin Nutr* (2012) 96(5):1204s–6s. doi: 10.3945/ajcn.112.034868

121. Trumbo P, Yates AA, Schlicker S, Poos M. Dietary reference intakes: vitamin a, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. *J Am Diet Assoc* (2001) 101(3):294–301. doi: 10.1016/S0002-8223(01)00078-5

122. Miller RK, Hendrickx AG, Mills JL, Hummler H, Wiegand UW. Periconceptional vitamin a use: how much is teratogenic? *Reprod Toxicol* (1998) 12 (1):75–88. doi: 10.1016/S0890-6238(97)00102-0

123. WHO. *Safe vitamin a dosage during pregnancy and lactation. recommendations and report of a consultation; world health organization, the micronutrient initiative*. Geneva: Switzerland (1998).

124. Chagas MH, Flores H, Campos FA, Santana RA, Lins EC. Vitamin a tertogenicity. *Rev Bras Saude Mater Infant* (2003) 3:247–52. doi: 10.1590/S1519-38292003000300003

125. OMS. Vitamina a na gestação e na lactação: recomendações e relatório de uma consultoria; série micronutrientes. WHO/NUT/98.4). In: *Centro colaborador de alimentação e nutrição do nordeste I*. Recife, Brazil: Organização Mundial da Saúde (2001).

126. Hill JO. Understanding and addressing the epidemic of obesity: an energy balance perspective. *Endocr Rev* (2006) 27(7):750–61. doi: 10.1210/er.2006-0032

127. Thomas-Valdés S, Tostes M, Anunciação PC, da Silva BP, Sant'Ana HMP. Association between vitamin deficiency and metabolic disorders related to obesity. *Crit Rev Food Sci Nutr* (2017) 57(15):3332–43. doi: 10.1080/10408398.2015.1117413

128. Blaner WS. Vitamin a signaling and homeostasis in obesity, diabetes, and metabolic disorders. *Pharmacol Ther* (2019) 197:153–78. doi: 10.1016/j.pharmthera.2019.01.006

129. Godala M, Materek-Kuśmierkiewicz I, Moczulski D, Rutkowski M, Szatko F, Gaszyńska E, et al. The risk of plasma vitamin a, c, e and d deficiency in patients with metabolic syndrome: a case-control study. *Adv Clin Exp Med* (2017) 26(4):581–6. doi: 10.17219/acem/62453

130. Song RH, Wang B, Yao QM, Li Q, Jia X, Zhang JA. The impact of obesity on thyroid autoimmunity and dysfunction: a systematic review and meta-analysis. *Front Immunol* (2019) 10:2349. doi: 10.3389/fimmu.2019.02349

131. Wang X, Liu H, Chen J, Huang Y, Li L, Rampersad S, et al. Metabolic characteristics in obese patients complicated by mild thyroid hormone deficiency. *Horm Metab Res* (2016) 48(5):331–7. doi: 10.1055/s-0042-105150

132. Zimmermann MB. Interactions of vitamin a and iodine deficiencies: effects on the pituitary-thyroid axis. *Int J Vitam Nutr Res* (2007) 77(3):236–40. doi: 10.1024/0300-9831.77.3.236

133. Zimmermann MB, Jooste PL, Mabapa NS, Schoeman S, Biebinger R, Mushaphi LF, et al. Vitamin a supplementation in iodine-deficient African children decreases thyrotropin stimulation of the thyroid and reduces the goiter rate. *Am J Clin Nutr* (2007) 86(4):1040–4. doi: 10.1093/ajcn/86.4.1040

134. Ma B, Yang P, Gao J, Du L, Sheng C, Usman T, et al. Relationship of vitamin a and thyroid function in individuals with obesity and after laparoscopic sleeve gastrectomy. *Front Nutr* (2022) 9:824193. doi: 10.3389/fnut.2022.824193

135. Morley JE, Melmed S, Reed A, Kasson BG, Levin SR, Pekary AE, et al. Effect of vitamin a on the hypothalamo-pituitary-thyroid axis. *Am J Physiol* (1980) 238(2):E174–9. doi: 10.1152/ajpendo.1980.238.2.E174

136. Milani A, Basirnejad M, Shahbazi S, Bolhassani A. Carotenoids: biochemistry, pharmacology and treatment. *Br J Pharmacol* (2017) 174(11):1290–324. doi: 10.1111/bph.13625

137. Novotny JA, Harrison DJ, Pawlosky R, Flanagan VP, Harrison EH, Kurilich AC. Beta-carotene conversion to vitamin a decreases as the dietary dose increases in humans. *J Nutr* (2010) 140(5):915–8. doi: 10.3945/jn.109.116947

138. Sommer A. Vitamin a deficiency, child health, and survival. *Nutrition* (1997) 13(5):484–5. doi: 10.1016/S0899-9007(97)00013-0

139. Biesalski HK, Chichili GR, Frank J, von Lintig J, Nohr D. Conversion of beta-carotene to retinal pigment. *Vitam Horm* (2007) 75:117–30. doi: 10.1016/S0083-6729(06)75005-1

140. Carlier C, Coste J, Etchepare M, Périquet B, Amédée-Manesme O. A randomised controlled trial to test equivalence between retinyl palmitate and beta carotene for vitamin a deficiency. *Bmj* (1993) 307(6912):1106–10. doi: 10.1136/bmj.307.6912.1106

141. Sommer A. New imperatives for an old vitamin (A). *J Nutr* (1989) 119(1):96–100. doi: 10.1093/jn/119.1.96

142. West KP, LeClerq SC, Shrestha SR, Wu LS, Pradhan EK, Khatry SK, et al. Effects of vitamin a on growth of vitamin a-deficient children: field studies in Nepal. *J Nutr* (1997) 127(10):1957–65. doi: 10.1093/jn/127.10.1957

143. Christian P, West KPJr., Khatry SK, Katz J, LeClerq S, Pradhan EK, et al. Vitamin a or beta-carotene supplementation reduces but does not eliminate maternal night blindness in Nepal. *J Nutr* (1998) 128(9):1458–63. doi: 10.1093/jn/128.9.1458

144. Borel P, Grolier P, Mekki N, Boirie Y, Rochette Y, Le Roy B, et al. Low and high responders to pharmacological doses of beta-carotene: proportion in the population, mechanisms involved and consequences on beta-carotene metabolism. *J Lipid Res* (1998) 39(11):2250–60. doi: 10.1016/S0022-2275(20)32480-9

145. von Lintig J, Hessel S, Isken A, Kiefer C, Lampert JM, Voolstra O, et al. Towards a better understanding of carotenoid metabolism in animals. *Biochim Biophys Acta* (2005) 1740(2):122–31. doi: 10.1016/j.bbadis.2004.11.010

146. Brubacher GB, Weiser H. The vitamin a activity of beta-carotene. *Int J Vitam Nutr Res* (1985) 55(1):5–15.

147. Tang G, Qin J, Dolnikowski GG, Russell RM. Vitamin a equivalence of beta-carotene in a woman as determined by a stable isotope reference method. *Eur J Nutr* (2000) 39(1):7–11. doi: 10.1007/s003940050070

148. McCarrison R. A GOITRE SURVEY IN ALBINO RATS. *Br Med J* (1930) 1 (3621):989–92. doi: 10.1136/bmj.1.3621.989

149. Drill VA. INTERRELATIONS BETWEEN THYROID FUNCTION AND VITAMIN METABOLISM. *Physiol Rev* (1943) 23(4):355–79. doi: 10.1152/physrev.1943.23.4.355

150. Morley JE, Damassa DA, Gordon J, Eugene Pekary A, Hershman JM. Thyroid function and vitamin a deficiency. *Life Sci* (1978) 22(21):1901–5. doi: 10.1016/0024-3205(78)90477-0

151. Di Bella L, Bianchini P. Oxygen consumption after thyroxine in rat hypovitaminosis a. *Arch Sci Biol (Bologna)* (1949) 33(1):60–76.

152. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet* (1994) 344(8924):721–4. doi: 10.1016/S0140-6736(94)92211-X

153. Dumitriu L, Bartoc R, Ursu H, Purice M, Ionescu V. Significance of high levels of serum malonyl dialdehyde (MDA) and ceruloplasmin (CP) in hyper-and hypothyroidism. *Endocrinologie* (1988) 26(1):35–8.

154. Paller MS, Sikora JJ. Hypothyroidism protects against free radical damage in ischemic acute renal failure. *Kidney Int* (1986) 29(6):1162–6. doi: 10.1038/ki.1986.122

155. Costantini F, Pierdomenico SD, Cesare DD, De Remigis P, Bucciarelli T, Bittolo-Bon G, et al. Effect of thyroid function on LDL oxidation. *Arteriosclerosis Thrombosis Vasc Biol* (1998) 18(5):732–7. doi: 10.1161/01.ATV.18.5.732

156. Swaroop A, Ramasarma T. Heat exposure and hypothyroid conditions decrease hydrogen peroxide generation in liver mitochondria. *Biochem J* (1985) 226 (2):403–8. doi: 10.1042/bj2260403

157. Sundaram V, Hanna AN, Koneru L, Newman HAI, Falko JM. Both hypothyroidism and hyperthyroidism enhance low density lipoprotein oxidation. *J Clin Endocrinol Metab* (1997) 82(10):3421–4. doi: 10.1210/jcem.82.10.4315

158. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. *Physiol Rev* (1979) 59(3):527–605. doi: 10.1152/physrev.1979.59.3.527

159. Castilho RF, Kowaltowski AJ, Vercesi AE. 3, 5, 3′-triiodothyronine induces mitochondrial permeability transition mediated by reactive oxygen species and membrane protein thiol oxidation. *Arch Biochem Biophysics* (1998) 354(1):151–7. doi: 10.1006/abbi.1998.0657

160. Morini P, Casalino E, Sblano C, Landriscina C. The response of rat liver lipid peroxidation, antioxidant enzyme activities and glutathione concentration to the thyroid hormone. *Int J Biochem* (1991) 23(10):1025–30. doi: 10.1016/0020-711X(91)90140-I

161. Asayama K, Dobashi K, Hayashibe H, Megata Y, Kato K. Lipid peroxidation and free radical scavengers in thyroid dysfunction in the rat: a possible mechanism of injury to heart and skeletal muscle in hyperthyroidism. *Endocrinology* (1987) 121 (6):2112–8. doi: 10.1210/endo-121-6-2112

162. Bianchi G, Solaroli E, Zaccheroni V, Grossi G, Bargossi AM, Melchionda N, et al. Oxidative stress and anti-oxidant metabolites in patients with hyperthyroidism: effect of treatement. *Hormone Metab Res* (1999) 31(11):620–4. doi: 10.1055/s-2007-978808

163. Choudhury S, Chainy GBN, Mishro MM. Experimentally induced hypo-and hyper-thyroidism influence on the antioxidant defence system in adult rat testis. *Andrologia* (2003) 35(3):131–40. doi: 10.1046/j.1439-0272.2003.00548.x

164. Ademoğlu E, De GÖKkuŞU C, Yarman S, Azizlerli H. The effect of methimazole on the oxidant and antioxidant system in patients with hyperthyroidism. *Pharmacol Res* (1998) 38(2):93–6. doi: 10.1006/phrs.1998.0336

165. Mancini A, De Marinis L, Calabrò F, Fiumara C, Goglia A, Littarru GP. Physiopathological relevance of coenzyme Q10 in thyroid disorders: CoQ10 concentrations in normal and diseased human thyroid tissue. *Biomed Clin Aspects Coenzyme Q* (1991) 6:441–8.

166. Baskol G, Atmaca H, Tanrıverdi F, Baskol M, Kocer D, Bayram F. Oxidative stress and enzymatic antioxidant status in patients with hypothyroidism before and after treatment. *Exp Clin Endocrinol Diabetes* (2007) 115(08):522–6. doi: 10.1055/s-2007-981457

167. Torun AN, Kulaksizoglu S, Kulaksizoglu M, Pamuk BO, Isbilen E, Tutuncu NB. Serum total antioxidant status and lipid peroxidation marker malondialdehyde levels in overt and subclinical hypothyroidism. *Clin Endocrinol* (2009) 70(3):469–74. doi: 10.1111/j.1365-2265.2008.03348.x

168. Zhang N, Wang L, Duan Q, Lin L, Ahmed M, Wang T, et al. Metallothionein-I/II knockout mice aggravate mitochondrial superoxide production and peroxiredoxin 3 expression in thyroid after excessive iodide exposure. *Oxid Med Cell Longevity* (2015) 2015:267027. doi: 10.1155/2015/267027

169. Vitale M, Di Matola T, D'Ascoli F, Salzano S, Bogazzi F, Fenzi G, et al. Iodide excess induces apoptosis in thyroid cells through a p53-independent mechanism involving oxidative stress. *Endocrinology* (2000) 141(2):598–605. doi: 10.1210/endo.141.2.7291

170. Corvilain B, Collyn L, Van Sande J, Dumont JE. Stimulation by iodide of H2O2generation in thyroid slices from several species. *Am J Physiol-Endocrinol Metab* (2000) 278(4):E692–E9. doi: 10.1152/ajpendo.2000.278.4.E692

171. Subudhi U, Das K, Paital B, Bhanja S, Chainy GB. Supplementation of curcumin and vitamin e enhances oxidative stress, but restores hepatic histoarchitecture in hypothyroid rats. *Life Sci* (2009) 84(11-12):372–9. doi: 10.1016/j.lfs.2008.12.024

172. Angioni A, Lania A, Cattaneo A, Beck-Peccoz P, Spada A. Effects of chronic retinoid administration on pituitary function. *J Endocrinol Invest* (2005) 28(2):961–4. doi: 10.1007/BF03345332

173. El-Eshmawy MM, Arafa MM, Elzehery RR, Elhelaly RM, Elrakhawy MM, El-Baiomy AA. Relationship between vitamin a deficiency and the thyroid axis in clinically stable patients with liver cirrhosis related to hepatitis c virus. *Appl Physiol Nutrition Metab* (2016) 41(9):985–91. doi: 10.1139/apnm-2016-0056

174. Ceresini G, Rebecchi I, Morganti S, Maggio M, Solerte SB, Corcione L, et al. Effects of vitamin a administration on serum thyrotropin concentrations in healthy human subjects. *Metabolism* (2002) 51(6):691–4. doi: 10.1053/meta.2002.32724

175. Ibrahim HS, Ghit MM, El-Azeem A, Niveen M. Assessment of nutritional status of women suffering from thyroid dysfunction and osteoporosis. *Egyptian J Nutr Health* (2020) 15(1):1–16. doi: 10.21608/ejnh.2020.117358

176. Mullur R, Liu Y-Y, Brent GA. Thyroid hormone regulation of metabolism. *Physiol Rev* (2014) 94(2):355–82. doi: 10.1152/physrev.00030.2013

177. Liu Y-C, Yeh C-T, Lin K-H. Molecular functions of thyroid hormone signaling in regulation of cancer progression and anti-apoptosis. *Int J Mol Sci* (2019) 20(20):4986. doi: 10.3390/ijms20204986

178. Olson CJH, Krois CR. Thyroid hormone regulation of retinoic acid synthesis in brown adipose tissue. *FASEB J* (2019) 33(S1):485.8–.8. doi: 10.1096/fasebj.2019.33.1_supplement.485.8

179. Gil-Ibáñez P, Bernal J, Morte B. Thyroid hormone regulation of gene expression in primary cerebrocortical cells: role of thyroid hormone receptor subtypes and interactions with retinoic acid and glucocorticoids. *PloS One* (2014) 9 (3):e91692. doi: 10.1371/journal.pone.0091692

180. Petkovich M, Chambon P. Retinoic acid receptors at 35 years. *J Mol Endocrinol* (2022) 69(4):T13–24. doi: 10.1530/JME-22-0097

181. Reay WR, Cairns MJ. The role of the retinoids in schizophrenia: genomic and clinical perspectives. *Mol Psychiatry* (2020) 25(4):706–18. doi: 10.1038/s41380-019-0566-2

182. Wang S, Moise AR. Recent insights on the role and regulation of retinoic acid signaling during epicardial development. *genesis* (2019) 57(7-8):e23303. doi: 10.1002/dvg.23303

183. Li S, Dong S, Xu W, Jiang Y, Li Z. Polymer nanoformulation of sorafenib and all-trans retinoic acid for synergistic inhibition of thyroid cancer. *Front Pharmacol* (2020) 10:1676. doi: 10.3389/fphar.2019.01676

184. Groener JB, Gelen D, Mogler C, Herpel E, Toth C, Kender Z, et al. BRAF V600E and retinoic acid in radioiodine-refractory papillary thyroid cancer. *Hormone Metab Res* (2019) 51(01):69–75. doi: 10.1055/a-0765-9078

185. Karooby E, Granpayeh N. Potential applications of nanoshell bow-tie antennas for biological imaging and hyperthermia therapy. *OptEn* (2019) 58(6):065102–. doi: 10.1117/1.OE.58.6.065102

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 07 June 2023
DOI 10.3389/fendo.2023.1042176

OPEN ACCESS

EDITED BY
Sutapa Mukherjee,
Visva-Bharati University, India

REVIEWED BY
Dipak Kumar Sahoo,
Iowa State University, United States
Kavindra Kumar Kesari,
Aalto University, Finland

*CORRESPONDENCE
Heriberto Rodríguez-Martínez
heriberto.rodriguez-martinez@liu.se

RECEIVED 12 September 2022
ACCEPTED 17 May 2023
PUBLISHED 07 June 2023

CITATION
Álvarez-Rodríguez M, Roca J, Martínez EA and Rodríguez-Martínez H (2023) Mating modifies the expression of crucial oxidative-reductive transcripts in the pig oviductal sperm reservoir: is the female ensuring sperm survival?
Front. Endocrinol. 14:1042176.
doi: 10.3389/fendo.2023.1042176

Mating modifies the expression of crucial oxidative-reductive transcripts in the pig oviductal sperm reservoir: is the female ensuring sperm survival?

Manuel Álvarez-Rodríguez[1,2], Jordi Roca[3], Emilio A. Martínez[3] and Heriberto Rodríguez-Martínez[1]*

[1]Department of Biomedical and Clinical Sciences (BKV), BKH/Obstetrics and Gynecology, Faculty of Medicine and Health Sciences, Linköping University, Linköping, Sweden, [2]Department of Animal Reproduction, Instituto Nacional de Investigación Agraria y Alimentaria (INIA)-CSIC, Madrid, Spain, [3]Department of Medicine and Animal Surgery, Faculty of Veterinary Medicine, University of Murcia, Murcia, Spain

Background: Mating induces large changes in the female genital tract, warranting female homeostasis and immune preparation for pregnancy, including the preservation of crucial oxidative status among its pathways. Being highly susceptible to oxidative stress, sperm survival and preserved function depend on the seminal plasma, a protection that is removed during sperm handling but also after mating when spermatozoa enter the oviduct. Therefore, it is pertinent to consider that the female sperm reservoir takes up this protection, providing a suitable environment for sperm viability. These aspects have not been explored despite the increasing strategies in modulating the female status through diet control and nutritional supplementation.

Aims: To test the hypothesis that mating modifies the expression of crucial oxidative-reductive transcripts across the entire pig female genital tract (cervix to infundibulum) and, particularly in the sperm reservoir at the utero-tubal junction, before ovulation, a period dominated by estrogen stimulation of ovarian as well as of seminal origin.

Methods: The differential expression of estrogen (ER) and progesterone (PR) receptors and of 59 oxidative-reductive transcripts were studied using a species-specific microarray platform, in specific segments of the peri-ovulatory sow reproductive tract in response to mating.

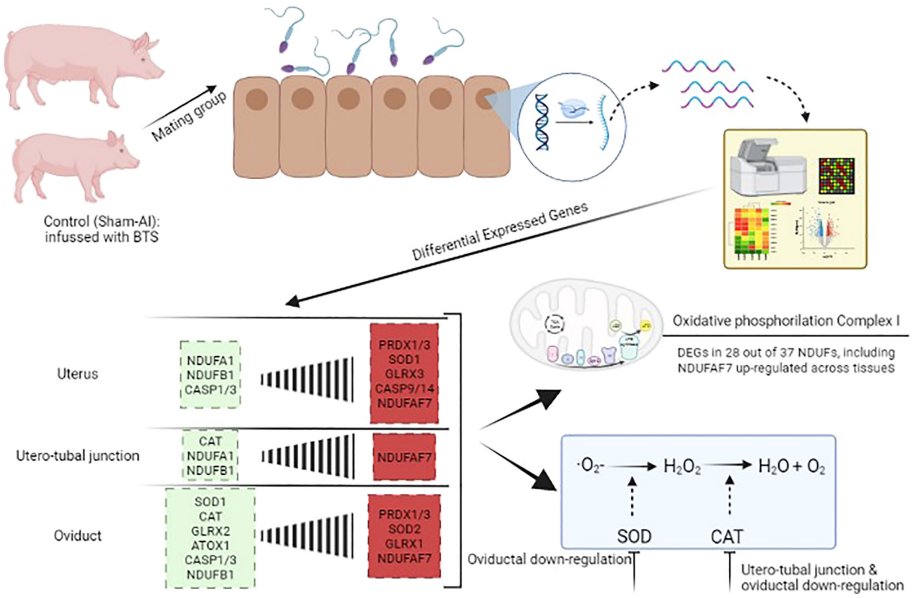
Results: Mating induced changes along the entire tract, with a conspicuous downregulation of both ER and PR and an upregulation of superoxide dismutase 1 (*SOD1)*, glutaredoxin (*GLRX3*), and peroxiredoxin 1 and 3 (*PRDX1*, *PRDX3)*, among other NADH Dehydrogenase Ubiquinone Flavoproteins, in the distal uterus segment. These changes perhaps helped prevent oxidative stress in the area adjacent to the sperm reservoir at the utero-tubal junction. Concomitantly, there were a downregulation of catalase (*CAT*) and NADH dehydrogenase (ubiquinone) oxidoreductases 1 beta subcomplex, subunit 1 (*NDUFB1*) in the utero-tubal junction alongside an overall downregulation of *CAT*, *SOD1*, and *PRDX3* in the ampullar and infundibulum segments.

10.3389/fendo.2023.1042176

Conclusions: Natural mating is an inducer of changes in the expression of female genes commanding antioxidant enzymes relevant for sperm survival during sperm transport, under predominant estrogen influence through the bloodstream and semen. The findings could contribute to the design of new therapeutics for the female to improve oxidative-reductive balance.

KEYWORDS

ROS, antioxidant, porcine, mating, periovulatory



GRAPHICAL ABSTRACT
Created with BioRender.com.

1 Introduction

Oxidative stress is critical to reproductive success and any distress of antioxidant capacity in the reproductive epithelia is capable of disturbing endocrine status in sows (1) Natural mating adds another dimension. On one hand, it imposes the transit and permanence of the foreign spermatozoa and of immunologically foreign proteins in the seminal plasma (SP). On the other hand, mating occurs solely under oestrus, a period dominated by estrogen influence, both humoral and local, considering the SP of the boar contains relevant concentrations of the hormone, up to 11.5 μg/ejaculate (2). These seminal estrogens condition the local release of prostaglandins, imposing dramatic increases in myometrial and myosalpingeal contractions ruling sperm transport (3). Mating calls for a decision by the female immune system; to maintain protection against pathogens and up to 80% of surplus foreign spermatozoa while at the same time allowing the survival of an aliquot of potentially fertile spermatozoa in the sperm reservoir (4). Such status of tolerance to male antigens is initiated at mating and maintained throughout pregnancy, as documented by several studies (5–9) including a large cohort of orchestrated events in the female, which includes preservation of cell homeostasis controlled by, among other factors, the correct oxidative-reductive balance of thousands of genes.

How do these genes react to endocrine changes and reproductive events such as mating? Mating in sows occurs during standing oestrus, a period of the estrous cycle dominated by estrogens of ovarian and seminal sources (2, 10), and mating is, per se, capable of affecting gene expression in tissues of the genital tract of the female (5) without considering the hormone levels present in the SP. It is well recognized that mating and deposition of semen modify the onset and the duration of ovulation in sows (11), effects also recognized as being affected by the SP (12), a composite fluid recently proposed as acting as a particular pheromone (13). Mating and deposition of semen also cause dramatic changes in the expression of genes, particularly of those related to immune function, in the internal genital tract of sows during the pre/peri ovulatory stage of oestrus (6), including the cortisol receptor and

some prostaglandins (9) and other complex molecules such as the RNA binding molecules, which have an active part of the immune response to the presence of spermatozoa (and seminal plasma) in the reproductive tract (8). The hypothesis behind these changes includes the existence of a tolerance status in the female, not yet fully understood, that allows the sperm to survive in the female genital tract, in particular, the sperm stored in the sperm storage site. If deprived of the antioxidant protection of the seminal plasma (14), the spermatozoa in the storage site are quite susceptible to oxidative stress, a common cause of sperm death (15).

Sperm transit through the female genital tract is quite rapid, and after only 1-2 h enough sperm is sequestered to ensure fertilization (16). Such quick transport is issued by myometrial and myosalpingeal contractions, under the stimulus of estrogens from the ovary and/or seminal plasma (2, 14, 15, 17). Under this period of estrogen influence, spermatozoa are stored in the utero-tubal junction (UTJ) for up to 36 h or more, being kept viable and fertile, expecting spontaneous ovulation (18–20) and the changes that progesterone issues on the UTJ, including sperm capacitation (21). Sperm capacitation, which is triggered by ionic changes in the sperm environment, is also related to the endocrine variations in the female (14), being partially regulated by estrogen (22) and progesterone (20).

However, is it possible to manage female tolerance through diet composition? This question remains yet unknown because most of the studies included the addition of antioxidants during pregnancy and not before, for preparation during the peri-ovulatory phase. Nutritional studies are usually focused on, for example, fiber addition that leads to increased oocyte maturation, prenatal survival, and litter size, being fluid hormones and metabolites, hypothalamic satiety center on gonadotropin secretion and epigenetics would affect strong candidates for the mechanism (23). Other examples of nutritional add-ons are lycopene, which improves maternal reproductive performance (24), and cysteamine, which alleviates oxidative stress and enhances angiogenesis in the porcine placenta (25). In addition, taurine supplementation to gilts during late gestation and lactation has a large effect on offspring growth and oxidative stress (26) as does resveratrol, which increased the oxidative status of offspring (27).

Most of the nutritional studies are oriented to map the oxidative-reductive balance in the organism, with little attention being paid to the expression in the female genital tract. An example of analytical parameters to be assayed in this regard is the superoxide dismutase (SOD) (SOD1: soluble, SOD2: mitochondrial, and SOD3: extracellular), which catalyzes the dismutation of the superoxide radical into a molecular oxygen and hydrogen peroxide. If abundant, hydrogen peroxide leads to many types of cell damage (28). Catalase (CAT) is an important second reactive oxygen species (ROS)-scavenger, converting hydrogen peroxide into water and oxygen. In addition, peroxiredoxins are a highly conserved family of cysteine-dependent peroxidases that reduce hydrogen peroxide, lipid hydroperoxides, and peroxynitrite and have emerged as one of the most important scavenging enzymes, together with CAT and glutathione peroxidases (29). Moreover, caspases are cysteine-aspartic proteases that are involved in several programmed cell death functions but act locally, with minimum effect on surrounding tissues (30). Finally, the oxidative phosphorylation pathway (OxPhos) is the primary pathway for energy production, but also to balance the oxidative-reductive balance in several cells and tissues. NADH:ubiquinone oxidoreductase (complex I) is the first of three large enzyme complexes located in the inner mitochondrial membrane which form the electron transport chain that carries electrons from NADH to molecular oxygen during oxidative phosphorylation (31). The NADH-ubiquinones, which are implicated in the respiratory chain complexes, participated in the NADH transfer of electrons and the oxidation of NDAH into its oxidized form (NAD+) (32) and, ultimately, are involved in the oxidation-reduction process, including post-translational protein modifications.

In the present study, we analyzed the effect of natural mating on the expression of genes relevant to the oxidative-reductive capacity of specific segments of the female internal genital tract of pigs pre-periovulation, relative to the basal expression of non-mated sows. We hypothesized that mating, on a particular endocrine milieu, modifies the expression of crucial oxidative-reductive transcripts across the entire pig female genital tract (cervix to infundibulum) that are of relevance for the survival of spermatozoa during sperm transit and particularly in the sperm reservoir (UTJ) during the lengthy pre-fertilization period.

2 Materials and methods

2.1 Ethics approval

Animal handling and experiments were carried out in accordance with the European Community Directive 2010/63/EU, 22/09/2010, and current Swedish legislation (SJVFS 2017:40). The study was accepted by the Regional Committee for Ethical Approval of Animal Experiments (Linköpings Djurförsöksetiska nämnd, Linköping, Sweden). Permits number 75-12 (10/02/2012), ID1400 (02/02/2018), and Dnr 03416-2020 (26/03/2020).

2.2 Tissue collection

Weaned sows (parity 1-3, n = 8) and young matured boars (9-11 months of age, n = 5) of the Swedish Landrace breed (*Sus scrofa domestica*) were held in individual pens at the Translational Medicine Centre (TMC/CBR-3) of Linköping University under temperature and light control. Animals were fed with commercial feedstuff, and water was provided ad libitum. Females were cervically infused with protein-free Beltsville thawing solution (Control group, n = 4) or mated with a single male (Mating group, n = 4), as previously described (5–7). After 24 h of each treatment, sows were subjected to general anesthesia during the tissue collection procedure. The following specific segments were retrieved: cervix (Cvx), distal uterus (DistUt), proximal uterus (ProxUt), UTJ, and the oviductal segments isthmus (Isth), ampulla (Amp), and infundibulum (Inf). Tissue samples were directly plunged into liquid nitrogen and stored in cryovials at

-80°C until mRNA expression analyses. Fixed and stained paraffin sections of complementary tissues confirmed the presence of spermatozoa in the UTJ of mated sows.

2.3 Oestradiol and progesterone concentrations in blood

Oestradiol (E2) and progesterone (P4) blood plasma concentrations were individually measured using porcine enzyme-linked immune sorbent assay (ELISA) kits (Cat#MBS700342 and Cat#MBS703577, MyBiosource Inc., San Diego, CA, USA), after preparation of a standard curve for the individual hormones. The optical density of each microplate well was determined using a microplate reader (TECAN, Sunrise GmbH, Grödig, Austria) set at 450 nm.

2.4 Transcriptome analysis and bioinformatics

Total RNA from reproductive samples was extracted following a TRIzol (Invitrogen, Carlsbad, CA, USA) modified protocol (25). RNA concentration, integrity evaluation, cDNA synthesis, and microarray analyses (GeneChip® Porcine Gene 1.0 ST Array, Affymetrix Inc., 3420 Central Expressway, Santa Clara, CA, USA) were performed according to methods previously described (25). Only samples with RNA values larger than 9 were employed for microarray hybridization. The GeneChip®Whole Transcript Plus reagent kit (Affymetrix, Santa Clara, CA, USA) was used to synthesize cDNA (250 ng/reaction). An initial incubation of the hybridization cocktail at 99°C for 5 min was done after a fall to 45°C before loading the array chip (GeneChip® Porcine Gene 1.0 ST Array, Affymetrix Inc., 3420 Central Expressway, Santa Clara, CA, USA). The cocktail hybridization solution (130 µL) was then put into each array chip and incubated for 16 h at 45°C under 60 rotations/min. The hybridized cartridge array was unloaded after incubation and washed and stained with the GeneChip® Fluidics Station 450 (Affymetrix, Santa Clara, CA, USA) before being scanned with the Affymetrix GeneChip® Scanner GCS3000 (Affymetrix, Santa Clara, CA, USA).

Transcriptomic results were processed as previously described (6, 33). Briefly, the array chip data were processed using robust multi-array average (RMA) normalization, computing average intensity values by background adjustment, quantile normalization among arrays, and finally, log2 transformation for extracting the expression values of each transcript in the probe set. The normalized mRNA expression data of the 60 selected transcripts were analyzed using the Transcription Analysis Console (TAC, Affymetrix). Differentially expressed transcripts were calculated using a linear model and the empirical Bayes' approach implemented in the package limma, included in the TAC console. A principal component analysis-based p-value correction was used, establishing a fold change (FC) >1 or < -1. GO terms and pathways were analyzed by PANTHER (34) based on the KEGG database (35). ClustVis (BETA) were used for the elaboration of the principal component analysis and the hierarchical clustering of the oxidative-reductive genes (36).

3 Results

3.1 The tissues explored were under estrogenic influence

Oestradiol concentrations (mean ± SD in pg/ml) were 376.50 ± 27.76 in controls versus 349.10 ± 62.19 in mated sows (ns). Progesterone concentrations (mean ± SD in ng/ml) were <0.68 ± 0.34 without significant differences between the sow groups. The hormone concentrations confirmed the animals were all in pre/peri-ovulatory oestrus, with a predominant estrogen influence.

3.2 The genes commanding progesterone and estrogen receptors showed a clear pattern of downregulation in the mated periovulatory sow

Gene expression of estrogen and progesterone receptors (ER and PR) showed a conspicuous pattern of down-regulation in all genital tissues studied, from Proximal Uterus to Infundibulum (Figure 1; Supplementary Table 1). The ER levels further confirmed the degree of tissue stimulation by estrogens.

3.3 Differential expression of the 59 oxidative-reductive transcripts

The differential expression of the 59 oxidative-reductive transcripts across tissues showed a significant number of down- and upregulation in response to natural mating, as depicted in the volcano plot (Figure 2). The principal component analysis explained more than 64% of the variation in the two components, tightly grouped the DistUt and ProxUt tissues, and showed the UTJ as the most distal group from the rest of the tissues, interestingly showing that the UTJ had more proximity to the Cvx expression than the oviductal tissues (Isth, Amp, and Inf) (Supplementary Figure 1). The hierarchical clustering analysis through a heap map representation (Supplementary Figure 2) showed a heterogeneous grouping pattern in some of the tissues, with the endometrial tissues being the ones showing a more discrete grouping pattern.

In addition, transcripts were classified following PANTHER and KEGG databases, according to molecular function (Figure 3A), particularly for catalytic activity and binding. In terms of protein class (Figure 3B), most transcripts belonged to metabolic interconversion enzymes. As for the cellular component

10.3389/fendo.2023.1042176

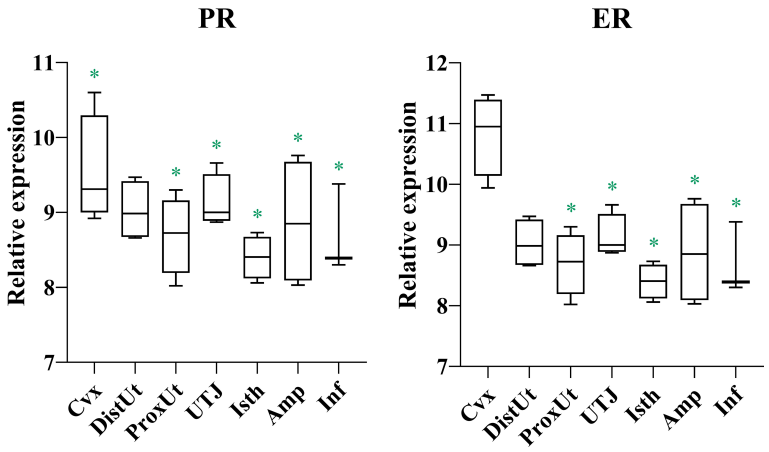


FIGURE 1
Summary of significant differential expression of progesterone and estrogen receptors. Box-plot representation of the log2 signal intensity of the selected transcripts by tissue, Cvx, cervix; DistUt, distal uterus; ProxUt, proximal uterus; UTJ, utero-tubal junction; Isth, isthmus; Amp, ampulla; and Inf, infundibulum. The green color "*" represents a relative decrease relative to the negative control ($p < 0.05$). PR, progesterone receptor; and ER, estrogen receptor.

(Figure 3C), all transcripts were classified into cellular anatomical entities and protein-containing complexes. Cellular and metabolic processes, followed by a response to stimulus and biological regulation, were the main categories inside biological process classification (Figure 3D). Finally, the four most abundant pathways of the oxidative-reductive transcripts were the FAS signaling pathway, apoptosis signaling pathway, Huntington's disease, and CCKR signaling map. (Figure 3E).

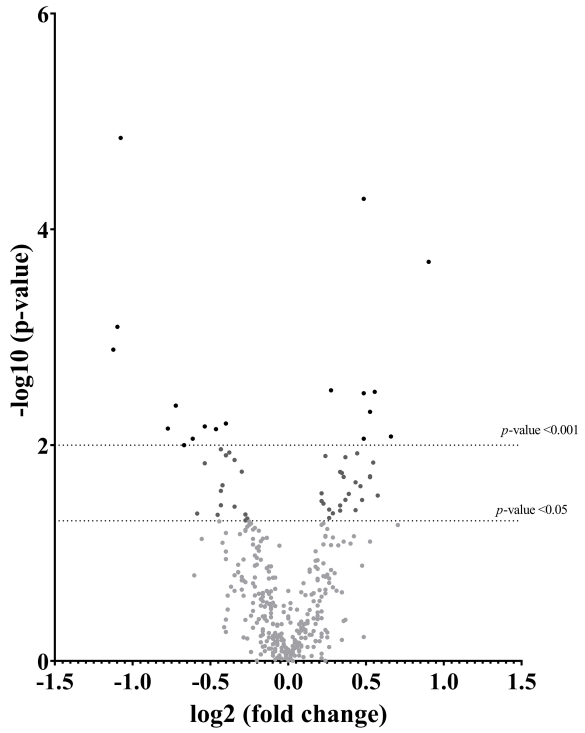


FIGURE 2
Volcano plot depicting a summary of the differential expression analyses of transcripts in the reproductive internal genital tract segments (cervix, distal uterus, proximal uterus, utero-tubal junction, isthmus, ampulla, and infundibulum), 24 h after natural mating *vs* unmated, Sham (infusion of BTS extender) controls. The x-axis shows the log2 fold-changes in expression and the y-axis the statistical significance (-log10 p-value). This figure depicts $p < 0.05$ and $p < 0.01$ relative to the control.

3.4 Peroxiredoxins 1 and 3 expression increases in the distal uterus in response to mating

The detailed analysis of the differential expression of peroxiredoxins (PRDX) showed upregulation in the DistUt (PRDX1 and PRDX3), and downregulation in both the Inf (PRDX1, PRDX3, and PRDX4) and the Amp (PRDX3) (Figure 4).

3.5 Classical oxidative-reduction markers showed a balanced up- and downregulation across tissues

The cytoplasmic subtype SOD1 is one of the members of the SOD protein family catalyzing the conversion of superoxide radicals into hydrogen peroxide and oxygen. Our results showed an upregulation in DistUt and a downregulation in Amp and Inf (Figure 5A). The mitochondrial subtype (SOD2) showed upregulation in the first part of the oviductal tissues (Inf) (Figure 5A). In contrast, no differences were found in SOD3, the extracellular subtype of SOD (Supplementary Table 1).

CAT, an oxidoreductase that together with SOD protects from radical attacks, converts hydrogen peroxide into oxygen and water. Our results showed a decrease in its expression in the UTJ, Isth, and Inf (Figure 5B).

Our findings revealed a differential pattern of expression of glutaredoxin (GLRX), a redox enzyme that employs glutathione as a cofactor and becomes essential to maintaining homeostasis and

10.3389/fendo.2023.1042176

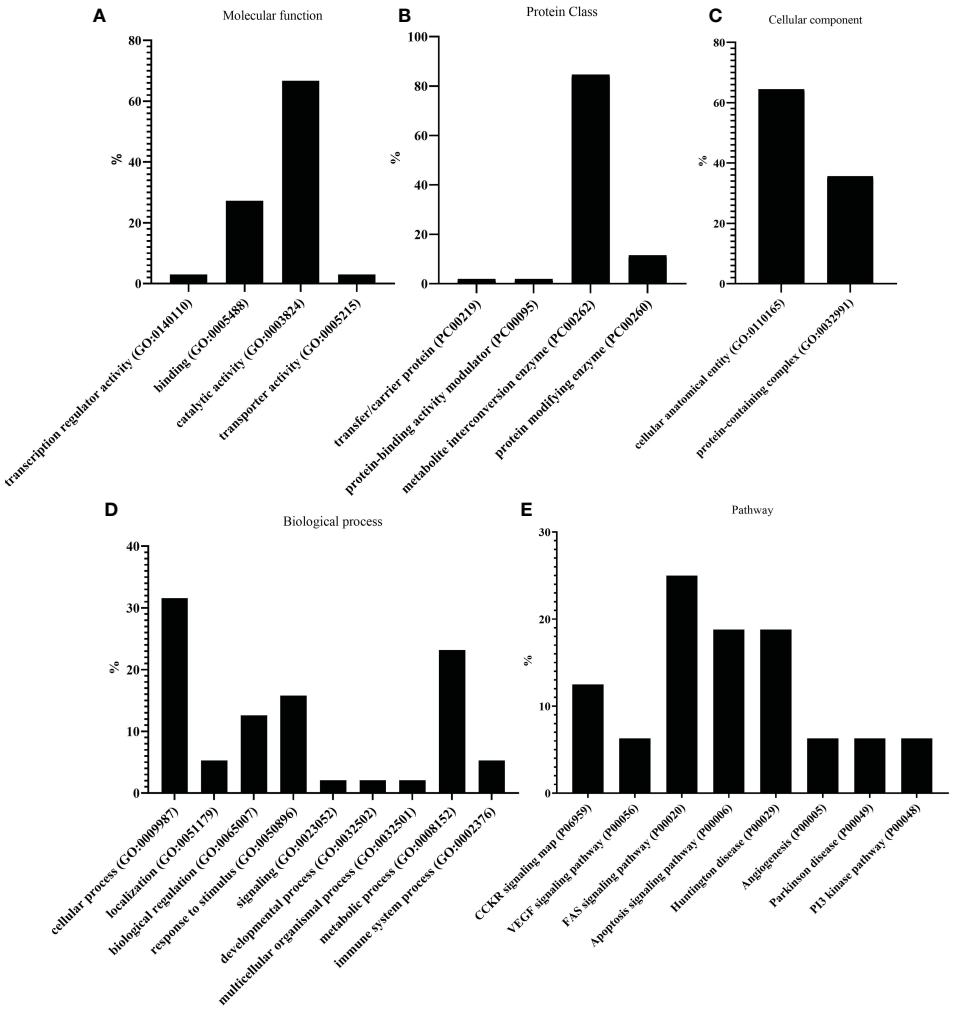


FIGURE 3
PANTHER classification showing differential gene expression (DEG) of the 59 oxidative-reductive (OR) genes. The bar plots represent different percentages of DEGs classified according to **(A)** molecular function, **(B)** protein class, **(C)** cellular component, **(D)** biological process, and **(E)** pathway.

oxidative equilibrium (Figure 5C). GLRX1 (in Inf) and GLRX3 (in DistUt) were upregulated, whereas GLRX2 was downregulated in Inf. No differences were found in GLRX5 expression among tissues (Supplementary Table 1).

Finally, the antioxidant protein 1 (ATOX1) showed a decrease in its expression in the Inf (Figure 5D).

3.6 Caspases 1 and 3 showed a high downregulation pattern across reproductive tissues, except in the UTJ

CASPs, cysteine-aspartic proteases involved in several programmed cell death functions, were mapped in our study (Figure 6). CASP1, with a pro-inflammatory function, was downregulated in DistUt, ProxUt, Isth, and Amp. In addition, CASP3, an initiator of apoptosis, was uniformly downregulated in all the tissues, except in the UTJ. CASP2, CASP8AP2, and CASP9 expression, all apoptosis initiators, were upregulated in DistUt. In contrast, no differences were found neither in CASP6, an apoptosis executioner, nor in the apoptosis and caspase activation inhibitor, in any of the collected tissues (Supplementary Table 1).

3.7 NADH dehydrogenase (ubiquinone) oxidoreductases showed a heterogeneous expression pattern in the distal uterus of the periovulatory sow

The 37 NADH dehydrogenase (ubiquinone) oxidoreductases (NDUF) included in the present study (Supplementary Table 1) showed a heterogeneous pattern of differential expression. Thus, 9 out of 16 NDUF (NDUFA2, NDUFA5, NDUFA8, NDUFA10, NDUFA12, NDUFAB1, NDUFAF1, NDUFAF6, and NDUFAF7) (Figure 7), and 5 out of 12 (NDUFB6, NDUFC2, NDUFS3, NDUFS6, and NDUFV1) (Figure 8) were upregulated in DistUt. In contrast, the pattern of downregulation was heterogeneously distributed across tissues, being the Inf the main tissue presenting this repression in 8 out of 16 (Figure 7) and 7 out of 12 (Figure 8) NDFUs.

10.3389/fendo.2023.1042176

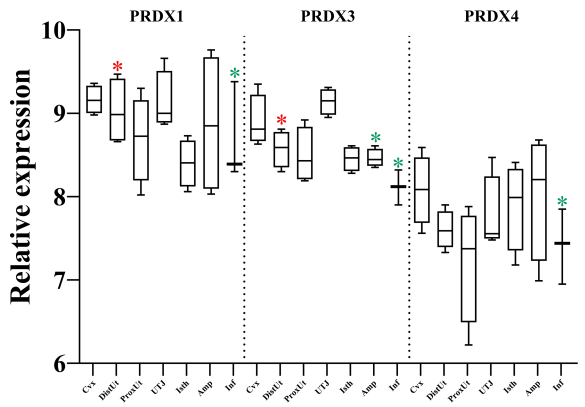


FIGURE 4
Summary of significant differential expression of peroxiredoxins (PRDX). Box-plot representation of the log2 signal intensity of the selected transcripts by tissue, Cvx, cervix; DistUt, distal uterus; ProxUt, proximal uterus; UTJ, utero-tubal junction; Isth, isthmus; Amp, ampulla; and Inf, infundibulum. The red color "*" represents a relative increase relative to the negative control ($p < 0.05$), whereas the green color "*" represents a relative decrease relative to the negative control ($p < 0.05$). PRDX1, peroxiredoxin 1; PRDX1, peroxiredoxin 3, and PRDX4, peroxiredoxin 4.

4 Discussion

The present study analyzed a particular timeframe, 24 h after natural mating when spermatozoa had colonized the female sperm reservoir in the UTJ (14). This particular period of the estrous cycle is dominated by estrogens, both of ovarian and seminal origin. The analyses of circulating estradiol and progesterone in the examined sows confirmed estrogens dominated, and the analyses of the estrogen (ER) and progesterone (PR) receptor transcripts indicate the dominance pressed the production of ERs. Certainly, semen was signaling to the female to activate its local immune system to counteract incoming micro-organisms, cells, and proteins from damaging effects (7). The female, nevertheless, tolerates a certain proportion of spermatozoa, those colonizing the UTJ-reservoir and further on the hemi-allogeneic embryos and their placentas, for the entire pregnancy (37). Estrogens, particularly oestradiol, exert clear effects on the immune system during this particular period (38) as well as on the genital tract epithelia (39) and the spermatozoa, cells provided with, among others, estrogen receptors (40–42).

Most current studies focus on improving the oxidative-reductive balance in sperm samples, but few of them focus on the environment that spermatozoa find when arriving at the sperm

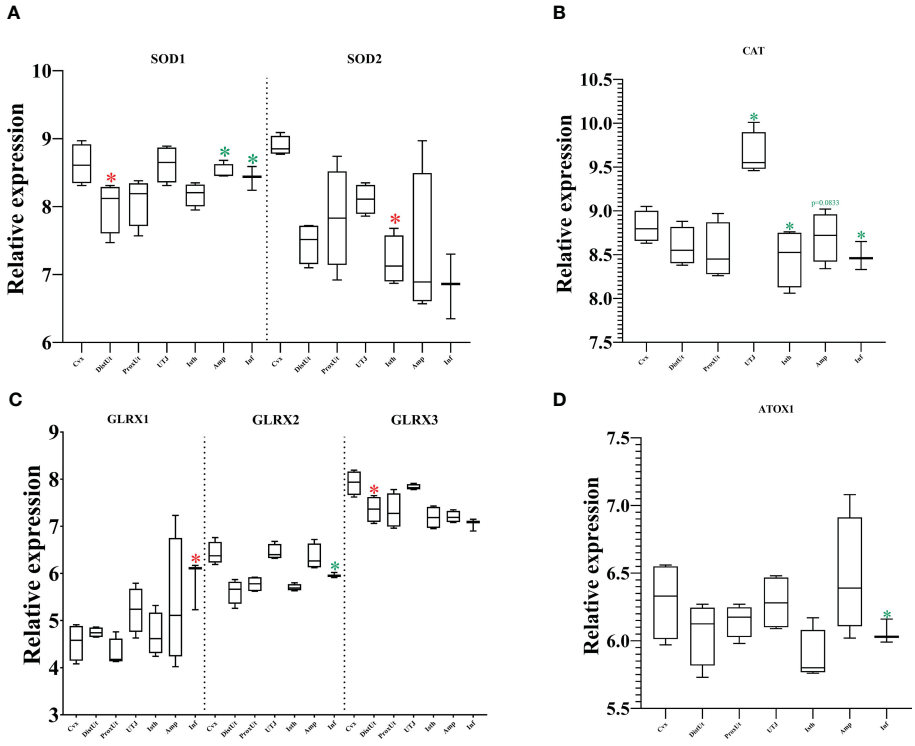


FIGURE 5
Summary of significant differential expression of superoxide dismutase (SOD); glutaredoxins (GLRX); and catalase (CAT). Box-plot representation of the log2 signal intensity of the selected transcripts by tissue, Cvx, cervix; DistUt, distal uterus; ProxUt, proximal uterus; UTJ, utero-tubal junction; Isth, isthmus; Amp, ampulla; and Inf, infundibulum. **(A)** SOD1, superoxide dismutase 1; cytoplasm; and SOD2, mitochondrial. **(B)** CAT, catalase. **(C)** GLRX1, glutaredoxin 1; GLRX2, glutaredoxin 2; and GLRX3, glutaredoxin 3. **(D)** ATOX1, antioxidant protein 1. The red color "*" represents a relative increase relative to the negative control ($p < 0.05$); whereas the green color "*" represents a relative decrease relative to the negative control ($p < 0.05$).

10.3389/fendo.2023.1042176

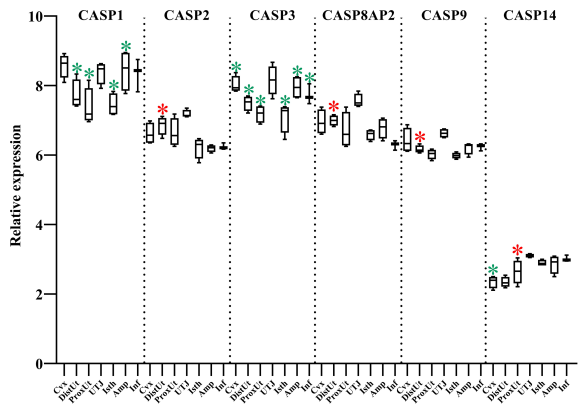


FIGURE 6
Summary of significant differential expression of caspases (CASP). Box-plot representation of the log2 signal intensity of the selected transcripts by tissue, Cvx, cervix; DistUt, distal uterus; ProxUt, proximal uterus; UTJ, utero-tubal junction; Isth, isthmus; Amp, ampulla; and Inf, infundibulum. The red color "*" represents a relative increase relative to the negative control ($p < 0.05$), whereas the green color "*" represents a relative decrease relative to the negative control ($p < 0.05$). CASP1, caspase 1; CASP2, caspase 2; CASP3, caspase 3; CASP8AP2, caspase 8 associated protein 2; CASP9, caspase 9; and CASP14, caspase 14.

storage place in the female and even further on during the transit towards the site where the fertilization takes place in the tubal ampulla. Furthermore, most of the antioxidants and other nutritional complements are used postpartum or during lactation but, to the best of our knowledge, little attention is paid to supplements before mating to favor another early reproductive event as the peri-ovulatory period. In pigs, the SP contains major antioxidants whose overall amount relates to fertility (43). Thus, in the present study, we aimed to analyze how crucial antioxidant biomarkers modify their expression within the pre/periovulatory phase. The rationale behind this was that this restricted period accounts for the main interaction and cross-talk between spermatozoa (and its eventual cargo, including the one inside the extracellular vesicles) and its sperm storage place (12). The survival of spermatozoa in the female genital tract depends on the seminal plasma and its antioxidant properties, but only before the spermatozoa reach the UTJ, the place where seminal plasma becomes scarce. So, the secretion produced by epithelial cells turns critical for sperm survival and fertilization capacity afterward.

PRDXs are thioredoxin-dependent peroxide reductases localized either in the cytoplasm (PRDX1 and PRDX2) or the mitochondria (PRDX3) and they protect the cell from ROS. PRDX1 controls ovulation in mice through a decrease in intracellular ROS (44), confirmed by the described role of PRDX1 in antioxidant scaffold during maternal to zygotic transition in mice (45). In the male counterpart, PRDXs in human testis, epididymis, and spermatozoa prevent H_2O_2-induced damage to spermatozoa (46), with PRDX1 being essential at the epididymis level to fight against the oxidative damage (47). Our results showed an increase of PRDX1 and PRDX3 in DistUt, maybe facilitating the passage of the spermatozoa toward the female reproductive tract. PRDX3 is involved in the thioredoxin pathway (48) and is involved in the protection of late events, as placental function, from oxidative stress occurring in mitochondria (49). Moreover, lower levels of PRDX3 were found in cumulus cells from higher-quality human embryos (50), establishing a negative correlation that could explain, at least in part, the downregulation that we found in Amp and Inf, as preparatory homeostasis for oocyte passage. Moreover, PRDX4 plays an important role in regulating male fertility, showing a positive correlation with litter size (51, 52). However, and in agreement with our results, the PRDX1 was upregulated in endometrial epithelial cells in response to trophectodermal small extracellular vesicles (53), so whether this upregulation is the starting point for preparation for embryo implantation requires further analysis. Overall, and in light of our findings, we suggest a concerted mechanism of both the PRDX1 and SOD1, PRDX1 and SOD1 are upregulated in DistUt and PRDX1 and SOD1 are downregulated in DistUT.

Linseed oil improved the antioxidant capacity of boars, including an increase in CAT abundance (54). In contrast, while CAT looks like a protective agent for the male gamete (54), our results showed a significant decrease in its expression in the UTJ and the oviductal tissues (except Amp ($p=0.0833$). Indeed, from the group of pure antioxidant enzymes, CAT, SOD, ATOX1, and GLRXs, only SOD1, GLRX1, and GLRX3 showed an increase in their expression locally: DistUt, Inf, and DistUT, respectively. In females, CAT supplementation improved fetal growth, modulating antioxidant capacity (55). In addition, resveratrol improved the antioxidant status of sows by increasing levels of CAT and SOD1, and also other molecules such as GPX4, agreeing with a recent research paper by our group where we found increased expression of GPX4 in high fertility boars (56). These results are supported by other studies using an antioxidant treatment on sows (from day 85 of gestation), where they confirmed an increase in the placental expression of SOD (57), as previously demonstrated in the human placenta, the increase in the expression of antioxidant enzymes was in response to oxidative stress (58).

The decreases in mRNA expression of the cytosolic antioxidant GLRX1 and the mitochondrial antioxidant PRDX3 are involved in age-related ovarian oxidative damage to lipid, protein, DNA, and other cellular components vital for maintaining ovarian function and fertility (59). GLRX2, a gene associated with oxidative stress, was downregulated in the presence of antioxidant supplementation during *in vitro* culture of mice oocytes (60). Our results showed a downregulation of GLRX2 in the Inf, which could be associated with the preparation of the receptacle to the soon-to-be ovulation. This idea could be supported by the aberrant redox gene expression patterns and disrupted redox homeostasis in prepubertal porcine oocytes that lead to a decrease in developmental competence (61). GLRX3 has a conserved function in protecting cells against oxidative stress and its deletion in mice causes early embryonic lethality, which may be associated with defective cell cycle progression (62). Our results highlighted the increase of GLRX3 mRNA in DistUt, perhaps relevant for the preparation of endometrium receptivity.

Concerning CASPs, CASP-2, 8, 9, 10, and 12 are classed as initiators or pro-apoptotic caspases, whereas Casp-3, 6, and 7 are classed as downstream effector caspases that are cleaved and activated by these initiators (30). In the murine oviduct, the CASP3, CASP6, and CASP12 were detected through the estrous cycle, as a plausible indicator of a certain level of basal apoptosis in this anatomical region (63). Results

10.3389/fendo.2023.1042176

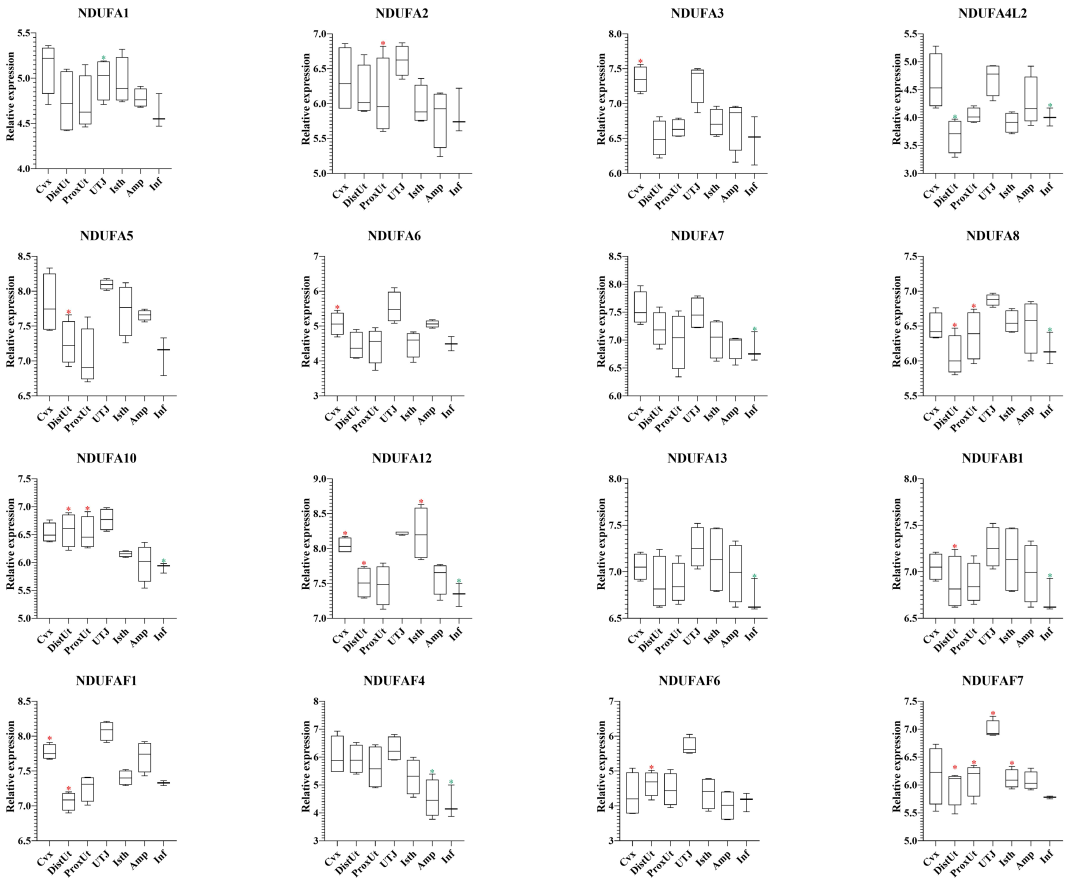


FIGURE 7
Summary of significant differential expression of NADH dehydrogenase (ubiquinone) oxidoreductases (NDUF) Part 2. Box-plot representation of the log2 signal intensity of the selected transcripts by tissue, Cvx, cervix; DistUt, distal uterus; ProxUt, proximal uterus; UTJ, utero-tubal junction; Isth, isthmus; Amp, ampulla; and Inf, infundibulum. The red color "*" represents a relative increase relative to the negative control ($p < 0.05$), whereas the green color "*" represents a relative decrease relative to the negative control ($p < 0.05$). NDUFA1, NDUF 1 alpha subcomplex, subunit 1; NDUFA2, NDUF 1 alpha subcomplex, ubunit 2; NDUFA3, NDUF 1 alpha subcomplex, subunit 3; NDUFA4L2, 1 alpha subcomplex, 4-like 2; NDUFA5, NDUF 1 alpha subcomplex, subunit 5; NDUFA6, NDUF 1 alpha subcomplex, subunit 6; NDUFA7, NDUF 1 alpha subcomplex, subunit 7; NDUFA8, NDUF 1 alpha subcomplex, subunit 8; NDUFA10, NDUF 1 alpha subcomplex, subunit 10; NDUFA12, NDUF 1 alpha subcomplex, subunit 12; NDUFA13, NDUF 1 alpha subcomplex, subunit 13; NDUFAB1, NDUF 1 alpha subcomplex, subunit 5; NDUFAF1, NDUF 1 alpha subcomplex assembly factor 1; NDUFAF4, NDUF complex assembly factor 4; NDUFAF6, NDUF complex assembly factor 6; and NDUFAF7, NDUF complex assembly factor 7.

from CASP3 in our study showed an interesting pattern, with the UTJ being the only one not showing downregulation on CASP3 expression in response to mating. We could hypothesize that the caspase activity through the female genital tract in response to the travel of spermatozoa is repressed, avoiding a high mobilization of macrophages, at least at this particular period. Interestingly, the dietary supplementation of CAT in sows leads to a dramatic reduction of CASP3 and CASP9 (64) which partially agrees with our results on CASP3, but not in the case of CASP9, since we found an increase in CASP9 in DistUt. This overexpression is consistent with the results in CASP2 and CASP8AP2, all of the apoptosis initiators, and may be involved in sperm clearance in the endometrium for later preparation for implantation. Previous studies have mapped the increase of mRNA abundance of CASP1, with a pro-inflammatory function, up to 18 days of gestation (65). Our results agree with the low level found by authors at 0 and 5 days, confirming the relevance of these low levels for the later reproductive success in this species. Since CASP1 acts by increasing the level of IL-1β, the lower levels obtained in our study relative to the un-mated control make sense. Finally, expression of mRNA for CASP14 was higher in oviducts collected from mice at dioestrus than metaestrus (63), and our results, during the periovulatory phase, showed an increase of expression in ProxUt, whereas showing a downregulation in the Cvx.

Oxidative phosphorylation pathway Complex I includes several subunits, and 28 out of 37 NDUFs included in the present study showed significant expression differences. Interestingly, 14 out of 28 NDUFs were upregulated in DistUt. The pattern of downregulation was heterogeneously distributed across tissues, being the Inf the main tissue presenting this repression in 15 out of 28 NDUFs. NADH: ubiquinone oxidoreductase (complex I) is the first of three large enzyme complexes located in the inner mitochondrial membrane which form the electron transport chain that carries electrons from NADH to molecular oxygen during oxidative phosphorylation (31). Antioxidant addition during *in vitro* culture of bovine embryos reduced the NDUFA2, improving resilience to stress (66). In contrast, maybe due to the matrix differences, our results showed an increase of NDUFA2 in DistUt, as well as so many other NDUFs analyzed in the present study. NDUFA8 was downregulated in ovine oocytes matured in the presence of lipopolysaccharide (67). However, whether there is a relation between

10.3389/fendo.2023.1042176

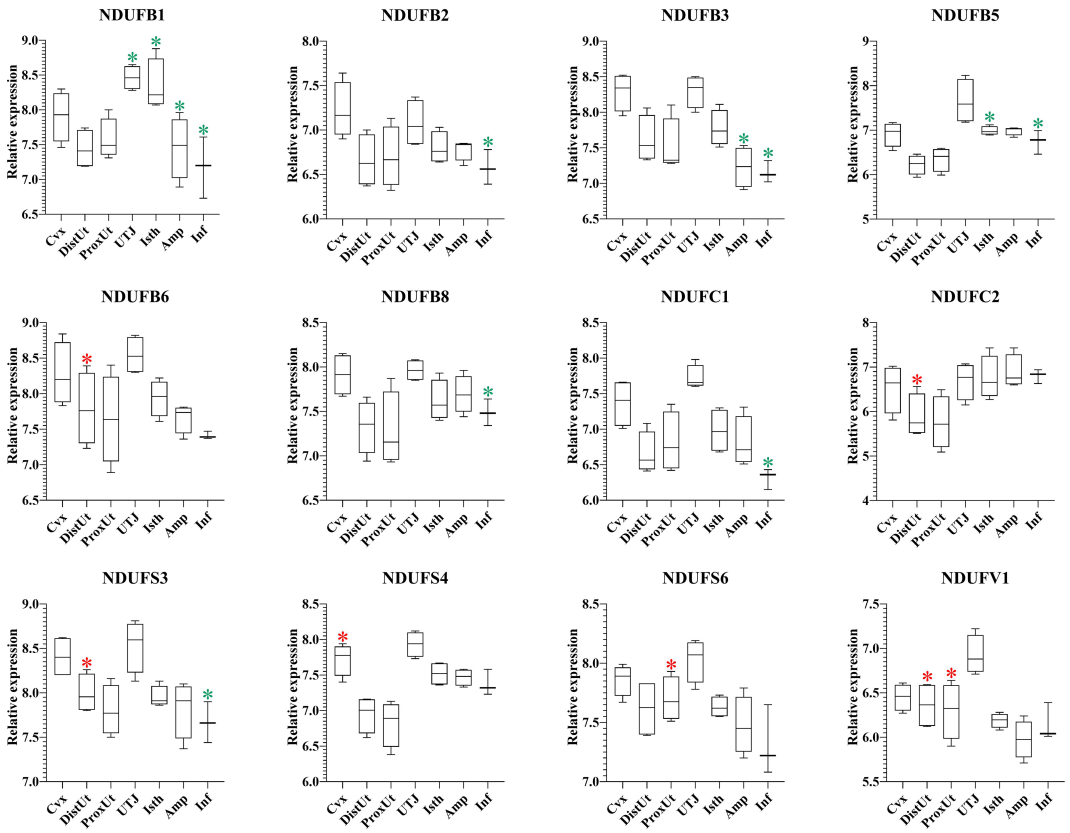


FIGURE 8
Summary of significant differential expression of NADH dehydrogenase (ubiquinone) oxidoreductases (NDUF) Part 2. Box-plot representation of the log2 signal intensity of the selected transcripts by tissue, Cvx, cervix; DistUt, distal uterus; ProxUt, proximal uterus; UTJ, utero-tubal junction; Isth, isthmus; Amp, ampulla; and Inf, infundibulum. The red color "*" represents a relative increase relative to the negative control ($p < 0.05$), whereas the green color "*" represents a relative decrease relative to the negative control ($p < 0.05$). NDUFB1, NDUF 1 beta subcomplex, subunit 1; NDUFB2, NDUF 1 beta subcomplex, ubunit 2; NDUFB3, NDUF 1 beta subcomplex, subunit 3; NDUFB5, 1 beta subcomplex, subunit 5; NDUFB6, NDUF 1 beta subcomplex, subunit 6; NDUBA8, NDUF 1 beta subcomplex, subunit 8; NDUFC1, NDUF 1 subunit C1; NDUFC2, NDUF 1 subunit C2; NDUFS3, NDUF iron-sulfur protein 3; NDUFS4, NDUF iron-sulfur protein 4; NDUFS6, NDUF iron-sulfur protein 6; and NDUFV1, NDUF flavoprotein 1.

the downregulation of its expression in Inf and upregulation in both DistUt and ProxUt in our study requires further research. NDUFAB1 is a promotor of ovarian follicle development by stimulating granulosa cell proliferation in hens (68) and establishing a direct link with the increased capacity in egg-laying production. We observed a decrease in the Inf and an increase in DistUt, the latter may be highlighting the necessity of a decrease in the apoptotic activity to the allowance of sperm travel towards the uterus. NDUFAF1 interacts to form the core mitochondrial respiratory complex I assembly complex (69). NDUFAF1 is indispensable for activation-induced IL-2 and IL-4 (70), both necessary for the establishment of cellular immunity memory. NDFUAF7 is essential for complex I assembly and early vertebrate embryogenesis (71), and it seems relevant in our experimental design, being upregulated in DistUt and ProxUt, as well as UTJ and Isth. In addition, the antioxidant curcumin, administrated orally in mice, can increase the abundance of several proteins, including the ones involved in protein phosphorylation, namely, NDUFB3, NDUFAB1, and NDUFA7 (72). Our results showed a significant decrease of these three genes in the Inf, suggesting the necessity of a controlled downregulation in this specific tissue may be related to the soon reception of the oocyte; however, further mechanistic studies are needed. Moreover, NDUFB3 plays a pivotal role in recurrent pregnancy loss in humans when it is upregulated (73). In addition, NDUFB3 has been identified as a candidate gene to climate adaptation in cattle (74) and plays an important role in development (75). Our results showed a reduced expression of NDUFB3 in Amp and Inf. As for NDUFB6, previous results in rats have demonstrated that NDUFB6 decreased in oocytes from preovulatory exposure to a low-protein diet compared to the control diet (76). Thus, its overexpression in DistUt may suggest a necessary role of this gene in the complex I formation at this level. The expression of NDUFB8 was reduced in an experimental model of androgen excess in rats (77), and these changes were also confirmed in a previous study on maternal nutrient restriction in baboon-cultured skin fibroblast (78). Thus, despite our results showing a decrease at the Inf level, which could be read as damage in the mitochondrial structure, it seems that the decrease could play another role at the Inf level, yet not fully understood. NDUFC1 is a key activator of cell proliferation and apoptosis (79). Therefore, our results showing downregulation in Inf could be linked to a low necessity of this relevant process in this tissue. Reduction of NDUFC2 was associated with mitochondrial impairment (80) and the overexpression in DistUt suggests a relevant function in this specific

tissue and at the periovulatory stage. NDUFS4 was reduced in advanced antral atretic follicles in the porcine (81). In contrast, our results confirmed an increase at the Cvx level, suggesting a healthy and relevant overexpression in this tissue. NDUFS6 was detected downregulated in adult mesenchymal stem cells (82), as a measurement of senescence. Our results suggested a specific role in the ProxUt, being downregulated after mating. Finally, maternal nutrition modulates fetal development in gilts, with the non-treated with high-energy diet sows overexpressing the NDUFV1 genes, which are involved in energy metabolism (69, 83). Our results, downregulation in DistUt and ProxUt, suggest the endometrium plays a relevant function in relation to the oxidative phosphorylation status.

Previous studies from our group (6–9, 84) highlighted the presence of a complex immunomodulatory expression of several genes in response to mating in pigs. In particular, semen-induced downregulation of cytokine, interleukine, interferon-gamma, and JAK/STAT pathways in the sperm reservoir (6). In particular, the anti-inflammatory IL-10 was upregulated in the UTJ (84), and the pro-inflammatory cytokine CXCL8 was downregulated in the cervix and proximal uterus (8). Even heat sock proteins, associated with several controlling physiological aspects, were downregulated in the female tract in response to mating (9). Considering all the aforementioned results, the present study specifically focused on oxidative-reductive transcripts, to discover the differential effect of mating on the expression of key targets of possibly designable nutritional additives. Maternal nutrition triggers thousands of different expression patterns and affects several different genes and complex pathways. Increasing our knowledge of the expression status in sows, before and after mating, is a relevant starting point of reproductive success and could lead to the development of new additives or procedures to increase the preparation of the female genital tract to succeed in decreasing embryo losses, which is a major concern in this species. Our results showed that a fine tune of the mRNA abundance was triggered during the pre-ovulatory stage, a period dominated by estrogenic influence, particularly by the upregulation of SOD1, SOD 2, CASP2, CASPAP2, CASP9, PRDX1, and PRDX3. These transcripts can become plausible targets when designing diet contents leading to an increase in the oxidative-reductive enzymes.

Data availability statement

The data presented in the study are deposited in the Harvard Dataverse public repository, accession number DVN/7U58F9_2022 (link: https://doi.org/10.7910/DVN/7U58F9).

Ethics statement

The animal study was reviewed and approved by Animal handling and experiments were carried out in accordance to the European Community Directive 2010/63/EU, 22/09/2010, and current Swedish legislation (SJVFS 2017:40). The study was accepted by the Regional Committee for Ethical Approval of Animal Experiments (Linköpings Djurförsöksetiska nämnd, Linköping, Sweden). Permits number 75-12 (10/02/2012), ID1400 (02/02/2018), and Dnr 03416-2020 (26/03/2020).

Author contributions

All authors provided contributions to the study conception and design, acquisition of data or analysis and interpretation of data, as well as drafting the article or revising it critically for important intellectual content, and final approval of the version to be published.

Funding

This research was funded by the Research Council FORMAS, Stockholm (Project 2017-00946 and Project 2019-00288) and by the Grant RyC2020-028715-I, PID2019-108320RJ-I00, IJCI-2015-24380 funded by MCIN/AEI/10.13039/501100011033 (Spain) and FEDER funds (EU).

Acknowledgments

The authors of this manuscript thank Annette Molbaek and Åsa Schippert from the Genomics Core Facility at LiU for their assistance with microarrays. To Jaume Gardela and Mateo Ruiz for their kind assistance with the graphical content. To QualiSperm (AKYmed, Cheseaux-sur-Lausanne, Switzerland) for assisting with computerized sperm motility and kinetics assessment.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1042176/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Principal Component Analysis of the target oxidative-reductive genes across female reproductive tissues. Cvx, cervix; DistUt, distal uterus; ProxUt, proximal uterus; UTJ, utero-tubal junction; Isth, isthmus; Amp, ampulla; and Inf, infundibulum.

SUPPLEMENTARY FIGURE 2
Hierarchical Clustering Analysis of the target oxidative-reductive genes across female reproductive tissues.

References

1. Lu J, Huang J, Zhao S, Xu W, Chen Y, Li Y, et al. FOXO1 is a critical switch molecule for autophagy and apoptosis of sow endometrial epithelial cells caused by oxidative stress. *Oxid Med Cell Longev* (2021) 2021:24. doi: 10.1155/2021/1172273

2. Claus R. Physiological role of seminal components in the reproductivetract of the female pig. *Biosci Proc* (2020) 13:117–31. doi: 10.1530/biosciprocs.13.009

3. Rodriguez-Martinez H, Petroni A, Einarsson S, Kindahl H. Concentrations of prostaglandin F2α in the pig oviductal fluid. *Prostaglandins* (1983) 25:413–24. doi: 10.1016/0090-6980(83)90045-X

4. Rodriguez-Martinez H, Nicander L, Viring S, Einarsson S, Larsson K. Ultrastructure of the uterotubal junction in preovulatory pigs. *Anat Histol Embryol* (1990) 19:16–36. doi: 10.1111/j.1439-0264.1990.tb00875.x

5. Alvarez-Rodriguez M, Martinez CA, Wright D, Rodriguez-Martinez H. Does the act of copulation per se, without considering seminal deposition, change the expression of genes in the porcine female genital tract? *Int J Mol Sci* (2020) 21:1–16. doi: 10.3390/ijms21155477

6. Álvarez-Rodríguez M, Martinez CA, Wright D, Rodríguez-Martinez H. The role of semen and seminal plasma in inducing large-scale genomic changes in the female porcine peri-ovulatory tract. *Sci Rep* (2020) 10:5061. doi: 10.1038/s41598-020-60810-z

7. Alvarez-Rodriguez M, Atikuzzaman M, Venhoranta H, Wright D, Rodriguez-Martinez H. Expression of immune regulatory genes in the porcine internal genital tract is differentially triggered by spermatozoa and seminal plasma. *Int J Mol Sci* (2019) 20:513. doi: 10.3390/ijms20030513

8. Gardela J, Ruiz-Conca M, Martinez CA, Wright D, López-Béjar M, Rodriguez-Martinez H, et al. The expression of cold-inducible RNA-binding protein mrna in sow genital tract is modulated by natural mating, but not by seminal plasma. *Int J Mol Sci* (2020) 21:1–23. doi: 10.3390/ijms21155333

9. Ruiz-Conca M, Gardela J, Martínez CA, Wright D, López-Bejar M, Rodríguez-Martínez H, et al. Natural mating differentially triggers expression of glucocorticoid receptor (Nr3c1)-related genes in the preovulatory porcine female reproductive tract. *Int J Mol Sci* (2020) 21:1–17. doi: 10.3390/ijms21124437

10. Knox R. 124 factors influencing follicle development in gilts and sows and management strategies used to regulate growth for control of estrus and ovulation. *J Anim Sci* (2018) 96:343. doi: 10.1093/jas/sky404.755

11. Signoret JP, Du Mesnil du Buisson F, Mauléon P. Effect of mating on the onset and duration of ovulation in the sow. *J Reprod Fertil* (1972) 31:327–30. doi: 10.1530/JRF.0.0310327

12. Rodriguez-Martinez H, Martinez EA, Calvete JJ, Peña Vega FJ, Roca J. Seminal plasma: relevant for fertility? *Int J Mol Sci* (2021) 22:4368. doi: 10.3390/ijms22094368

13. Robertson SA, Martin GB. Perspective: re-defining "Pheromone" in a mammalian context to encompass seminal fluid. *Front Vet Sci* (2021) 8:819246. doi: 10.3389/fvets.2021.819246

14. Rodriguez-Martinez H, Saravia F, Wallgren M, Tienthai P, Johannisson A, Vazquez JM, et al. Boar spermatozoa in the oviduct. *Theriogenology* (2005) 63:514–35. doi: 10.1016/j.theriogenology.2004.09.028

15. Rodríguez-Martínez H, Kvist U, Saravia F, Wallgren M, Johannisson A, Sanz L, et al. The physiological roles of the boar ejaculate. *Soc Reprod Fertil Suppl* (2009) 66:1–21. doi: 10.1530/biosciprocs.18.0001

16. Hunter RHF. Sperm transport and reservoirs in the pig oviduct in relation to the time of ovulation. *J Reprod Fertil* (1981) 63:109–17. doi: 10.1530/JRF.0.0630109

17. Rodriguez-Martinez H, Einarsson S. Influence of prostaglandins on the spontaneous motility of pig oviducts. *Anim Reprod Sci* (1985) 8:259–79. doi: 10.1016/0378-4320(85)90031-4

18. Hunter RHF. Pre-ovulatory arrest and peri-ovulatory redistribution of competent spermatozoa in the isthmus of the pig oviduct. *J Reprod Fertil* (1984) 72:203–11. doi: 10.1530/jrf.0.0720203

19. Mburu JN, Einarsson S, Lundeheim N, Rodriguez-Martinez H. Distribution, number and membrane integrity of spermatozoa in the pig oviduct in relation to spontaneous ovulation. *Anim Reprod Sci* (1996) 45:109–21. doi: 10.1016/S0378-4320(96)01566-7

20. Tienthai P, Johannisson A, Rodríguez-Martínez H. Sperm capacitation in the porcine oviduct. *Anim Reprod Sci* (2004) 80:131–46. doi: 10.1016/S0378-4320(03)00134-9

21. Rodriguez-Martinez H. Role of the oviduct in sperm capacitation. *Theriogenology* (2007) 68 Suppl 1:S138–46. doi: 10.1016/j.theriogenology.2007.03.018

22. Ded L, Dostalova P, Dorosh A, Dvorakova-Hortova K, Peknicova J. Effect of estrogens on boar sperm capacitation *in vitro*. *Reprod Biol Endocrinol RBE* (2010) 8:87. doi: 10.1186/1477-7827-8-87

23. Jarrett S, Ashworth CJ. The role of dietary fibre in pig production, with a particular emphasis on reproduction. *J Anim Sci Biotechnol* (2018) 9:59. doi: 10.1186/S40104-018-0270-0

24. Sun S, Meng Q, Bai Y, Cao C, Li J, Cheng B, et al. Lycopene improves maternal reproductive performance by modulating milk composition and placental antioxidative and immune status. *Food Funct* (2021) 12:12448–67. doi: 10.1039/D1FO01595H

25. Huang S, Wu Z, Huang Z, Hao X, Zhang L, Hu C, et al. Maternal supply of cysteamine alleviates oxidative stress and enhances angiogenesis in porcine placenta. *J Anim Sci Biotechnol* (2021) 12:91. doi: 10.1186/S40104-021-00609-8

26. Xu M, Che L, Gao K, Wang L, Yang X, Wen X, et al. Effects of dietary taurine supplementation to gilts during late gestation and lactation on offspring growth and oxidative stress. *Animals* (2019) 9:220. doi: 10.3390/ANI9050220

27. Meng Q, Sun S, Bai Y, Luo Z, Li Z, Shi B, et al. Effects of dietary resveratrol supplementation in sows on antioxidative status, myofiber characteristic and meat quality of offspring. *Meat Sci* (2020) 167:108176. doi: 10.1016/J.MEATSCI.2020.108176

28. Hayyan M, Hashim MA, Alnashef IM. Superoxide ion: generation and chemical implications. *Chem Rev* (2016) 116:3029–85. doi: 10.1021/acs.chemrev.5b00407

29. Vazquez-Medina JP. Redox signaling and the onset of the inflammatory cascade. Emerging roles of Nutraceuticals and Functional Foods in Immune Support. *Immunity and Inflammation in Health and Disease* (2018) 37–42. doi: 10.1016/B978-0-12-805417-8.00003-2

30. Shi Y. Caspase activation: revisiting the induced proximity model. *Cell* (2004) 117:855–8. doi: 10.1016/J.CELL.2004.06.007

31. Nicolaou KC, Pfefferkorn JA, Schuler F, Roecker AJ, Cao GQ, Casida JE. Combinatorial synthesis of novel and potent inhibitors of NADH:ubiquinone oxidoreductase. *Chem Biol* (2000) 7:979–92. doi: 10.1016/S1074-5521(00)00047-8

32. Galemou Yoga E, Schiller J, Zickermann V. Ubiquinone binding and reduction by complex I–open questions and mechanistic implications. *Front Chem* (2021) 9:672851. doi: 10.3389/fchem.2021.672851

33. Alvarez-rodriguez M, Martinez C, Wright D, Barranco I, Roca J, Rodriguez-martinez H. The transcriptome of pig spermatozoa, and its role in fertility. *Int J Mol Sci* (2020) 21:1572. doi: 10.3390/ijms21051572

34. Mi H, Muruganujan A, Casagrande JT, Thomas PD. Large-Scale gene function analysis with the PANTHER classification system. *Nat Protoc* (2013) 8:1551–66. doi: 10.1038/nprot.2013.092

35. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* (2000) 28:27–30. doi: 10.1093/nar/28.1.27

36. Metsalu T, Vilo J. ClustVis: a web tool for visualizing clustering of multivariate data using principal component analysis and heatmap. *Nucleic Acids Res* (2015) 43:W566–70. doi: 10.1093/NAR/GKV468

37. Martinez CA, Rodriguez-Martinez H. Context is key: maternal immune responses to pig allogeneic embryos. *Mol Reprod Dev* (2022) 89:316–24. doi: 10.1002/mrd.23624

38. Cooke PS, Nanjappa MK, Ko C, Prins GS, Hess RA. Estrogens in male physiology. *Physiol Rev* (2017) 97:995–1043. doi: 10.1152/physrev.00018.2016

39. Sahlin L, Rodriguez-Martinez H, Stanchev P, Dalin A -M, Norstedt G, Eriksson H. Regulation of the uterine expression of messenger ribonucleic acids encoding the oestrogen receptor and IGF–I peptides in the pig uterus. *J Vet Med Ser A* (1990) 37:795–800. doi: 10.1111/j.1439-0442.1990.tb00974.x

40. Rago V, Aquila S, Panza R, Carpino A. Cytochrome P450arom, androgen and estrogen receptors in pig sperm. *Reprod Biol Endocrinol* (2007) 5:23. doi: 10.1186/1477-7827-5-23

41. Dostalova P, Zatecka E, Dvorakova-Hortova K. Of oestrogens and sperm: a review of the roles of oestrogens and oestrogen receptors in Male reproduction. *Int J Mol Sci* (2017) 18:E904. doi: 10.3390/ijms18050904

42. Vicente Carrillo A. *Sperm membrane channels, receptors and Kinematics : using boar spermatozoa for drug toxicity screening* (2016). Available at: http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-131862 (Accessed September 19, 2022).

43. Barranco I, Rubio CP, Tvarijonaviciute A, Rodriguez-Martinez H, Roca J. Measurement of oxidative stress index in seminal plasma can predict *In vivo* fertility of liquid-stored porcine artificial insemination semen doses. *Antioxid Basel Switz* (2021) 10:1203. doi: 10.3390/antiox10081203

44. Park HJ, Kim B, Koo DB, Lee DS. Peroxiredoxin 1 controls ovulation and ovulated cumulus-oocyte complex activity through TLR4-derived ERK1/2 signaling in mice. *Int J Mol Sci* (2021) 22:9437. doi: 10.3390/IJMS22179437

45. Morita K, Tokoro M, Hatanaka Y, Higuchi C, Ikegami H, Nagai K, et al. Peroxiredoxin as a functional endogenous antioxidant enzyme in pronuclei of mouse zygotes. *J Reprod Dev* (2018) 64:161–71. doi: 10.1262/JRD.2018-005

46. Shi H, Liu J, Zhu P, Wang H, Zhao Z, Sun G, et al. Expression of peroxiredoxins in the human testis, epididymis and spermatozoa and their role in preventing H2O2-induced damage to spermatozoa. *Folia Histochem Cytobiol* (2018) 56:141–50. doi: 10.5603/FHC.A2018.0019

47. Liu Y, O'flaherty C. *In vivo* oxidative stress alters thiol redox status of peroxiredoxin 1 and 6 and impairs rat sperm quality. *Asian J Androl* (2017) 19:73–9. doi: 10.4103/1008-682X.170863

48. Deroo BJ, Hewitt SC, Peddada SD, Korach KS. Estradiol regulates the thioredoxin antioxidant system in the mouse uterus. *Endocrinology* (2004) 145:5485–92. doi: 10.1210/EN.2004-0471

49. Shibata E, Nanri H, Ejima K, Araki M, Fukuda J, Yoshimura K, et al. Enhancement of mitochondrial oxidative stress and up-regulation of antioxidant

protein peroxiredoxin III/SP-22 in the mitochondria of human pre-eclamptic placentae. *Placenta* (2003) 24:698–705. doi: 10.1016/S0143-4004(03)00083-3

50. Hammond ER, Stewart B, Peek JC, Shelling AN, Cree LM. Assessing embryo quality by combining non-invasive markers: early time-lapse parameters reflect gene expression in associated cumulus cells. *Hum Reprod Oxf Engl* (2015) 30:1850–60. doi: 10.1093/HUMREP/DEV121

51. Ryu D-Y, Pang W-K, Rahman MS, Park Y-J, Pang M-G. Peroxiredoxin 4 as potential fertility marker in boars. *Res Sq* (2020). doi: 10.21203/rs.3.rs-19365/v1

52. Pang WK, Kang S, Ryu DY, Rahman MS, Park YJ, Pang MG. Optimization of sperm RNA processing for developmental research. *Sci Rep* (2020) 10:11606. doi: 10.1038/S41598-020-68486-1

53. Poh QH, Rai A, Carmichael II, Salamonsen LA, Greening DW. Proteome reprogramming of endometrial epithelial cells by human trophectodermal small extracellular vesicles reveals key insights into embryo implantation. *Proteomics* (2021) 21:2000210. doi: 10.1002/PMIC.202000210

54. Singh M, Mollier RT, Pongener N, Bordoloi LJ, Kumar R, Chaudhary JK, et al. Linseed oil in boar's diet during high temperature humidity index (THI) period improves sperm quality characteristics, antioxidant status and fatty acid composition of sperm under hot humid sub-tropical climate. *Theriogenology* (2022) 189:127–36. doi: 10.1016/J.THERIOGENOLOGY.2022.06.012

55. Guo G, Zhou T, Ren F, Sun J, Deng D, Huang X, et al. Effect of maternal catalase supplementation on reproductive performance, antioxidant activity and mineral transport in sows and piglets. *Anim Open Access J MDPI* (2022) 12:828. doi: 10.3390/ANI12070828

56. Alvarez-Rodriguez M, Martinez CA, Roca J, Rodriguez-Martinez H. mRNA expression of oxidative-reductive proteins in boars with documented different fertility can identify relevant prognostic biomarkers. *Res Vet Sci* (2021) 141:195–202. doi: 10.1016/j.rvsc.2021.10.022

57. Su G, Zhao J, Luo G, Xuan Y, Fang Z, Lin Y, et al. Effects of oil quality and antioxidant supplementation on sow performance, milk composition and oxidative status in serum and placenta. *Lipids Health Dis* (2017) 16:107. doi: 10.1186/S12944-017-0494-6

58. Lappas M, Mittion A, Permezel M. In response to oxidative stress, the expression of inflammatory cytokines and antioxidant enzymes are impaired in placenta, but not adipose tissue, of women with gestational diabetes. *J Endocrinol* (2010) 204:75–84. doi: 10.1677/JOE-09-0321

59. Lim J, Luderer U. Oxidative damage increases and antioxidant gene expression decreases with aging in the mouse ovary. *Biol Reprod* (2011) 84:775–82. doi: 10.1095/BIOLREPROD.110.088583

60. Silva E, Greene AF, Strauss K, Herrick JR, Schoolcraft WB, Krisher RL. Antioxidant supplementation during *in vitro* culture improves mitochondrial function and development of embryos from aged female mice. *Reprod Fertil Dev* (2015) 27:975–83. doi: 10.1071/RD14474

61. Yuan Y, Wheeler MB, Krisher RL. Disrupted redox homeostasis and aberrant redox gene expression in porcine oocytes contribute to decreased developmental competence. *Biol Reprod* (2012) 87:1–10. doi: 10.1095/BIOLREPROD.112.099952

62. Cheng NH, Zhang W, Chen WQ, Jin J, Cui X, Butte NF, et al. A mammalian monothiol glutaredoxin, Grx3, is critical for cell cycle progression during embryogenesis. *FEBS J* (2011) 278:2525–39. doi: 10.1111/J.1742-4658.2011.08178.X

63. Jeoung M, Bridges PJ. Cyclic regulation of apoptotic gene expression in the mouse oviduct. *Reprod Fertil Dev* (2011) 23:638–44. doi: 10.1071/RD11011

64. Sun X, Piao L, Jin H, Margarette K, Nogoy C, Zhang J, et al. Effects of dietary supplementation of glucose oxidase, catalase, or both on reproductive performance, oxidative stress, fecal microflora and apoptosis in multiparous sows. *Anim Biosci* (2022) 35:75–86. doi: 10.5713/AB.20.0839

65. Ashworth MD, Ross JW, Stein DR, White FJ, DeSilva UW, Geisert RD. Endometrial caspase 1 and interleukin-18 expression during the estrous cycle and peri-implantation period of porcine pregnancy and response to early exogenous estrogen administration. *Reprod Biol Endocrinol RBE* (2010) 8:33. doi: 10.1186/1477-7827-8-33

66. Chowdhury MMR, Mesalam A, Khan I, Joo MD, Lee KL, Xu L, et al. Improved developmental competence in embryos treated with lycopene during *in vitro* culture system. *Mol Reprod Dev* (2018) 85:46–61. doi: 10.1002/MRD.22937

67. Rasekhi M, Mohammadi-Sangcheshmeh A, Daliri M, Bakhtiarizadeh M, Shariati V, Rahimi M, et al. Transcriptional profile of ovine oocytes matured under lipopolysaccharide treatment *in vitro*. *Theriogenology* (2020) 157:70–8. doi: 10.1016/J.THERIOGENOLOGY.2020.07.034

68. Sun X, Chen X, Zhao J, Ma C, Yan C, Liswaniso S, et al. Transcriptome comparative analysis of ovarian follicles reveals the key genes and signaling pathways implicated in hen egg production. *BMC Genomics* (2021) 22:899. doi: 10.1186/S12864-021-08213-W

69. Xia C, Lou B, Fu Z, Mohsen AW, Shen AL, Vockley J, et al. Molecular mechanism of interactions between ACAD9 and binding partners in mitochondrial respiratory complex I assembly. *iScience* (2021) 24:103153. doi: 10.1016/J.ISCI.2021.103153

70. Kamiński MM, Sauer SW, Klemke C-D, Süss D, Okun JG, Krammer PH, et al. Mitochondrial reactive oxygen species control T cell activation by regulating IL-2 and IL-4 expression: mechanism of ciprofloxacin-mediated immunosuppression. *J Immunol Baltim Md 1950* (2010) 184:4827–41. doi: 10.4049/JIMMUNOL.0901662

71. Zurita Rendón O, Silva Neiva L, Sasarman F, Shoubridge EA. The arginine methyltransferase NDUFAF7 is essential for complex I assembly and early vertebrate embryogenesis. *Hum Mol Genet* (2014) 23:5159–70. doi: 10.1093/HMG/DDU239

72. Silva-Gaona OG, Hernández-Ortiz M, Vargas-Ortiz K, Ramírez-Emiliano J, Garay-Sevilla ME, Encarnación-Guevara S, et al. Curcumin prevents proteins expression changes of oxidative phosphorylation, cellular stress response, and lipid metabolism proteins in liver of mice fed a high-fructose diet. *J Proteomics* (2022) 263:104595. doi: 10.1016/J.JPROT.2022.104595

73. Yin XJ, Hong W, Tian FJ, Li XC. Proteomic analysis of decidua in patients with recurrent pregnancy loss (RPL) reveals mitochondrial oxidative stress dysfunction. *Clin Proteomics* (2021) 18:9. doi: 10.1186/S12014-021-09312-2

74. Flori L, Moazami-Goudarzi K, Alary V, Araba A, Boujenane I, Boushaba N, et al. A genomic map of climate adaptation in Mediterranean cattle breeds. *Mol Ecol* (2019) 28:1009–29. doi: 10.1111/MEC.15004

75. Alston CL, Howard C, Oláhová M, Hardy SA, He L, Murray PG, et al. A recurrent mitochondrial p.Trp22Arg NDUFB3 variant causes a distinctive facial appearance, short stature and a mild biochemical and clinical phenotype. *J Med Genet* (2016) 53:634–41. doi: 10.1136/JMEDGENET-2015-103576

76. Schutt AK, Blesson CS, Hsu JW, Valdes CT, Gibbons WE, Jahoor F, et al. Preovulatory exposure to a protein-restricted diet disrupts amino acid kinetics and alters mitochondrial structure and function in the rat oocyte and is partially rescued by folic acid. *Reprod Biol Endocrinol RBE* (2019) 17:12. doi: 10.1186/S12958-019-0458-Y

77. Song L, Yu J, Zhang D, Li X, Chen L, Cai Z, et al. Androgen excess induced mitochondrial abnormality in ovarian granulosa cells in a rat model of polycystic ovary syndrome. *Front Endocrinol* (2022) 13:789008. doi: 10.3389/FENDO.2022.789008

78. Salmon AB, Dorigatti J, Huber HF, Li C, Nathanielsz PW. Maternal nutrient restriction in baboon programs later-life cellular growth and respiration of cultured skin fibroblasts: a potential model for the study of aging-programming interactions. *GeroScience* (2018) 40:269–78. doi: 10.1007/S11357-018-0024-0

79. Xu L, Chen X, Jiang H, Xu J, Wang L, Sun Y. NDUFC1 is upregulated in gastric cancer and regulates cell proliferation, apoptosis, cycle and migration. *Front Oncol* (2021) 11:709044. doi: 10.3389/FONC.2021.709044

80. Raffa S, Chin XLD, Stanzione R, Forte M, Bianchi F, Cotugno M, et al. The reduction of NDUFC2 expression is associated with mitochondrial impairment in circulating mononuclear cells of patients with acute coronary syndrome. *Int J Cardiol* (2019) 286:127–33. doi: 10.1016/J.IJCARD.2019.02.027

81. Meng L, Wu Z, Zhao K, Tao J, Chit T, Zhang S, et al. Transcriptome analysis of porcine granulosa cells in healthy and atretic follicles: role of steroidogenesis and oxidative stress. *Antioxid Basel Switz* (2020) 10:1–17. doi: 10.3390/ANTIOX10010022

82. Zhang Y, Guo L, Han S, Chen L, Li C, Zhang Z, et al. Adult mesenchymal stem cell ageing interplays with depressed mitochondrial Ndufs6. *Cell Death Dis* (2020) 11:1075. doi: 10.1038/S41419-020-03289-W

83. Che L, Yang ZG, Xu MM, Xu SY, Che LQ, Lin Y, et al. Maternal nutrition modulates fetal development by inducing placental efficiency changes in gilts. *BMC Genomics* (2017) 18:213. doi: 10.1186/S12864-017-3601-1

84. Gardela J, Ruiz-Conca M, Wright D, López-Béjar M, Martínez CA, Rodríguez-Martínez H, et al. Semen modulates cell proliferation and differentiation-related transcripts in the pig peri-ovulatory endometrium. *Biology* (2022) 11:616. doi: 10.3390/biology11040616

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 25 July 2023
DOI 10.3389/fendo.2023.1201198

OPEN ACCESS

EDITED BY
Dipak Kumar Sahoo,
Iowa State University, United States

REVIEWED BY
Ahmad Perwez,
Case Western Reserve University, United States
Suvranil Ghosh,
University of Texas Southwestern Medical Center, United States

*CORRESPONDENCE
Vinod K. Nelson
vinod.kumar457@gmail.com
Niraj Kumar Jha
niraj.jha@sharda.ac.in
Petr Slama
petr.slama@mendelu.cz
Shubhadeep Roychoudhury
shubhadeep1@gmail.com

†These authors have contributed equally to this work and share first authorship

‡These authors have contributed equally to this work and share second authorship

RECEIVED 06 April 2023
ACCEPTED 28 June 2023
PUBLISHED 25 July 2023

CITATION
Nelson VK, Nuli MV, Mastanaiah J, Saleem T. S. M, Birudala G, Jamous YF, Alshargi O, Kotha KK, Sudhan HH, Mani RR, Muthumanickam A, Niranjan D, Jain NK, Agrawal A, Jadon AS, Mayasa V, Jha NK, Kolesarova A, Slama P and Roychoudhury S (2023) Reactive oxygen species mediated apoptotic death of colon cancer cells: therapeutic potential of plant derived alkaloids. *Front. Endocrinol.* 14:1201198. doi: 10.3389/fendo.2023.1201198

Reactive oxygen species mediated apoptotic death of colon cancer cells: therapeutic potential of plant derived alkaloids

Vinod K. Nelson[1*†], Mohana Vamsi Nuli[1†], Juturu Mastanaiah[2‡], Mohamed Saleem T. S.[3‡], Geetha Birudala[4], Yahya F. Jamous[5], Omar Alshargi[3], Kranthi Kumar Kotha[6], Hari Hara Sudhan[1], Ravishankar Ram Mani[7], Alagusundaram Muthumanickam[8], Divya Niranjan[8], Nem Kumar Jain[8], Ankur Agrawal[8], Arvind Singh Jadon[9], Vinyas Mayasa[10], Niraj Kumar Jha[11,12,13,14*], Adriana Kolesarova[15], Petr Slama[16*] and Shubhadeep Roychoudhury[17*]

[1]Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, India, [2]Department of Pharmacology, Balaji College of Pharmacy, Anantapur, India, [3]College of Pharmacy, Riyadh ELM University, Riyadh, Saudi Arabia, [4]Faculty of Pharmacy, Dr. M.G.R. Educational and Research Institute, Chennai, India, [5]Vaccines and Bioprocessing Centre, King Abdulaziz City for Science and Technology (KACST), Riyadh, Saudi Arabia, [6]Department of Pharmaceutics, College of Pharmaceutical Sciences, Dayananda Sagar University, Bengaluru, India, [7]Faculty of Pharmaceutical Sciences, UCSI University, Kuala Lumpur, Malaysia, [8]School of Pharmacy, ITM University, Gwalior, India, [9]Amity Institute of Pharmacy, Amity University, Gwalior, India, [10]GITAM School of Pharmacy, GITAM University Hyderabad Campus, Rudraram, India, [11]Department of Biotechnology, School of Engineering and Technology, Sharda University, Greater Noida, India, [12]Department of Biotechnology, School of Applied & Life Sciences (SALS), Uttaranchal University, Dehradun, India, [13]School of Bioengineering & Biosciences, Lovely Professional University, Phagwara, India, [14]Department of Biotechnology Engineering and Food Technology, Chandigarh University, Mohali, India, [15]Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovakia, [16]Laboratory of Animal Immunology and Biotechnology, Department of Animal Morphology, Physiology and Genetics, Faculty of AgriSciences, Mendel University in Brno, Brno, Czechia, [17]Department of Life Science and Bioinformatics, Assam University, Silchar, India

Colorectal cancer (CRC) is one of the most deaths causing diseases worldwide. Several risk factors including hormones like insulin and insulin like growth factors (e.g., IGF-1) have been considered responsible for growth and progression of colon cancer. Though there is a huge advancement in the available screening as well as treatment techniques for CRC. There is no significant decrease in the mortality of cancer patients. Moreover, the current treatment approaches for CRC are associated with serious challenges like drug resistance and cancer re-growth. Given the severity of the disease, there is an urgent need for novel therapeutic agents with ideal characteristics. Several pieces of evidence suggested that natural products, specifically medicinal plants, and derived phytochemicals may serve as potential sources for novel drug discovery for various diseases including cancer. On the other hand, cancer cells like colon cancer require a high basal level of reactive oxygen species (ROS) to maintain its own cellular functions. However, excess production of intracellular ROS leads to cancer cell death *via* disturbing cellular redox homeostasis. Therefore, medicinal

plants and derived phytocompounds that can enhance the intracellular ROS and induce apoptotic cell death in cancer cells *via* modulating various molecular targets including IGF-1 could be potential therapeutic agents. Alkaloids form a major class of such phytoconstituents that can play a key role in cancer prevention. Moreover, several preclinical and clinical studies have also evidenced that these compounds show potent anti-colon cancer effects and exhibit negligible toxicity towards the normal cells. Hence, the present evidence-based study aimed to provide an update on various alkaloids that have been reported to induce ROS-mediated apoptosis in colon cancer cells *via* targeting various cellular components including hormones and growth factors, which play a role in metastasis, angiogenesis, proliferation, and invasion. This study also provides an individual account on each such alkaloid that underwent clinical trials either alone or in combination with other clinical drugs. In addition, various classes of phytochemicals that induce ROS-mediated cell death in different kinds of cancers including colon cancer are discussed.

KEYWORDS

oxidative stress, mutation, IGF-1, colon cancer progression, alkaloids, HIF-1α, IGFBP-3, apoptosis

1 Introduction

Globally, cancer is one of the highest mortality-causing diseases. Though there is a drastic improvement in the current treatment schedules followed for various cancers, there is no marked decrease of death rate (1, 2). Among the various dreadful cancers, colorectal cancer (CRC) stood in third and second globally regarding prevalence and death rates, respectively (3, 4). Moreover, a rapid rise in the cases and death rates of CRC has been predicted soon (5). By 2035, a global rise of 2.5 million cases has been estimated (6). However, the CRC incidence varies from country to country, and the maximum number of cases have been reported from the developed world (7). Colorectal tumors were more influenced by gender and sex, where male populations have been affected more significantly than the females (8). In addition, the other risk factors, such as age, consumption of alcohol, diet with high fat and low fruits, vegetables, and maintaining low physical activities, influences the level of progression of the disease (5). Growing evidence also suggests that mutations in the growth factors such as insulin-like growth factor 1 (IGF-1) also shows a massive impact on the development of colon cancer *via* activating rat sarcoma/mitogen-activated protein kinase/phosphatidylinositol-3 kinase/protein kinase B (RAS/MAPK/PI3K/AKT) pathways (4, 9). On the other hand, hormonal alterations also show significant role in production of ROS, which plays an important role in CRC development. Various studies have revealed that hormones like thyroid hormones, corticosteroids and catecholamines are also involved in the generation of ROS by modulating various signaling molecules and pathways like NADPH oxidase (NOX) pathway, mitochondrial electron transport chain (ETC), nitric oxide (NO), and nuclear factor erythroid 2-related factor 2 (Nrf2), apart from their normal functions (10, 11). In addition, some diseases like inflammatory bowel disorder (IBD) also serves as significant risk factors for colon cancer (12, 13).

Recently, there has been a huge advancement in the screening and treatment techniques of CRC. Among the various available treatments, surgery is the preferred treatment option for colon cancer patients (14). However, these treatments cannot effectively reach low-income patients and can only extend the survival time of CRC patients (4, 14). Furthermore, many effective chemotherapeutic drug candidates such as 5-fluorouracil, oxaliplatin, capecitabine, and irinotecan that are clinically recommended for colon cancer treatment either individually or in combination exhibit severe toxicity to normal cells (15, 16). Therefore, there is an urgent need for novel drug treatment with ideal characteristics to treat colorectal carcinoma. From ancient days natural products, specifically medicinal plants and herbal products, have played a significant role in identifying novel treatments against various diseases (17–24). Moreover, several studies have reported plant-derived components as safe to use, and they exhibited minimal toxicity towards non-cancerous cells (2, 25). Hence, these phytocompounds have also been suggested for use together with other clinically recommended chemotherapy drugs to reduce toxicity.

In general, cancer cells require a high level of reactive oxygen species (ROS) compared to normal cells due to elevated metabolic rate and mitochondrial dysfunction, and this makes the cancer cells more vulnerable to oxidative stress. Hence, an increased level of ROS than required can trigger the death-inducing signals in cancer cells *via* irreversible damage to various biomolecules. In contrast to cancer cells, normal cells develop potent antioxidant proteins that can nullify the toxic effects of ROS (26). Therefore, minimal elevation of intracellular ROS than the threshold limit *via* intervention with external agents can make the cancer cells more

sensitive to oxidative stress than the normal cells (27–29). There are several phytocompounds, such as curcumin, capsaicin, sulforaphane, alpha-lipoic acid, and piperine belonging to the class of alkaloids, flavonoids, terpenoids, and phenolic compounds that are known to initiate ROS-mediated apoptotic cell death. Among the various chemical classes, alkaloids are the most divergent and vigorously investigated class of phytochemicals in different diseases including cancer. Various alkaloids like vincristine, vinblastine, evodiamine, sanguinarine, matrine, tetrandrine, camptothecin, and berberine have already proved their anti-cancer potentials *via* targeting different kinds of essential signaling molecules such as MAPK, extracellular signal-related kinases 1/2 (ERK1/2), tumor suppressor protein (p53), p38 mitogen-activated protein kinase, c-Jun N-terminal kinase (JNK), and PI3K/Akt. Hence, forced activation of ROS in the cancer cells through small molecules like alkaloids derived from medicinal plants and altering the signaling molecules that initiate the death process can improve the cancer condition. Moreover, several kinds of alkaloids like berberine, camptothecin and epigallocatechin as such or as derivatives have been under clinical trials at different stages (30; 31). Some of them have till now exhibited a good safety profile as compared to other recommended drugs (32, 33). Hence, this evidence-based study focused on the alkaloids and their ROS-mediated apoptotic signaling pathways. In addition, other classes of phytocompounds that induce ROS-mediated apoptosis and have been used in clinical trials either individually or in combination with other clinical drugs have also been looked at. Several studies have revealed that the synergetic or combination effect of medicinal plants or phytocompounds provides a novel therapeutic tool. Moreover, combinations of herbal compounds with existing clinical drugs have also been reported to increase the specificity and minimize the toxic effects of existing chemotherapy drugs. In this way, the current review acts as a standalone reference to all the classes of herbal compounds that involve ROS-mediated apoptotic cell death.

2 Methodology

The most relevant literature to conduct the study was extracted from electronic databases SCOPUS, and PubMed. The key words and the phrases used for collecting the highly relevant articles included colorectal cancer, alkaloids, ROS-mediated apoptosis, medicinal plants, phytochemicals, current therapies, side effects, hormones and growth factors, clinical trials, and related terms. Later, the findings from the selected studies were summarized by focusing on the alkaloids that induce ROS mediated apoptosis in colon cancer. The articles not published in English language were excluded.

3 ROS: sources and role in cancer development

ROS are highly active and most unstable oxygen containing metabolic products, generally produced by various endogenous and exogenous sources. Among the multiple endogenous sources, mitochondria-based metabolic pathways, p450 metabolism, endoplasmic reticulum, peroxisome, activated macrophages, and neutrophils mainly contribute to ROS generation (Figure 1). Besides, there are other resources such as monoamine oxidase, sirtuins, forkhead box O3 (FOXO3), alpha-ketoglutarate dehydrogenase (α-KGDH), nuclear factor erythroid 2-related factor 2 (Nrf2) and mitochondrial $p66^{shc}$ (proapoptotic protein) which also contribute to ROS generation (34). In addition, the innate immune response shown by the host against the pathogens also generates ROS as a defense mechanism. However, persistent or chronic inflammation leads to excessive production of ROS and develops various kinds of diseases including colon cancer. In a recent study, Sahoo and his group revealed that lipopolysaccharides (LPS)-induced chronic inflammation in the intestine may also lead to cancer in the gut. They observed that LPS treatment can enhance the levels of pro-cancer genes and help in cell proliferation in the colonoids obtained from the dogs suffering from IBD. Therefore, the persistent or chronic inflammatory diseases like IBD also play a part in the development of colon cancer though oxidative stress (13, 35).

In addition, there are many exogenous sources like tobacco smoking, radiation, chemotherapeutic agents, anti-inflammatory drugs, chemicals containing quinones, organic solvents, pesticides, and other heavy metals (like cadmium, chromium, and arsenic) that also produce ROS intermediates by interacting with other molecules. In general, ROS like hydroxyl radical ($^{\bullet}OH$), superoxide radical ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2) at a limited level are crucial to cellular activities (Figure 1). They act as secondary messengers, promoting various essential cellular activities such as signal transduction, immune responses, and gene transcription. However, when the levels of ROS increase beyond the limit, it leads to oxidative stress and damages various vital biomolecules such as lipids, proteins, deoxyribonucleic acids (DNA), and ribonucleic acids (RNA). Moreover, ROS also initiate the development of various diseases, such as cardiovascular diseases, cataracts, neurodegenerative diseases, inflammation, diabetes mellitus, and cancer (Figure 1). Furthermore, ROS plays an important role in the initiation, development, and progression of cancer. Besides, the cancer cells themselves require a high level of ROS in contrast to the normal cells due to the high metabolic rate and increased energy demand. These increased levels of ROS *via* multiple sources interact with various essential signaling molecules thereby activating various growth factors like epidermal growth factor (EGF), and IGF-1, and inducing mutation in their receptors.

Mutated EGF receptor (EGFR) elevates the expression of its downstream signaling molecules such as rat sarcoma - rapidly accelerated fibrosarcoma (RAS-RAF), MAPK, AKT, PI3K and mammalian target of rapamycin (mTOR). In the initial stage of the pathway, the EGFR initiates son of sevenless homolog 1 (Sos 1) gene-mediated activation of RAS-RAF molecules. Then the activated RAS-RAF initiates the phosphorylation of MAPK, which later activates ERK1 and ERK2. The activated ERK immediately phosphorylates the various components of cytoskeleton like MAP 1, 2, and 4 in the cytoplasm, which controls the cell morphology and other functions. In addition, the activated ERK also shifts to the nucleus and helps in the transcription of various signaling molecules like proto-oncogene

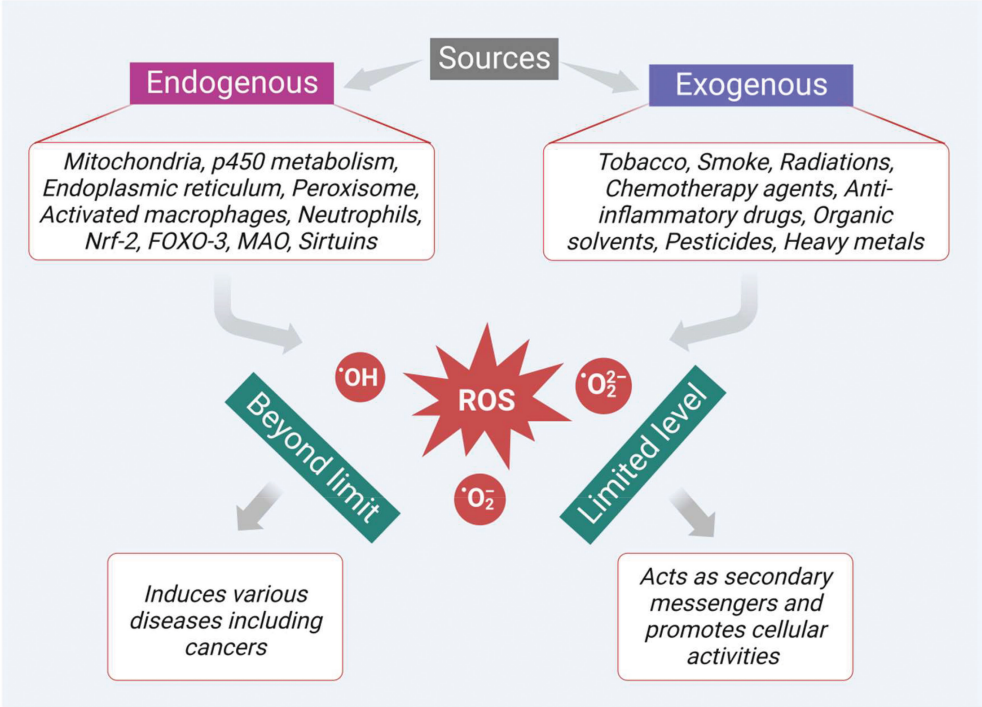


FIGURE 1
Sources of ROS generation which play dual role as secondary messengers that promote cellular activities at limited levels and as inducers of various diseases including cancer at excessive levels.

fos proto-oncogene (c-Fos), transcription factor Jun (c-Jun), cellular myelocytomatosis oncogene (c-Myc), erythroblast transformation specific (ETS) domain-containing protein ETS domain transcription factor ELK1 (Elk-1) and cyclic adenosine monophosphate (cAMP) dependent transcription factor ATF2 (activating transcription factor 2) (36). Mitogen-activated extracellular signal-regulated kinase (MEK) and ERK signaling molecules, proto-oncogene B-Raf (BRAF) (a downstream proto-oncogene of the RAF family) play a significant role in the activation of RAS. On the other hand, RAS also triggers the activation of PI3K signaling molecules, which promotes the conversion of PI-3, phosphatidylinositol 4,5-bisphosphate (PIP_2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP_3). This PIP_3 activates protein kinase B/Akt *via* phosphoinositide-dependent kinase 1 (PDK1) dependent phosphorylation. Then the activated Akt inactivates the transcription activity of FOXO and its interlinked downstream signals *via* phosphorylation and finally promotes the survival, proliferation, and growth of cancer cells (37). Besides Akt activation, it also inhibits glycogen synthase kinase-3 (GSK-3) *via* phosphorylation. This inactivation of GSK-3 protein leads to the activation of various signaling molecules such as (wingless gene) WNT/β-catenin, nuclear factor kappa B (NF-κB), mTOR and its respective downstream signals (Figure 2).

In addition, activated EGFR directly phosphorylates and activates phosphoinositide-specific phospholipase C gamma 1 (PLC-γ1). This PLC-γ1 facilitates the generation of PIP_3 and diacylglycerol (DAG) from PIP_2. DAG further triggers PKC activation and enhances intracellular calcium (Ca^{+2}) release, ultimately leading to carcinogenesis (38). Moreover, EGFR also promotes transcriptional activation of signal transducer and activator of transcription 3 (STAT3) in the nucleus *via* direct interaction and enhances its biological functions such as tumor growth, differentiation, and apoptosis (Figure 2). On the other hand, angiogenesis is another critical factor that plays a prominent role in the colon cancer progression.

Besides, ROS also initiate the activation of vascular endothelial growth factor (VEGF) *via* upregulation of the activity of hypoxia-inducible factor 1-alpha (HIF-1α). This activated VEGF binds to its respective receptor to further activate its downstream signaling molecules (39). There are three types of VEGF receptors such as VEGF receptors 1, 2, and 3 (VEGFR-1, -2, and -3). Moreover, they are all different in their activities and sites of action (40). Among the various VEGFR receptors, VEGFR-1 is found explicitly in various cancers, epithelial and inflammatory cells and promotes biological functions *via* interacting with VEGF. This receptor predominately helps the cancer cells to migrate during angiogenesis (41). Furthermore, it also shows a significant contribution to inflammatory disease conditions, including various cancers, through the activation of downstream signals such as PI3K/Akt/MAPK/ERK, resulting in the release of various inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), and interleukins (IL-1β, IL-6, and IL-8). As shown in Figure 2, these cytokines finally promote tumor growth in respective tissues (42).

In addition, ROS also induce transforming growth factor-beta (TGF-β), which plays a vital role in various fibrotic diseases.

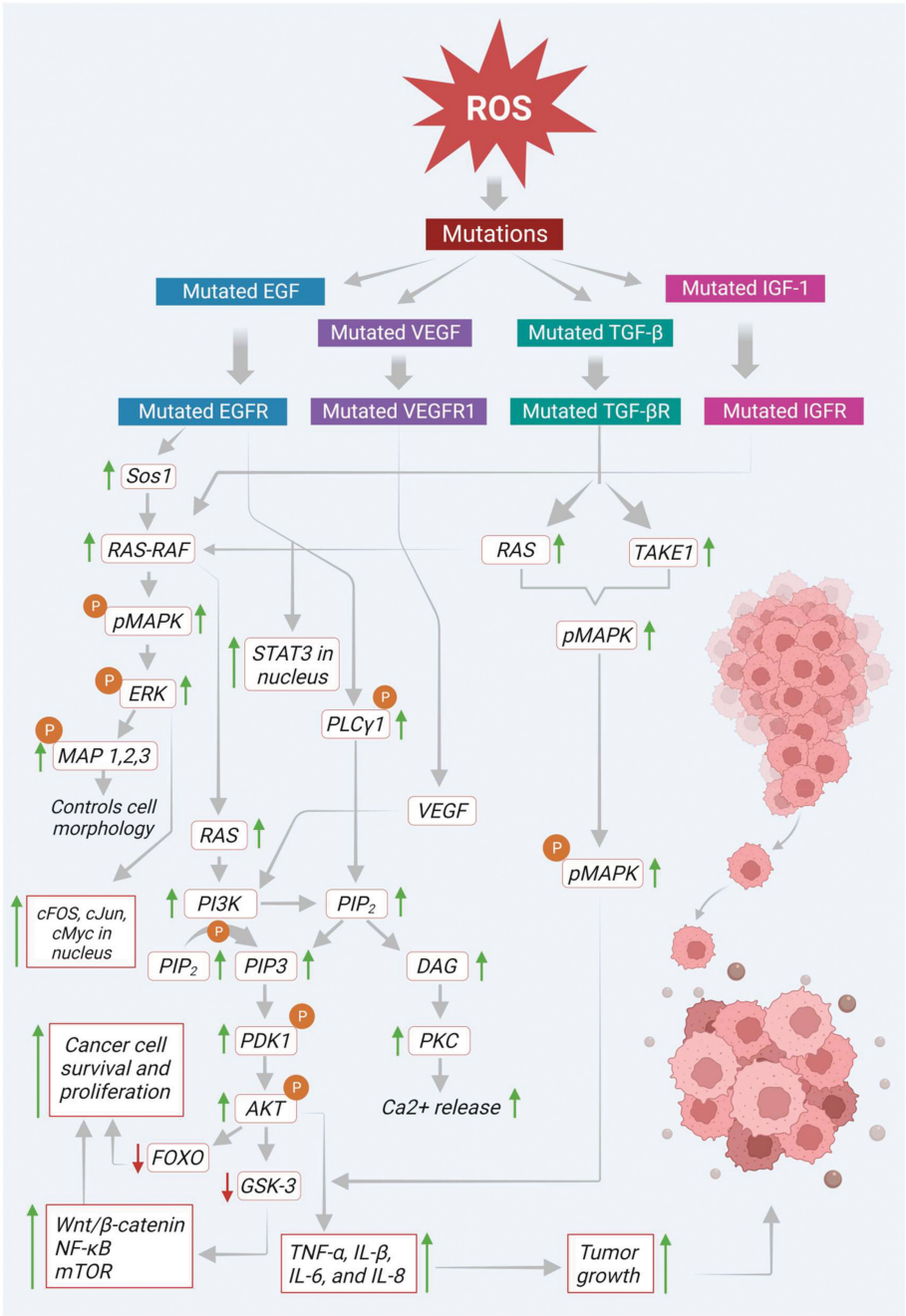


FIGURE 2
Basal levels of ROS initiate mutation in various transmembrane receptors thereby modulating multiple signaling molecules/pathways that involve tumor growth and progression.

Growing evidence suggests that TGF-β also shows impact on tumor growth and progression, which is mediated by ROS. In cancer cells, ROS initiated TGF-β was reported to induce the MAPK signaling pathway *via* activating Ras and TGF-β-activated kinase 1 (TAK1) (34). The activated MAPK further triggers its downstream signaling molecules like ERK1 and ERK2. Finally, this MAPK and TGF-β together with other cytokine molecules, recommend epithelial-mesenchymal transition (EMT), where the tumor epithelial cells adopt mesenchymal-like characteristics that can help in tumor progression and metastasis (Figure 2).

Furthermore, ROS also induce IGFs that play a pivotal role in cancer survival and progression. Several studies revealed that ROS-mediated IGF-I activation also activates its corresponding receptor IGF-1 *via* phosphorylation. As shown in Figure 2, this activated IGF-1 turns on its downstream signaling, such as MAPK, Akt, mTOR and NF-κB *via* induction of Ras-Raf proto-oncogenes (9). The pathways mentioned above are involved in ROS-mediated signaling in cancer development. The subsequent sections highlight growth factors and corresponding signaling in cancer progression.

4 ROS mediated apoptotic signaling

ROS are short-lived metabolic by-products that generally possess an unpaired electron in their respective outermost shells. Because of the unpaired electron, the ROS are highly reactive and show both dangerous and beneficial effects (43, 44). In fact, at the level or below the threshold limit, ROS assist in the development of various cancers *via* initiation of mutations in various signaling molecules like p53 as well as upregulation of oncogenes like Ras and c-Myc. However, cancer cells initiate apoptotic cell death mechanisms due to ROS accumulation beyond the threshold limit (45). Normally, ROS-associated apoptotic cell death can be triggered *via* activation of intrinsic or mitochondrial signaling and upregulation of extrinsic or death receptor signaling.

In the intrinsic pathway, excessive ROS production (e.g., by H_2O_2) modulates mitochondrial permeability transition (MPT) pore and mitochondrial membrane depolarization. As a result, two very essential groups that consist of inactive pro-apoptotic molecules are released in the cytoplasm. One such group contains signaling molecules like cytochrome c, second mitochondria-derived activator of caspase (Smac)/DIABLO (a direct IAP binding protein with low PI), and serine protease Htr (with high-temperature requirements) A2 (Omi) (nuclear-encoded mitochondrial serine protease). Significantly, all these molecules are involved in caspase-dependent apoptotic pathways (43). To be specific, cytochrome c facilitates the proteolytic maturation of both caspases-3 and 9 *via* allosteric activation of apoptotic peptidase activating factor 1 (APAF-1). This activation finally leads to the formation of apoptosomes (46, 47). In a similar manner, as shown in Figure 3, other pro-apoptotic proteins like Smac/DIABLO as well as HtrA2/Omi also initiate the apoptotic process *via* inhibition of IAP like XIAP (47, 48). The second group released from the mitochondrial membrane comprises signaling molecules like apoptosis-inducing factor (AIF), endonuclease G, and caspase-activated DNAse (CAD). After immediate release AIF, endonuclease G, and CAD proteins slide inside the nucleus and initiate caspase-dependent apoptotic features in the cells like DNA fragmentation as well as chromatin condensation (47, 49). Besides, the Bcl-2 family proteins also control the release of various apoptotic signaling molecules including cytochrome c. In the Bcl-2 family, proteins such as Bax, Bak, Bid, Bad, Bim, Bik, and Blk facilitate apoptosis. The other remaining proteins like B-cell leukemia/lymphoma proteins (Bcl-2, Bcl-x, Bcl-XL, Bcl-XS, Bcl-w) and BAG (Bcl-2–associated athanogene), favor inhibition of apoptosis signaling (50, 51). Interestingly, these Bcl-2 family proteins can be significantly tuned by tumor suppressor protein p53. Several studies revealed that p53 directly interacts with Bcl-2 family proteins and helps in mitochondrial membrane depolarization and increases the release of apoptotic signals. To be specific, p53 plays a role in promoting p53 upregulated modulator of apoptosis (Puma) and Bcl2 homology domain 3 (BH3) (Noxa), the two essential proteins of the Bcl2 family (Figure 3). A previous report reveals that Puma, in particular, enhances Bcl-2-associated X protein (BAX) and Noxa supports p53 mediated apoptotic signaling (3, 52). Additionally, excessive ROS upregulation influences apoptosis through the elevation of Ca^{2+} levels, which increases mitochondrial permeability (52).

Furthermore, an elevated level of ROS or prolonged oxidative stress induces endoplasmic reticulum (ER) stress *via* increasing protein misfolding. This intrinsic pathway promotes the unfolded protein response and activates C/EBP homologous protein (CHOP) by triggering the induction of IRE1α, ASK, and p38 MAPK proteins. This finally leads to apoptosis by upregulation of Bcl-2 apoptotic protein. At the same time, excess ROS also prompts the discharge of Ca^{2+} from the ER lumen. Furthermore, due to the proximity of mitochondria to the ER, a maximum amount of Ca^{2+} is absorbed into mitochondria. This condition leads to Ca^{2+} overload in mitochondria and initiates the opening of MPTs, and facilitates the release of molecules like ATP and cytochrome c. These molecules further enhance ROS production and apoptotic signaling, as shown in Figure 3.

On the other hand, ROS also induces apoptosis *via* the regulation of signaling molecules involved in the extrinsic pathway. In this pathway, several death receptors like death receptor 1 (DR1) or tumor necrosis factor receptor 1 (TNF-R1), death receptor 2 (DR2) or type-II transmembrane protein (Fas), death receptor 3 (DR3) or TNF receptor family (TRAMP), death receptor 4 (DR4) or TNF-related apoptosis-inducing ligand-receptor-1 (TRAIL-R1), death receptor 5 (DR5) or TNF-related apoptosis-inducing ligand-receptor-1(TRAIL-R2) and death receptor 6 (DR6) or TNF receptor superfamily member 21(TNFRSF21), that are derived from the TNF-R superfamily, have been found to be involved prominently in the apoptosis process. Generally, the extrinsic apoptotic pathway gets triggered *via* induction of transmembrane death receptors through interacting with various receptor-specific ligands such as Fas ligand (FasL), TNF-α, Apo3 ligand (Apo3L), and Apo2 ligand (Apo2L) (53). However, ligands like FasL, TNF-α, and Apo are mainly produced by activated macrophages and interact with specific death receptors, thereby initiating the apoptosis process in the cancer cells (54, 55). In the first step, the FasL and TNF-α interact with transmembrane proteins Fas or DR2 and TNFR1 or DR1 that consist of respective death domains. Both death receptors initiate apoptosis signaling in different manners.

In the TNF-α/TNFR1 pathway, the immediate interaction of TNF-α ligand to its receptor initiates the activation of TNFR1 *via* trimerization (Figure 4). This step engages other corresponding adapter molecules like TNFR type 1-associated DEATH domain protein (TRADD) and forms a complex in the cytoplasm. This complex activates and attracts a few more partners or signaling molecules, which decides the fate of the pathway (either cell survival or cell death). In this process, the TRADD complex attracts and activates other signaling molecules like tumor necrosis factor receptor 2 (TRAF2) and receptor-interacting protein kinases (RIP). This, in turn, triggers NFκB and MAPK-mediated cell survival process by increasing the levels of antioxidants as well as anti-apoptotic proteins such as Bcl-XL, X-linked inhibitor of apoptosis protein (XIAP), and inhibitors of apoptosis proteins (cIAP 1, 2) (53, 56) (Figure 4). On the other hand, the TRADD complex also stimulates apoptosis *via* recruiting FAS-associated death domain (FADD) protein. This complex promotes procaspases 8 and 10, which regulate the expression of caspases 3, 6 and 7 as shown in Figure 4.

10.3389/fendo.2023.1201198

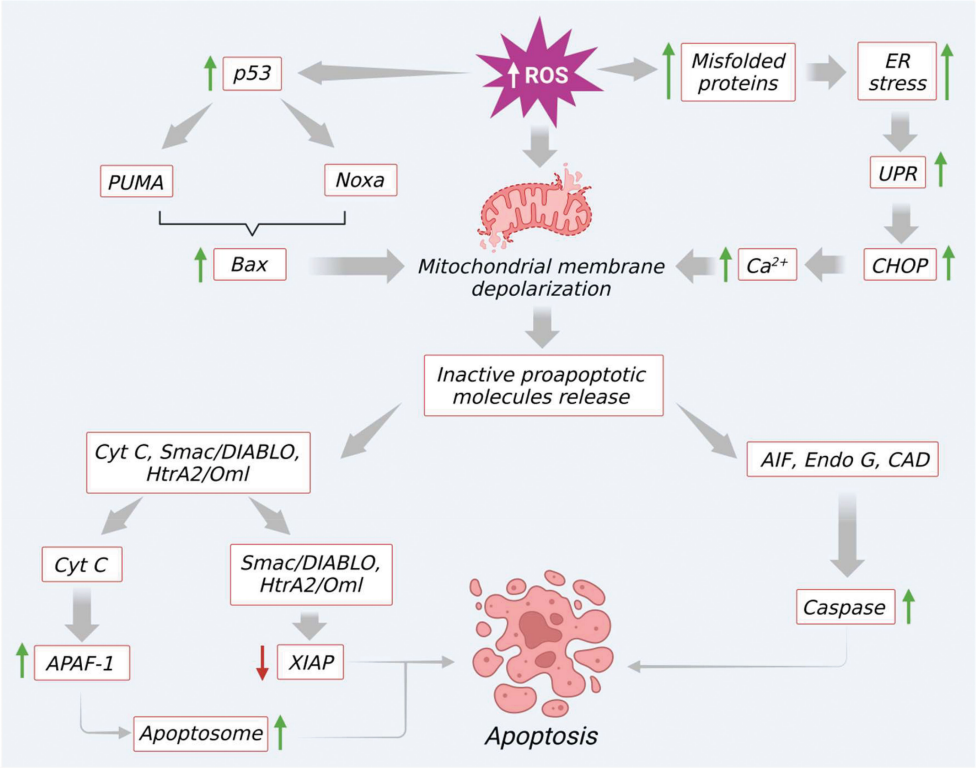


FIGURE 3
Excessive ROS generation induce mitochondrial mediated apoptosis through modulation of various signaling molecules.

Similarly, FasL activates apoptosis by binding to its corresponding receptor DR2 or Fas. This step activates the DR2 or Fas receptor *via* trimerization and recruits the other adaptor molecules like FADD. These linkages further recruit pro-caspase-8 through DED. This finally promotes the generation of DISC and triggers the downstream caspases such as caspases 3 and 6 and initiates the apoptotic signals in the cancer cells by inducing apoptotic features like nuclear condensation, DNA damage, and membrane blebbing (Figure 4).

In the same way, excessive ROS levels also initiate apoptosis *via* activation of PI3K/Akt/mTOR signaling pathway by altering the binding activity of various hormones and growth factors to their respective receptors. This evidence-based study specifically highlights the IGF-1 and its role in apoptosis. Several reports suggest that excessive intracellular ROS production trigger HIF-1α mediated apoptosis in cancer cells *via* activation of IGF-1 binding protein 3 (IGFBP-3) and inhibition of IGF-1 signaling. This step blocks the binding of IGF-1 to its receptor and subsequently inactivates the downstream signaling molecules like PI3K, Akt, and mTOR that help in tumor growth and progression, shown in Figure 5. Finally, this inhibits the NF-κB protein and promotes apoptosis. Therefore, the phytocompounds like alkaloids that induce ROS and modulate different kinds of signaling molecules, including hormone-related growth factors, can be exploited in colon cancer treatment (57).

5 Current treatment strategies of colon cancer and their limitations

Several studies revealed that colon cancer treatment depends on the tumor stage and location. In the early stage, a tumor can be surgically eliminated from the site. However, in the advanced stage, chemotherapy as a single drug or combined with other drugs or radiation is the only option. Among the chemotherapy drugs, fluoropyrimidines have been highly recommended for the treatment of colon cancer. However, fluoropyrimidine derivatives like 5-fluorouracil alone have not been found to be effective. In addition, it also exhibited multiple side effects such as neutropenia, stomatitis, nausea, alopecia, photosensitivity, cardiotoxicity, and sub-acute multifocal leukoencephalopathy. Therefore, its usage as a single drug is limited for colon cancer treatment (58). Subsequently, a platinum derivative named oxaliplatin has been introduced in the standard colon cancer treatment, and it was co-administered with 5-fluorouracil and leucovorin (folinic acid) to reduce the toxicity. This drug has specifically been used in the advanced stage of colon cancer. However, in clinical studies, oxaliplatin drug exhibited characteristic side effects as compared to other platinum derivatives including generation of neurotoxicity, hematotoxicity, gastrointestinal tract toxicity, neurolopathy, thrombocytopenia and nephrotoxicity. In addition to these toxicities, oxaliplatin increases CRC patients' death rate when

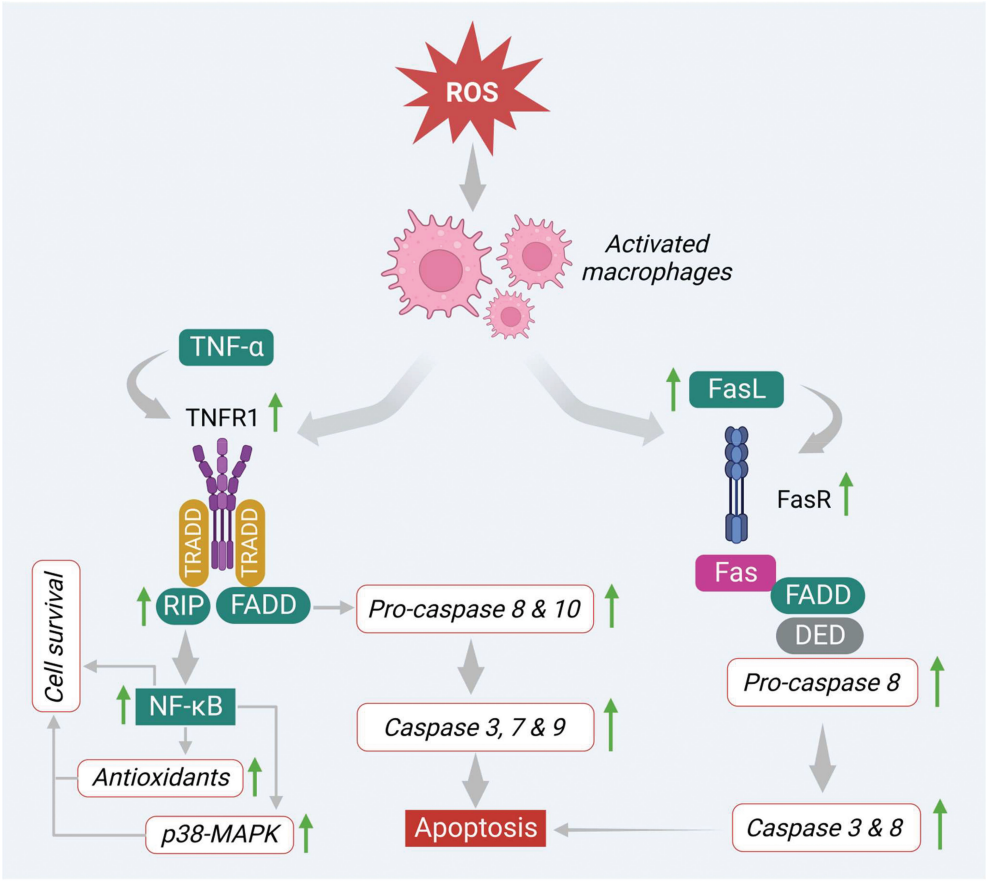


FIGURE 4
ROS-mediated extrinsic apoptosis *via* alteration of numerous intermediate signals.

included in the treatment regimen (58, 59). Irinotecan hydrochloride, a camptothecin derivative, was introduced to improve the CRC treatment. This drug has been recommended in combination with 5-fluorouracil, leucovorin, and oxaliplatin to treat metastatic CRC. Interestingly, this combination increased the survival time by more than 30 months. However, the active metabolite of irinotecan (SN-38), an active metabolite of irinotecan hydrochloride showed severe toxicity, including neutropenia, by developing polymorphic glucuronidation. Additionally, two monoclonal antibodies such as cetuximab and

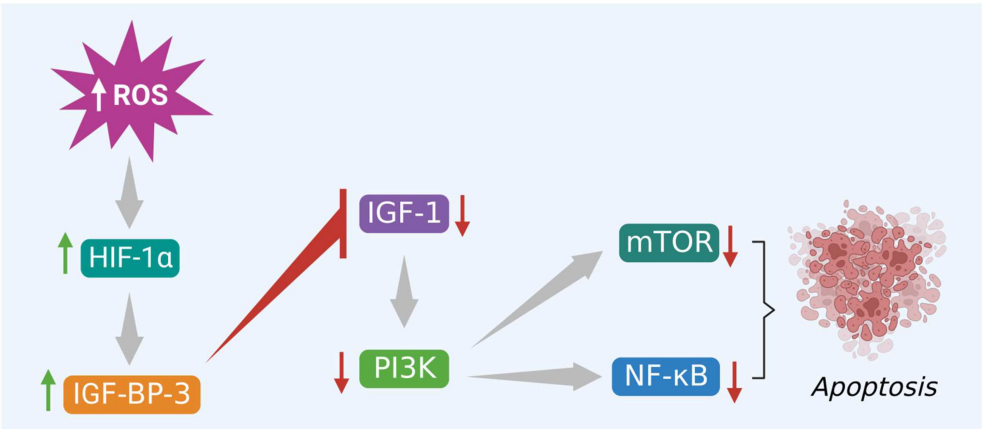


FIGURE 5
Excessive ROS levels induce apoptosis of cancer cells *via* activation of HIF-1α and its interlinked IGFBP-3 protein. IGFBP-3 inhibits PI3K/Akt/mTOR and triggers apoptosis.

bevacizumab, belonging to the class of EGFR and angiogenesis antagonists, respectively, have been added to the irinotecan hydrochloride treatment strategy. Cetuximab was also reported to specifically bind to and inhibit EGFR, whereas bevacizumab inhibited VEGF. Together with an irinotecan hydrochloride treatment regimen (comprising 5-fluorouracil/leucovorin), both cetuximab and bevacizumab are considered helpful in managing metastatic CRC patients. The inclusion of cetuximab can significantly reduce the progression of metastatic CRC. On the other hand, the inclusion of bevacizumab in the irinotecan hydrochloride treatment regimen can increase the survival rate of CRC patients by over 4 months as compared to other standard treatments. However, cetuximab, as a single drug or combined with irinotecan treatment, was associated with various adverse effects such as asthenia, leukopenia, abdominal pain, and neutropenia (60). Similarly, adverse effects like gastrointestinal hemorrhage and bowel ischemia have been associated with bevacizumab (61). Besides, panitumumab, another monoclonal antibody, and an EGFR inhibitor, have recently been introduced specifically to treat metastatic CRC patients with RAS wild type *via* interaction with PI3K/Akt/mTOR signaling molecules. This drug also showed severe toxicity related to skin including dermatitis, pruritus, paronychia, and erythema (62).

In addition, capecitabine, the first oral prodrug of 5-fluorouracil, was used in combination with intravenous irinotecan. However, this combination was administered with or without bevacizumab to treat metastatic CRC. Capecitabine administration was associated with toxicities such as hand-foot syndrome, leukopenia, and proteinuria. In addition, this drug has also shown toxicities like 5-fluorouracil (63, 64). Another prodrug of 5-fluorouracil, known as tegafur, is orally used to treat colorectal cancer liver metastases in combination with 5-fluorouracil. This combination blocks the crucial metabolizing enzyme of 5-fluorouracil and increases the serum concentration of 5-fluorouracil. Hence, in the current treatment regimen, this combination along with oral leucovorin has been included as a typical treatment for stage III colon cancer. Moreover, tegafur also shows serious side effects like neutropenia, thrombocytopenia, mucositis, and asthenia (65, 66). Recently, regorafenib, a kinase inhibitor has been approved for the treatment of metastatic CRC. This drug inhibits various kinases such as fms-related receptor tyrosine kinase 1 (FLT1), TEK receptor tyrosine kinase, rapidly accelerated fibrosarcoma (Raf-1) proto-oncogene, KIT proto-oncogene receptor tyrosine kinase, serine/threonine kinases, and kinase insert domain receptor (KDR). This drug has also proved its efficacy by increasing the survival time by 2.5 months. Moreover, this drug shows few side effects compared to other currently used chemotherapeutic drugs (64). Recently, another monoclonal antibody named dostarlimab, an anti-programmed cell death protein PD-1, has been introduced in colon cancer treatment regimen. The clinical study results show that dostarlimab can completely eradicate colon cancer. Moreover, this drug has also exhibited minimal toxicity in the CRC patients (67). Another monoclonal antibody, a PD-1 inhibitor, nivolumab was introduced to treat microsatellite instability high CRC. The study results revealed that nivolumab possess a better disease control rate of microsatellite instability high CRC (68). At the same time, when co-administrated with ipilimumab (a CTLA4 inhibitor), nivolumab improved cancer conditions and showed 80% disease control rate (69–71). Similarly, another monoclonal antibody named prembrolizumab was introduced to efficiently treat microsatellite instability high defective DNA mismatch repair CRC with a much higher rate of overall response and progression free survival rate. In the study, prembrolizumab has been found to increase the survival time by 8.3 months and the overall survival rate by 43.8% (72). However, almost all existing drugs, including immunotherapy, sooner or later have been becoming less effective, mainly due to resistance, associated toxicities, and immune escape (73, 74), as detailed in Table 1. Hence, there is an urgent need to develop a potential therapeutic tool against CRC. Among the various sources, medicinal plants and their derived compounds play essential roles in drug discovery. They show various pharmacological functions, including anti-cancer and immunomodulatory effects. Several plant derived compounds as such or combined with other clinical drugs have been used to treat various cancers. Phytocompounds like berberine, epigallocatechin, and lycopene have recently undergone clinical trials to manage colon cancer at different stages. Hence, this

TABLE 1 Problems associated with current drug therapies for colon cancer.

Drug	Side effects/Adverse effects	Reference
Fluoropyrimidine (5-fluorouracil)	Neutropenia, Stomatitis, Nausea, Alopecia, Photosensitivity, Cardiotoxicity, and Sub-acute multifocal leukoencephalopathy	(58)
Oxaliplatin	Neurotoxicity, Hematotoxicity, Gastrointestinal tract toxicity, Neurolopathy, Thrombocytopenia and Nephrotoxicity. Increases the death rate of CRC patient.	(58, 59)
Irinotecan hydrochloride	SN-38, an active metabolite of irinotecan shows side effects like Neutropenia due to polymorphic glucuronidation	(58, 59)
Cetuximab	Asthenia, Leukopenia, Abdominal pain, and Neutropenia	(60)
Bevacizumab	Gastrointestinal hemorrhage and Bowel ischemia	(61)
Panitumumab	Severe skin toxicities including Dermatitis, Pruritus, paronychia, and Erythema	(62)
Capecitabine	Hand-foot syndrome, Leukopenia, and Proteinuria	(63, 64)
Tegafur	Neutropenia, Thrombocytopenia, Mucositis, and Asthenia	(65, 66)

evidence-based study highlights phytochemicals, specifically the alkaloids that induce ROS-mediated apoptosis.

6 Role of phytocompounds in inducing ROS mediated apoptosis in colon cancer

Cancer is the second leading cause of mortality and a substantial global health concern. Despite significant advancements in treatment modalities, cases of various cancers and deaths have still been on the rise. In 2020, nearly 19.3 million new cancer cases and 10 million new deaths have been reported, accounting for the extreme increase of cases and deaths worldwide (75). Among the various cancers, CRC stood second in terms of cancer-related deaths (6, 75). This continuous rise in CRC cases is due to the lack of efficient screening and treatment methods. In addition, colon cancers were being detected at an advanced stage or at metastasis stage in most cases, which made it difficult to treat the patients. Moreover, currently recommended treatments such as surgery, radiation, chemotherapy, and combination treatments also exhibit serious challenges like non-specificity to cancer cells, side effects as well as drug resistance, thus limiting the efficacy of various treatment options (76).

Given the severity of the disease, there is an urgent need to find a novel treatment with minimal toxicity to the CRC patients. Due to the never-matched chemical library and other favorable factors, medicinal plants and derived phytochemicals act as potential resources for novel drug discovery against various diseases, including cancer (77, 78). In fact, plant secondary metabolites such as alkaloids, terpenoids, flavonoids, steroids, saponins, and phenolic compounds have been held responsible for their antitumor activity as well as other biological functions of the medicinal plants (76, 79, 80). For example, taxol, vincristine, vinblastine, and podophyllotoxin form the primary secondary metabolites with proven anticancer potentials *via* modulating various signaling molecules of death pathways (78, 81, 82). However, studies revealed that in most cases these anticancer phytochemicals also show toxicity towards non-cancerous cells, which is a serious challenge to the treatment (83). Hence, there is an urgent need for unique and ideal therapeutic tools that can selectively target cancer cells alone.

Generally, cancer cells require a high level of ROS to run normal biological functions such as differentiation and development and maintain an increased metabolic rate. In addition, ROS at the required level also help in tumor progression, metastasis, and angiogenesis. However, the level of ROS exceeds the limit, leading to oxidative damage to the cells, specifically in cancer cells (27). This discrete damage to cancer cells alone is due to the increased mitochondrial dysfunction, which makes the cancer cells more sensitive to oxidative stress than the normal cells. Hence, a slight increase in ROS levels by using external agents can induce cell death through various death mechanisms in cancer cells. Among the different kinds of programmed cell deaths, apoptosis is a well-studied and vital pathway involved in several diseases, including cancer. In cancer, the malignant cells maintain shallow levels of apoptosis, which result in tumor growth and progression. Hence, forced activation of apoptotic signaling molecules *via* up-regulation of ROS through introduction of small molecules from various sources can help to treat cancer better. Mounting evidence also suggests that medicinal plants and their derived components are better sources for novel drug discovery because of their minimal toxic effects.

Among the several classes of phytocompounds, alkaloids serve as a vital source for identification of novel drug candidates against various diseases including cancer. In addition, they can also induce ROS-mediated cell death by regulating various intrinsic and extrinsic apoptotic signaling molecules in different cancers, including colon cancer (24, 84). Alkaloid compounds that induce ROS-mediated apoptosis in colorectal cancer have been discussed in detail. Additionally, various classes of phytocompounds that initiate ROS-mediated apoptosis in different cancer cells are mentioned in Supplementary Tables 1–6. In this way, the current evidence-based review serves as a complete reference for all kinds of phytochemicals that promote ROS-associated apoptosis *via* targeting various signaling molecules, including IGF-1 in cancer cells.

6.1 Alkaloids as anti-colon cancer agent

Alkaloids are plant-derived naturally occurring secondary metabolites. These compounds mostly contain nitrogen as the heteroatom, which imparts basic nature to these molecules. There are several kinds of alkaloids based on heterocyclic rings that show numerous medicinal potentials against malaria, neurodegenerative diseases, viral infections, arrhythmia, hepatitis, asthma, bacterial infections, hypertension, eye infections as well as different kinds of cancers, including colon cancer (85). Various kinds of alkaloids that induce ROS-mediated apoptosis in colon cancer, either alone or in combination with other drugs, are discussed below.

6.1.1 Isoquinoline alkaloids

These compounds are known to show potential anti-colon cancer activity *via* targeting cancer cells alone through initiating ROS-dependent apoptosis. Evidence suggests that benzophenanthridine alkaloids belonging to the class of isoquinoline type of alkaloids can trigger ROS-mediated cell death in cancer cells. Among this class of compounds, sanguinarine is a naturally derived alkaloid isolated from *Macleaya cordata*, belonging to the family Papaveraceae. This compound killed the colon cancer cells like human colon adenocarcinoma (SW480) and human colon cancer (HCT116), as well as reduced the SW480 and HCT-116 cell-generated tumor growth in an orthotopic model of nude BALB/c mice. Sanguinarine also induced intrinsic apoptotic cell death in colon cancer cells *via* increasing ROS-mediated mitochondrial permeabilization and releasing important signaling molecules like cytochrome c and ATP. In fact, apoptosis was initiated through the activation of Bax (dependent), which disrupted the association of serine-threonine kinase receptor-associated protein (STRAP) and maternal embryonic leucine zipper kinase (MELK) proteins. Then ultimately activated the caspase 3 protein and its function (86). In another study,

sanguinarine induced ROS mediated apoptotic cell death in colon cancer cells through DNA damage wherein the apoptotic activity was p53 independent (87). In addition, 6-methoxydihydrosanguinarine, a natural sanguinarine derivative, induces apoptotic cell death in various cancer cells *via* ROS production (88). Berberine, another isoquinoline type of alkaloid isolated from the Berberis genus, also produces an anti-colon cancer effect by upregulating ROS and apoptotic signals. In a study, this compound was reported to trigger apoptosis through ROS generation in colon cancer cells like human colorectal adenocarcinoma (HT-29) and HCT-116 cells *via* increasing the expression of lnc RNA cancer susceptibility candidate 2 (lncRNAs CASC2). lncRNA CASC2 later interacts with AU-rich element RNA-binding factor 1 (AUF1) to block the translation of Bcl-2 triggering apoptosis (89). This compound also initiated apoptotic mediated cell death in mouse conditionally immortalized epithelial (IMCE) cells that were inherited with adenomatous polyposis coli (Apc) multiple intestinal neoplasias (min) mutation. This investigation also revealed that berberine kills IMCE *via* inducing ROS, which initiates the release of AIF from mitochondria and triggers apoptosis in caspase-independent manner. Moreover, berberine did not show toxicity towards normal colon epithelial cells i.e., young adult mouse colon epithelial cells (90). In addition, berberine inhibits the migration of SW480 and HCT-116 cells *via* activation of AMPK through upregulation of ROS and downregulation of glucose levels. Thus activated AMPK decreases the activity of integrin β1 protein and initiates cell death (91). In another investigation, berberine showed ROS-mediated apoptosis in human colon carcinoma (SW620) cells by increasing JNK, c-Jun, and p38 MAPK phosphorylation. On the other hand, these compounds also enhance the release of cytochrome c and activate caspases 3 and 8 to support Fas-mediated apoptosis (92). Li and the team also studied the effect of berberine on azoxymethane or dextran sulfate sodium-induced colon tumors in mice. In their study, berberine reduced the tumor size to 60% *via* activation of AMPK and reduction of mTOR activities. They concluded that the tumor regression activity is associated with reduced activity of cyclin D1, nonhistone nuclear protein (Ki-67), cyclooxygenase-2 (COX-2), survivin, NF-κB, and increased activity of caspase-3 function. This compound also inhibits the migration and metastasis of SW620 and human colon cancer cell line (LoVo) cells *via* down-regulating the expression of COX-2, prostaglandin E2 (PGE2), Janus kinase 2 (JAK2), and STAT3 signaling molecules. Besides, it also suppresses the solid tumor growth in BALB/c mice and lung metastasis developed by SW620 and LoVo cells (93, 94). On the other hand, berberine inhibits colon tumor growth *via* targeting signaling molecules like TNF-α, EGFR, and Wnt/β-catenin signals (95–97). Furthermore, recent combination studies of berberine with *Andrographis paniculata* extract also showed an excellent anti-colon cancer effect, where they inhibited the growth of HT-29 cells and colon carcinoma cell line (RKO) cells and their corresponding *in vivo* tumor growth. This activity was initiated by an increase in the ROS levels, which finally altered the genes involved in DNA replication (98). Furthermore, when combined with evodamine an indole alkaloid, berberine enhances the anti-tumor activity. This combination promotes apoptosis in cancer coli-2 (Caco-2) and HT-29 cells *via* modulation of Nrf2-mediated pathways (99). Cepharanthine, a biscoclurine or bis benzylisoquinoline alkaloid extracted from *Stephania cepharantha Hayanta*, also significantly induced oxidative stress-mediated apoptosis in p53 mutated colon cancer cells HT-29 and SW620 cells. Cepharanthine initiated apoptosis in HT-29 cells, which is linked with cell cycle arrest at growth 1 (G1) phase and cyclin-dependent kinase inhibitor 1 or CDK-interacting protein 1 (p21Waf1/Cip1) level increase (100). Coptisine, another isoquinoline alkaloid extracted from *Coptis chinensis Franch*, reportedly triggered ROS-dependent apoptosis in HCT-116 cells. It reduced the growth and migration and finally promoted caspase-mediated apoptosis in HCT-116 cells by altering mitochondrial membrane potential as well as modulating various signaling molecules like Bcl-2, Bcl-XL, XIAP, Bax, Bad, cytochrome c, Apaf-1, AIF, caspase-3, PI3K and Akt. In addition, coptisine reduces the HCT-116 generated tumor growth by inducing apoptosis in nude BALB/c mice (101). In another study, this compound was reported to induce cell cycle arrest at G1 phase as well as inhibit HCT-116 xenograft tumor growth *via* targeting Milk Fat Globule EGF And Factor V/VIII Domain Containing (MFG-E8) gene and rat sarcoma-extracellular signal-regulated kinase (RAS-ERK) pathways (101–103). Moreover, alkaloids like neferine and isoliensinine isolated from *Nelumbo nucifera* (lotus) plants have shown significant anticancer potential by enhancing the chemo- sensitivity of colon cancer cells towards cisplatin treatment. These two alkaloids and cisplatin increased the ROS levels in human CRC (HCT-15) cells, leading to caspase-3 dependent apoptosis through the deactivation of MAPK/PI3K/Akt/mTOR signaling molecules. In addition, this combination was also found to decrease the resistance towards cisplatin and increase apoptotic markers in colon cancer stem cells *via* uplifting Ca^{2+} level and damaging mitochondrial membrane integrity (104, 105). Recent studies revealed that palmatine, another isoquinoline alkaloid, induces apoptosis in colon cancer cells like HCT-116, SW480, HT-29, and, in HCT-116 cell xenograft tumor *via* increasing ROS levels and decreasing mitochondrial membrane potential. This leads to release of cytochrome c protein. Palmatine is believed to target various signaling molecules such as aurora kinase A (AURKA), Bcl-xl, Bcl2, caspase 3 and 9 to induce apoptosis (106). Tetrandrine, a bisbenzylisoquinoline alkaloid derived from the roots of *Stephania tetrandra*, also triggers oxidative stress-mediated cell death in HCT-116 cells through modulation of E2F transcription factor 1 (E2F1) and p53/p21Cip1 levels (107, 108). Nano formulation of this alkaloid showed an enhanced anticancer effect against Lovo cells through ROS-mediated upregulation of JNK and caspase-3 activity (109). Similarly, xylopine, an isoquinoline alkaloid isolated from the stem of *Xylopia laevigata*, also induces oxidative stress-mediated apoptosis in HCT-116 cells by triggering apoptosis in p53 independent manner *via* induction of cell cycle arrest at growth 2 (G2)/mitosis and cytokinesis (M) phase, and caspase-3 activity (110). For more clarification, the above-mentioned compounds are presented in the Supplementary Table 7.

6.1.2 Indole alkaloids

Indole alkaloids also show a prominent anticancer effect. Camptothecin, a well-studied compound under the class of indole

alkaloids, extracted from *Camptotheca acuminata* belonging to the family Nyssaceae showed potent anticancer effects (111). Evidence suggests that this molecule exhibits anti-tumor activity against various cancer cells, including colon cancer. Notably, the anticancer effect of camptothecin is associated with the apoptosis mechanism, which is mediated by ROS (112). A study by Park and colleagues reported that camptothecin kills the HCT-116 cells through activating apoptosis signaling associated with TNF-related apoptosis-inducing ligand (TRAIL). This study also found that camptothecin induces DNA damage and activates Bax, p53, and p21 proteins. This step finally leads to the death of colon cancer cells. Their investigation also proved that hypoxia condition reduces the apoptotic cell death of HCT 116 cells *via* decreasing Bax. The study concluded that ROS are essential for camptothecin's anti-tumor activity (113). In addition, camptothecin, along with other chemotherapeutic drugs, initiates apoptosis in colorectal adenocarcinoma (DLD1) and HCT-116 colon cancer cells *via* increasing mitochondrial fission and decreasing complex I activity as well as mitochondrial size. This mitochondrial change provokes excess ROS production, which helps to activate apoptotic signaling (114). Furthermore, Wenzel and coworkers found that camptothecin at 50μM can induce apoptotic cell death in HT-29 colon cancer cells through upregulating caspase-3 activity *via* increasing mitochondrial superoxide ROS production. This caspase-3 activation triggers the apoptotic characteristics, like loss of plasma membrane integrity and nuclear fragmentation, in the HT-29 cells. In addition, this study also revealed that camptothecin modulates various signaling molecules like Bcl-2, Bax, Bak, p21, COX-2 and NF-κB in HT-29 cells, which promote apoptosis. However, ascorbic acid treatment at 50uM reverses the changes made by camptothecin in various signals and inhibits the apoptosis process *via* scavenging the superoxide radicals in HT-29 cells (115). In another study, Guo et al. showed that prodrug formulation of camptothecin *via* conjugating with lysine or arginine amino acids enhances water solubility, tumor penetration capacity as well as pharmacokinetic properties. This study also proved that prodrug formulation of camptothecin increases the mitochondrial ROS production much better than the camptothecin alone, thereby decreasing the murine colorectal carcinoma (CT-26) cells induced tumor size in Bagg albino (BALB/c) mice *via* initiating apoptosis process (116). Besides, the semi-synthetic derivative of camptothecin like irinotecan hydrochloride (IH) also shows anti-colon cancer activity. Moreover, SN-38, the active metabolite of IH, significantly improves the patients' colon cancer condition and survival rate up to 30 months. Nowadays, IH is highly recommended to treat metastatic CRC in combination with other clinical drugs like 5-fluorouracil and oxaliplatin (117). A study conducted by Britten and his colleagues showed that irinotecan reduces the growth of HT-29 cells generated tumors drastically when co-administered with other compounds like 6-hydroxymethylacylfulvene and 5-fluorouracil (118). In another experiment, Allegrini and his team demonstrated that irinotecan, when co-administered with the antiangiogenic drug thrombospondin-1 (TS-1) to nude mice bearing HT-29 cells generated tumor, a significant reduction in the size of the tumor occurs as compared to the control as well as individual treatment. At the same time combination of TS-1 with irinotecan did not affect much growth of HT-29 cells (119, 120). Moreover, this combination is non-toxic to the normal cells. In another study, an anti-folate agent used in combination with SN-38, an active metabolite of irinotecan to treat 5-fluorouracil resistant HT-29 cells and found a significant growth inhibition of the cells. This combination also potentially reduced the HT-29 cell-oriented solid tumor in BALB/c nude mice (119, 120). Results from the clinical study also revealed that when irinotecan was given in combination with oxaliplatin, capecitabine, and bevacizumab for 8 cycles to a patient suffering from advanced tumor provided promising results by increasing the survival time. Similarly, irinotecan co-administered with 5-fluorouracil and oxaliplatin also showed a significant recovery in advanced colon cancer patients (121, 122). Additionally, all the indole alkaloids that induce ROS mediated apoptosis in colon cancer are presented in Supplementary Table 7 for detailed clarification.

6.1.3 Capsaicinoids

Capsaicinoids are another alkaloid class that induce ROS-mediated apoptosis in colon cancer cells. Capsaicin is a significant alkaloid that belongs to the class of capsaicinoids, reported to up-regulate apoptotic signals in colon cancer cells through increasing intercellular ROS (123). In a study conducted by Yang and his team, capsaicin was found to reduce the viability of colon cancer cells like human colon carcinoma (Colo320DM) and LoVo cells through initiating caspase-dependent apoptosis, which is linked with an increase in intracellular ROS production and altering mitochondrial membrane potentials (124). In another investigation, a group of researchers reported that capsaicin can trigger apoptosis in HT-29 colon cancer cells *via* activating peroxisome proliferator-activated receptor –γ (PPAR- γ) as well as AMPK but not the vanilloid receptor (125). Lu et al. showed that this compound induces apoptotic cell death in colo 205 cells as well as its corresponding tumor xenograft through targeting various signaling molecules like Fas, cytochrome c, Bax, Bcl-2 p53, p21, and caspases 3, 8 and 9. In addition, this apoptotic activity is strongly associated with increased intracellular ROS, Ca^{2+} production, and damage to mitochondrial membrane integrity (125–127). Furthermore, capsaicin in combination with 3,3′- diindolylmethane enhances the anti-cancer potential by inducing apoptosis in colon cancer cells like HCT116, SW480, LoVo, Caco-2, and HT-29 *via* targeting apoptotic markers like Fas, NF-κB, p53 and other proteins (128). Capsaicin, combined with resveratrol, have also been reported to induce apoptotic cell death in HCT-116 cells by increasing nitric oxide (NO) concentration and p53 level. The increase in p53 level decreases murine double minute 2 (Mdm2) and increases Bax expression to promote apoptosis (129). Supplementary Table 7 provides further information on capsaicinoids.

6.1.4 Carbazole alkaloids

Carbazole alkaloids constitute another plant-based alkaloid class, exhibiting an anti-colon cancer effect. Clausenidin is the

one belonging to the carbazole alkaloid class, isolated from the roots of *Clausena excavata* and also reported to initiate caspase-9 dependent apoptosis in HT-29 cells through cell cycle arrest at quiescent phase (G0)/G1 phase, triggered by increasing ROS mediated mitochondrial membrane depolarization (130). More details can be found in Supplementary Table 7.

6.1.5 Diterpenoid alkaloids

Besides, diterpenoid alkaloids too exhibit anti-colon cancer effect (Supplementary Table 7). Lappaconitine hydrochloride (LH) is one such compound and a derivative of C 18-diterpenoid alkaloid. Song and colleagues suggested that this compound demonstrates an anti-colon cancer effect by inhibiting proliferation and increasing apoptosis in HCT-116 cells. Moreover, this activity is associated with increased ROS and decreased mitochondrial membrane potential. They also found that LH reduces the tumor volume of the HCT-116 cells-oriented xenograft model *via* altering the MAPK pathway (131).

6.1.6 Piperidine alkaloids

Piperine, a piperidine alkaloid obtained from black pepper also reported to trigger apoptotic cell death in HT-29 cells through cell cycle arrest at the G1 phase (Supplementary Table 7). This activity is achieved *via* targeting various markers, including cyclin D1, D2, and cyclin-dependent kinase inhibitor ($p21^{WAF1}$ and $p27^{KIP1}$) (132).

6.1.7 Amide alkaloids

Amide alkaloids are an essential group of alkaloids derived from plants. They also show potent anti-colon cancer effects (Supplementary Table 7). Piperlongumine is a critical compound from this class, extracted from the fruits of *Piper* species. Reports suggested this compound also induces apoptosis in HCT-116 *via* increasing ROS and decreasing antioxidant enzyme glutathione S-transferase. It targets JNK, MEK, and ERK proteins but not Bax, p21, and p53 to induce selective apoptosis in colon cancer cells. In addition, piperlongumine is also used in combination with oxaliplatin, a first-line drug of choice for metastatic colon cancer. This combination enhanced anti-colon cancer activity by increasing the level of ROS-mediated apoptosis, which is linked with upregulation of mitochondrial dysfunction and ER related apoptotic markers in HCT-116 and LoVo cells (133, 134).

6.1.8 Carboline alkaloids

Carboline alkaloids also show prominent anti-cancer potential (Supplementary Table 7). Kim et al. (135) reported another alkaloid harmine, a carboline derivative, extracted from *Peganum harmala*. This compound can induce ROS-dependent apoptosis in HCT-116 cells. In a study, they investigated the anti-colon cancer potential of harmine hydrochloride. This compound found found to activate apoptotic cell death in HCT-116 cells through increasing apoptotic [capases 3 and 9, poly (ADP-ribose) polymerases (PARP)] as well as pro-apoptotic proteins (Bax) and decreasing anti-apoptotic protein (Bcl-2) markers. These investigations also revealed that the harmine hydrochloride compound mainly alters ERK/PI3K/Akt/mTOR signaling pathway to initiate apoptosis. In addition, this compound also triggered apoptosis in SW620 cells by inducing arrest at the sub-G1 phase and decreasing mitochondrial membrane potential. Notably, harmine promotes caspase-dependent apoptotic cell death in SW620 cells through downregulation of ERK/PI3K/Akt/mTOR-related pathways (45, 135, 136).

6.1.9 Quinolizidine alkaloids

Likewise, a study revealed the anti-colon cancer effect of oxymatrine (Supplementary Table 7), a quinolizidine alkaloid extracted from the roots of *Sophora flavescens* – a Chinese traditional medicine. These compounds induce apoptosis in colon cancer cells *via* ROS generation. Moreover, earlier reports suggests that co-administration of oxymatrine with doxorubicin produces a better anti-colon cancer effect *via* enhancing apoptotic signals (cleaved caspase-3, cleaved caspase-9 and Bax/Bcl-2 ratio) in HT-29 and SW620 cells mediated by ROS generation. In addition, this combination also reduces the growth of HT-29 generated tumor xenograft in a dose dependent way *via* modulating E-cadherin and N-cadherin level. Besides, oxymatrine also increases the sensitivity of 5-fluorouracil resistant cells like HCT-8 and initiates apoptosis by promoting inhibition of EMT and NF-κB activity (137, 138).

6.1.10 Proto alkaloids

In a few studies, researchers reported colchicine, a proto alkaloid, to show an anti-colon cancer effect through up-regulation of ROS and its associated apoptotic markers. They showed that colchicine induces apoptosis in HT-29 cells by regulating ROS and decreasing mitochondrial membrane integrity. This compound was ultimately reported to trigger apoptosis by activating BAX, caspase 3, p38 and deactivating AKT signaling molecules. In another investigation, this compound reportedly initiated ROS-mediated apoptosis in HCT-116 and Colo-205 cells by depolymerizing microtubules and arresting the cell cycle at the G2/M transition state (139, 140).

6.2 Alkaloids and other phytocompounds in clinical trials against colorectal cancer

Existing drugs for colon cancer treatment have been showing various side effects, including resistance. Therefore, there is a rise in demand for novel therapeutics to overcome the toxicities and limitations (2). Multiple pieces of evidence support medicinal plants and their derived phytochemicals as a suitable and potent source of drug discovery against various diseases. In this evidence-based study, various classes of phytocompounds have been discussed, including the alkaloids that are of clinical value for colon cancer treatment. Such compounds include berberine, epigallocatechin-3-gallate (EGCG), curcumin, lycopene, fisetin,

and resveratrol. These compounds have recently been used in different phases of clinical trials against CRC. In a clinical study, a patient suffering from CRC was advised to take 0.3 g of berberine daily two times, and there was no sign of a tumor in the later stage. Berberine was strongly recommended to decrease the chances of recurrence of colorectal adenoma and polypoid lesions. Moreover, this drug was safe and showed no severe side effect (33). In addition, EGCG is also in the early stage of CRC clinical trials. The chemopreventive effect of Teavigo (purified tea extract with 94% EGCG) has been evaluated. CRC patients with curative resections were administered orally with 450 mg of EGCG twice daily. Blood was drawn from the patients at 0, 3, 6, 9, 12, 15, and 18 months and a colonoscopy was done after a year (141). Furthermore, lycopene, a carotenoid, was also tested against metastatic CRC and was found to decrease skin toxicity associated disease as a single drug or in combination with panitumumab (31). Likewise, resveratrol, a stilbenoid compound, was also studied against metastatic CRC with liver metastases. A 5.0 g daily dose of the compounds administered for two weeks, could markedly induce caspase-dependent apoptosis in metastatic liver cells of CRC patients (142, 143).

Similarly, curcumin, a polyphenol compound, was also effective against various cancers, including CRC. In addition, some other phytocompounds that act as immune boosters or immune modulators help in blocking colon tumor progression. One such compound is fisetin, a flavonoid compound, evaluated in patients suffering from CRC for its anti-inflammatory effect. CRC patients under chemotherapy were fed with 100 mg of fisetin for 7 weeks. At the end of chemotherapy, various inflammatory markers were evaluated, and fisetin was found to reduce the levels of IL-8 and high-sensitivity C-reactive protein (hs-CRP) in CRC patients. Hence, fisetin may be suggested as an adjuvant treatment to the conventional therapies followed to CRC patients (32). Other details regarding the phytocompounds that are under clinical trials aere provided in Table 2.

7 Conclusions and future perspective

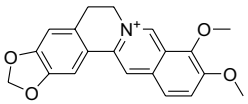
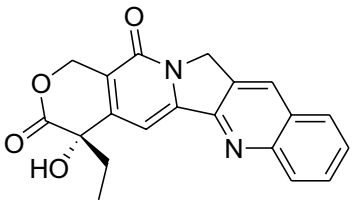
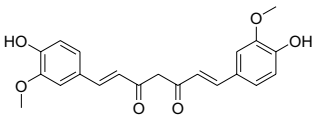
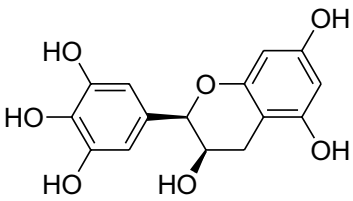
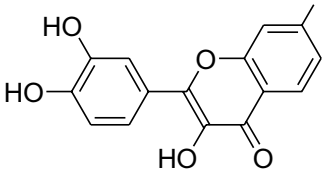
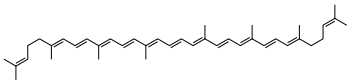
Colon cancer globally occupies the third and second positions regarding of prevalence and death rate, respectively. However, there is a vast improvement in the screening and early detection techniques as well as treatment schedules. Therefore, the CRC death rate has slightly decreased in recent times. Yet the incidence of the disease is increasing. This is due to the rise of multiple side effects and limitations in the existing therapies that are followed against CRC. Hence, there is an immediate need for typical, potential, and alternate treatment regimens that can overcome the existing limitations. Several documented evidence suggests that phytocompounds that induce ROS-mediated apoptosis in cancer cells could pose a better and alternative cancer treatment. Moreover, these compounds exhibit minimal toxicity and high specificity to cancer cells in most cases. Their selective cytotoxicity of ROS induced on cancer cells are due to the features like continuous mitochondrial dysfunction and compromised antioxidant defense mechanism. This leads the cancer cells to become more susceptible to oxidative stress. Therefore, slight increase of ROS than the threshold limit by using external agents like phytocompounds can lead the cancer cells to oxidative stress induced apoptotic cell death. However, normal cells can nullify the minimal ROS due to its proper antioxidant defense mechanism and escape from the ROS mediated cell death. Hence, researchers now-a-days focus on plants and their derived compounds that induce ROS for identifying novel drug candidates against various diseases including CRC.

This evidence-based study discusses important plant-based alkaloids that induce cell death *via* ROS-mediated apoptosis in colon cancer. Alkaloids can induce ROS-associated apoptosis *via* altering multiple signaling molecules that involve various vital characters like proliferation (Ki-67, STRAP, MELK, JNK, PI3K/Akt/mTOR/Wnt/β-catenin, ERK, MEK, MAPK-p38), cell cycle (cyclin D1, D2, surviving, c-Jun), inflammation (TNF-α, COX-2, NF-κB), oxidative stress (Nrf2, GSK-3β), tumor suppression (p53, E2F1, AMPK, PPAR- γ), metastasis or angiogenesis (IGF-1, MFG-E8, integrin β1, ERK, EGFR, E-cadherin, N-cadherin, PGE2) and apoptosis (cytochrome C, AIF, Bax, p21, p21WAF1, p27KIP1, p53/p21Cip1, caspases 3 and 9, XIAP, Bcl-2, IncRNA CASC2, AUF1, PARP) of colon cancer cells (Figure 6). Many of these alkaloids have shown promising results in various colon cancer preclinical studies. For example, alkaloids like coptisine, camptothecin, capsaicin, lappaconitine, oxymatrine, sophoridine, aleutianamine, and palmatine have shown positive results in different kinds of preclinical models and effectively reduced colon tumor growth. Similarly, preclinical and clinical data suggest berberine (an isoquinoline alkaloid) as a future drug candidate to treat colon cancer. Moreover, berberine works well against CRC and shows negligible toxicity. Furthermore, most alkaloids currently available in the market against various diseases are largely extracted from medicinal plants. Therefore, plant-derived alkaloids provide a potential and novel therapeutic source for effectively managing CRC.

Recent evidence suggests that diet and its related factors (hormonal alteration) are also the leading causes for the generation of 70 to 90% of CRC cases. Food material influences the gut microbiome composition (bacterial strains), which plays a vital role in the colon cancer progression. The gut microbiome is known to be occupied with two significant strains Bacteroides and Prevotella. However, a diet containing fruits and vegetables increases the Prevotella strain in the gut, which can break down the dietary phytochemicals in the lumen into tiny fatty acids like butyric acid. Multiple pieces of evidence revealed that butyric acid exhibits promising anti-cancer and anti-inflammatory effects. Taken together, dietary phytochemicals like alkaloids, which can modulate the gut microbiome, improve the host's immunity, up regulate the intracellular ROS, and thus could be of promising

10.3389/fendo.2023.1201198

TABLE 2 Phytocompounds assessed in clinical trials for colon cancer treatment.

Phytocompound	Biological source and Class of the compound	Structure	Information (type of clinical trial, number of patients, period, and outcome)	Status of the study	Reference
Berberine	Plant: *Berberis vulgaris*, *Berberis thunbergii*, *Berberis darwinii*. Family: Berberidaceae. Plant: *Rhizoma coptidis*, Family: Ranunculaceae. Type: Isoquinoline alkaloid		In Phase II clinical study, fixed dose of berberine hydrochloride, reduces the chances of reoccurrence and generation of another new colorectal adenomas. This study was done with 1000 participants for 3 years	Completed	(33), NCT03281096
Camptothecin	Plant: *Camptotheca acuminate*. Family: Nyssaceae. Type: Alkaloid		In clinical trials phase II, 100 mg administration of Camptothecin to metastatic colon cancer patients shows partial response in patients with liver metastasis. In this study 67 patients with metastatic colorectal cancer were participated for nearly 2 years.	Completed	(30)
Curcumin	Plant: Curcuma longa. Family: Zingiberaceae. Type: Polyphenol compound		In phase I clinical study, curcumin capsules containing 3.6g of curcumin administered to patients suffering with advanced CRC that shows resistance to available chemotherapy drugs. This study was done on 23 patients suffering with advanced colon tumor were for 4 months.	Completed	(144)
Epigallocatechin	Plant: *Camellia sinensis*. Family: Theaceae. Type: Polyphenol compound		In early phase I clinical trials, Teavigo™ a natural extra purified green tea extract with 94% Epigallocatechin induces alteration in methylation pattern as compared to control. In this study fifty after surgery colon cancer patients were taken part for 1 year study.	Study in progress (estimated date of completion-June, 2024)	(141), NCT02891538
Fisetin	Plant: *Fragaria ananassa*, Family: Rosaceae. Plant: *Malus domestica*, Family: Rosaceae. Type: Flavonoid		In double-blind, randomized placebo-controlled clinical trial, the fisetin was evaluated for its anti-inflammatory effect in the colon cancer patient receiving chemotherapy treatment. In this study 100mg of fisetin administered to the patient prior to the chemotherapy and found the decreased level of inflammatory markers. This study was done on 37 colon cancer patients for seven weeks.	Completed	(32), IRCT2015110511288N9
Lycopene	Plant: *Solanum lycopersicum*. Family: Solanaceae.		In clinical trials phase II, 20 mg of lycopene reduced the skin toxicity of metastasis CRC patients with panitumumab	Completed	(31), NCT03167268

(Continued)

TABLE 2 Continued

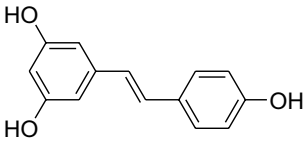
Phytocompound	Biological source and Class of the compound	Structure	Information (type of clinical trial, number of patients, period, and outcome)	Status of the study	Reference
	Type: Carotenoids		treatment. In this study 28 patients received with anti-EGFR inhibitor were participated for 12 weeks.		
Resveratrol	Plant: *Vaccinium uliginosum*, Family: Ericaceae. Plant: *Vitis vinifera*, Family: Vitaceae. Plant: *Ribes nigrum*, Family: Grossulariaceae. Type: Polyphenol compound		In Phase I clinical study, resveratrol of 20 mg in the form of tablet was administered to the patient with CRC. This compound found to alter Wnt signaling in colon cancer. 11 colon cancer patients were involved in this study for 14 days.	Completed	(142, 143), NCT00256334

FIGURE 6
Various sources that promote colon cancer progression. Effects of alkaloids in reducing the colon cancer growth *via* increasing ROS production and modulating the important signals that trigger apoptosis are also shown.

therapeutic value against CRC. Hence, it may be concluded that consuming diet that contains fruits and vegetables that possess secondary metabolites like alkaloids can better control various kinds of cancers including colon cancer.

Author contributions

Conceptualization: VKN, SR; Writing—original draft: VKN, MN, JM, MS, NeKJ; Writing—review and editing: VKN, GB, YJ, OA, HS, AM, DN, AA, AJ, KKK, RRM, VM, NiKJ, AK, PS, SR; Illustrations: NiKJ; Supervision: PS, SR. All authors contributed to the article and approved the submitted version.

Acknowledgments

The authors thank Raghavendra Institute of Pharmaceutical Education & Research (Autonomous), Anantapuramu, Andhra Pradesh, India, and Assam University, Silchar, India for providing support. The authors would like to acknowledge BioRender (BioRender.com) for the illustrations.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1201198/full#supplementary-material

References

1. Ma X, Yu H. Global burden of cancer. *Yale J Biol Med* (2006) 79(3-4):85–94.

2. Nelson VK, Sahoo NK, Sahu M, Sudhan HH, Pullaiah CP, Muralikrishna KS. *In vitro* anticancer activity of eclipta alba whole plant extract on colon cancer cell HCT-116. *BMC Complement Med Ther* (2020) 20(1):355. doi: 10.1186/s12906-020-03118-9

3. Abane R, Mezger V. Roles of heat shock factors in gametogenesis and development. *FEBS J* (2010) 277(20):4150–72. doi: 10.1111/j.1742-4658.2010.07830.x

4. Zaytseva Y. Lipid metabolism as a targetable metabolic vulnerability in colorectal cancer. *Cancers (Basel)* (2021) 13(2):1–5. doi: 10.3390/cancers13020301

5. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. *Lancet* (2019) 394(10207):1467–80. doi: 10.1016/s0140-6736(19)32319-0

6. Sawicki T, Ruszkowska M, Danielewicz A, Niedźwiedzka E, Arłukowicz T, Przybyłowicz KE. A review of colorectal cancer in terms of epidemiology, risk factors, development, symptoms and diagnosis. *Cancers (Basel)* (2021) 13(9):1–23. doi: 10.3390/cancers13092025

7. Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. *Transl Oncol* (2021) 14(10):101174. doi: 10.1016/j.tranon.2021.101174

8. White A, Ironmonger L, Steele RJC, Ormiston-Smith N, Crawford C, Seims A. A review of sex-related differences in colorectal cancer incidence, screening uptake, routes to diagnosis, cancer stage and survival in the UK. *BMC Cancer* (2018) 18(1):906. doi: 10.1186/s12885-018-4786-7

9. Brahmkhatri VP, Prasanna C, Atreya HS. Insulin-like growth factor system in cancer: novel targeted therapies. *BioMed Res Int* (2015) 2015:538019. doi: 10.1155/2015/538019

10. Chainy GBN, Sahoo DK. Hormones and oxidative stress: an overview. *Free Radic Res* (2020) 54(1):1–26. doi: 10.1080/10715762.2019.1702656

11. Sahoo DK, Chainy GBN. Hormone-linked redox status and its modulation by antioxidants. *Vitam Horm* (2023) 121:197–246. doi: 10.1016/bs.vh.2022.10.007

12. Lukas M. Inflammatory bowel disease as a risk factor for colorectal cancer. *Dig Dis* (2010) 28(4-5):619–24. doi: 10.1159/000320276

13. Sahoo DK, Borcherding DC, Chandra L, Jergens AE, Atherly T, Bourgois-Mochel A, et al. Differential transcriptomic profiles following stimulation with lipopolysaccharide in intestinal organoids from dogs with inflammatory bowel disease and intestinal mast cell tumor. *Cancers (Basel)* (2022) 14(14):1–59. doi: 10.3390/cancers14143525

14. Gavrilas LI, Cruceriu D, Mocan A, Loghin F, Miere D, Balacescu O. Plant-derived bioactive compounds in colorectal cancer: insights from combined regimens with conventional chemotherapy to overcome drug-resistance. *Biomedicines* (2022) 10 (8):1–14. doi: 10.3390/biomedicines10081948

15. Braun MS, Seymour MT. Balancing the efficacy and toxicity of chemotherapy in colorectal cancer. *Ther Adv Med Oncol* (2011) 3(1):43–52. doi: 10.1177/1758834010388342

16. Pardini B, Kumar R, Naccarati A, Novotny J, Prasad RB, Forsti A, et al. 5-fluorouracil-based chemotherapy for colorectal cancer and MTHFR/MTRR genotypes. *Br J Clin Pharmacol* (2011) 72(1):162–3. doi: 10.1111/j.1365-2125.2010.03892.x

17. Dutta N, Ghosh S, Nelson VK, Sareng HR, Majumder C, Mandal SC, et al. Andrographolide upregulates protein quality control mechanisms in cell and mouse through upregulation of mTORC1 function. *Biochim Biophys Acta Gen Subj* (2021) 1865(6):129885. doi: 10.1016/j.bbagen.2021.129885

18. Ghosh S, Hazra J, Pal K, Nelson VK, Pal M. Prostate cancer: therapeutic prospect with herbal medicine. *Curr Res Pharmacol Drug Discovery* (2021) 2:100034. doi: 10.1016/j.crphar.2021.100034

19. Pullaiah CP, Nelson VK, Rayapu S, Nk G V, Kedam T. Exploring cardioprotective potential of esculetin against isoproterenol induced myocardial toxicity in rats: *in vivo* and *in vitro* evidence. *BMC Pharmacol Toxicol* (2021) 22 (1):43. doi: 10.1186/s40360-021-00510-0

20. Dutta N, Pemmaraju DB, Ghosh S, Ali A, Mondal A, Majumder C, et al. Alkaloid-rich fraction of ervatamia coronaria sensitizes colorectal cancer through modulating AMPK and mTOR signalling pathways. *J Ethnopharmacol* (2022) 283:114666. doi: 10.1016/j.jep.2021.114666

21. Nelson VK, Paul S, Roychoudhury S, Oyeyemi IT, Mandal SC, Kumar N, et al. Heat shock factors in protein quality control and spermatogenesis. *Adv Exp Med Biol* (2022) 1391:181–99. doi: 10.1007/978-3-031-12966-7_11

22. Nelson VK, Pullaiah CP, Saleem Ts M, Roychoudhury S, Chinnappan S, Vishnusai B, et al. Natural products as the modulators of oxidative stress: an herbal approach in the management of prostate cancer. *Adv Exp Med Biol* (2022) 1391:161–79. doi: 10.1007/978-3-031-12966-7_10

23. De S, Paul S, Manna A, Majumder C, Pal K, Casarcia N, et al. Phenolic phytochemicals for prevention and treatment of colorectal cancer: a critical evaluation of *In vivo* studies. *Cancers (Basel)* (2023) 15(3):1–66. doi: 10.3390/cancers15030993

24. Badavenkatappa Gari S, Nelson VK, Peraman R. Tinospora sinensis (Lour.) merr alkaloid rich extract induces colon cancer cell death *via* ROS mediated, mTOR dependent apoptosis pathway: "an *in-vitro* study". *BMC Complement Med Ther* (2023) 23(1):33. doi: 10.1186/s12906-023-03849-5

25. Nelson VK, Ali A, Dutta N, Ghosh S, Jana M, Ganguli A, et al. Azadiradione ameliorates polyglutamine expansion disease in drosophila by potentiating DNA binding activity of heat shock factor 1. *Oncotarget* (2016) 7(48):78281–96. doi: 10.18632/oncotarget.12930

26. Liou GY, Storz P. Reactive oxygen species in cancer. *Free Radic Res* (2010) 44 (5):479–96. doi: 10.3109/10715761003667554

27. Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat Rev Drug Discovery* (2009) 8(7):579–91. doi: 10.1038/nrd2803

28. Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy. *Nat Rev Drug Discovery* (2013) 12(12):931–47. doi: 10.1038/nrd4002

29. NavaneethaKrishnan S, Rosales JL, Lee KY. Targeting Cdk5 for killing of breast cancer cells *via* perturbation of redox homeostasis. *Oncoscience* (2018) 5(5-6):152–4. doi: 10.18632/oncoscience.431

30. Shimada Y, Yoshino M, Wakui A, Nakao I, Futatsuki K, Sakata Y, et al. Phase II study of CPT-11, a new camptothecin derivative, in metastatic colorectal cancer. CPT-11 gastrointestinal cancer study group. *J Clin Oncol* (1993) 11(5):909–13. doi: 10.1200/jco.1993.11.5.909

31. Kapała A, Szlendak M, Motacka E. The anti-cancer activity of lycopene: a systematic review of human and animal studies. *Nutrients* (2022) 14(23):1–44. doi: 10.3390/nu14235152

32. Farsad-Naeimi A, Alizadeh M, Esfahani A, Darvish Aminabad E. Effect of fisetin supplementation on inflammatory factors and matrix metalloproteinase enzymes in colorectal cancer patients. *Food Funct* (2018) 9(4):2025–31. doi: 10.1039/c7fo01898c

33. Jiang X, Jiang Z, Jiang M, Sun Y. Berberine as a potential agent for the treatment of colorectal cancer. *Front Med (Lausanne)* (2022) 9:886996. doi: 10.3389/fmed.2022.886996

34. Aggarwal V, Tuli HS, Varol A, Thakral F, Yerer MB, Sak K, et al. Role of reactive oxygen species in cancer progression: molecular mechanisms and recent advancements. *Biomolecules* (2019) 9(11):1–26. doi: 10.3390/biom9110735

35. Sahoo DK, Allenspach K, Mochel JP, Parker V, Rudinsky AJ, Winston JA, et al. Synbiotic-IgY therapy modulates the mucosal microbiome and inflammatory indices in dogs with chronic inflammatory enteropathy: a randomized, double-blind, placebo-controlled study. *Vet Sci* (2022) 10(1):1–18. doi: 10.3390/vetsci10010025

36. Guo YJ, Pan WW, Liu SB, Shen ZF, Xu Y, Hu LL. ERK/MAPK signalling pathway and tumorigenesis. *Exp Ther Med* (2020) 19(3):1997–2007. doi: 10.3892/etm.2020.8454

37. Zhang Y, Gan B, Liu D. & paik J FoxO family members in cancer. *Cancer Biol Ther* (2011) 12:4. doi: 10.4161/cbt.12.4.15954

38. Dai L, Zhuang L, Zhang B, Wang F, Chen X, Xia C, et al. DAG/PKCδ and IP3/Ca^{2+}/CaMK IIβ operate in parallel to each other in PLCγ1-driven cell proliferation and migration of human gastric adenocarcinoma cells, through Akt/mTOR/S6 pathway. *Int J Mol Sci* (2015) 16(12):28510–22. doi: 10.3390/ijms161226116

39. Hwang AB, Lee SJ. Regulation of life span by mitochondrial respiration: the HIF-1 and ROS connection. *Aging* (2011), 304–10.

40. Guba M, Seeliger H, Kleespies A, Jauch KW, Bruns C. Vascular endothelial growth factor in colorectal cancer. *Int J Colorectal Dis* (2004) 19(6):510–7. doi: 10.1007/s00384-003-0576-y

41. Lee YJ, Karl DL, Maduekwe UN, Rothrock C, Ryeom S, D'Amore PA, et al. Differential effects of VEGFR-1 and VEGFR-2 inhibition on tumor metastases based on host organ environment. *Cancer Res* (2010) 70(21):8357–67. doi: 10.1158/0008-5472.CAN-10-1138

42. Lan T, Chen L, Wei X. Inflammatory Cytokines in cancer: comprehensive understanding and clinical progress in gene therapy. *Cells* (2021) 10(1):100. doi: 10.3390/cells10010100

43. NavaneethaKrishnan S, Rosales JL, Lee KY. ROS-mediated cancer cell killing through dietary phytochemicals. *Oxid Med Cell Longev* (2019) 2019:9051542. doi: 10.1155/2019/9051542

44. Sreevalsan S, Safe S. REACTIVE OXYGEN SPECIES AND COLORECTAL CANCER. *Curr Colorectal Cancer Rep* (2013) 9(4):350–7. doi: 10.1007/s11888-013-0190-5

45. Ahmed K, Zaidi SF, Cui ZG, Zhou D, Saeed SA, Inadera H. Potential proapoptotic phytochemical agents for the treatment and prevention of colorectal cancer. *Oncol Lett* (2019) 18(1):487–98. doi: 10.3892/ol.2019.10349

46. Hill MM, Adrain C, Duriez PJ, Creagh EM, Martin SJ. Analysis of the composition, assembly kinetics and activity of native apaf-1 apoptosomes. *EMBO J* (2004) 23(10):2134–45. doi: 10.1038/sj.emboj.7600210

47. Garrido C, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G. Mechanisms of cytochrome c release from mitochondria. *Cell Death Differ* (2006) 13(9):1423–33. doi: 10.1038/sj.cdd.4401950

48. Silke J, Meier P. Inhibitor of apoptosis (IAP) proteins-modulators of cell death and inflammation. *Cold Spring Harb Perspect Biol* (2013) 5(2). doi: 10.1101/cshperspect.a008730

49. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol* (2007) 35(4):495–516. doi: 10.1080/01926230701320337

50. Bruckheimer EM, Cho SH, Sarkiss M, Herrmann J, McDonnell TJ. The bcl-2 gene family and apoptosis. *Adv Biochem Eng Biotechnol* (1998) 62:75–105. doi: 10.1007/BFb0102306

51. Hardwick JM, Soane L. Multiple functions of BCL-2 family proteins. *Cold Spring Harb Perspect Biol* (2013) 5(2). doi: 10.1101/cshperspect.a008722

52. Hempel N, Trebak M. Crosstalk between calcium and reactive oxygen species signaling in cancer. *Cell Calcium* (2017) 63:70–96. doi: 10.1016/j.ceca.2017.01.007

53. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim Biophys Acta* (2016) 1863(12):2977–92. doi: 1016/j.bbamcr.2016.09.012

54. Park DR, Thomsen AR, Frevert CW, Pham U, Skerrett SJ, Kiener PA, et al. Fas (CD95) induces proinflammatory cytokine responses by human monocytes and monocyte-derived macrophages. *J Immunol* (2003) 170(12):6209–16. doi: 10.4049/jimmunol.170.12.6209

55. Volpe E, Sambucci M, Battistini L, Borsellino G. Fas-fas ligand: checkpoint of T cell functions in multiple sclerosis. *Front Immunol* (2016) 7:382. doi: 10.3389/fimmu.2016.00382

56. Pobezinskaya YL, Liu Z. The role of TRADD in death receptor signaling. *Cell Cycle* (2012) 11(5):871–6. doi: 10.4161/cc.11.5.19300

57. Huang YT, Liu CH, Yang YC, Aneja R, Wen SY, Huang CY, et al. ROS- and HIF1α-dependent IGFBP3 upregulation blocks IGF1 survival signaling and thereby mediates high-glucose-induced cardiomyocyte apoptosis. *J Cell Physiol* (2019) 234 (8):13557–70. doi: 10.1002/jcp.28034

58. Akhtar R, Chandel S, Sarotra P, Medhi B. Current status of pharmacological treatment of colorectal cancer. *World J Gastrointest Oncol* (2014) 6(6):177–83. doi: 10.4251/wjgo.v6.i6.177

59. Cassidy J, Misset JL. Oxaliplatin-related side effects: characteristics and management. *Semin Oncol* (2002) 29(5 Suppl 15):11–20. doi: 10.1053/sonc.2002.35524

60. Reynolds NA, Wagstaff AJ. Cetuximab: in the treatment of metastatic colorectal cancer. *Drugs* (2004) 64(1):109–18. doi: 10.2165/00003495-200464010-00007

61. Willems E, Gerne L, George C, D'Hondt M. Adverse effects of bevacizumab in metastatic colorectal cancer: a case report and literature review. *Acta Gastroenterol Belg* (2019) 82(2):322–5.

62. Battaglin F, Puccini A, Ahcene Djaballah S, Lenz HJ. The impact of panitumumab treatment on survival and quality of life in patients with RAS wild-type metastatic colorectal cancer. *Cancer Manag Res* (2019) 11:5911–24. doi: 10.2147/cmar.S186042

63. Hirsch BR, Zafar SY. Capecitabine in the management of colorectal cancer. *Cancer Manag Res* (2011) 3:79–89. doi: 10.2147/cmr.S11250

64. Van der Jeught K, Xu HC, Li YJ, Lu XB, Ji G. Drug resistance and new therapies in colorectal cancer. *World J Gastroenterol* (2018) 24(34):3834–48. doi: 10.3748/wjg.v24.i34.3834

65. Hasegawa K, Saiura A, Takayama T, Miyagawa S, Yamamoto J, Ijichi M, et al. Adjuvant oral uracil-tegafur with leucovorin for colorectal cancer liver metastases: a randomized controlled trial. *PloS One* (2016) 11(9):e0162400. doi: 10.1371/journal.pone.0162400

66. Hata T, Hagihara K, Tsutsui A, Akamatsu H, Ohue M, Shingai T, et al. Administration method of adjuvant tegafur-uracil and leucovorin calcium in patients with resected colorectal cancer: a phase III study. *Oncologist* (2021) 26(5):e735–41. doi: 10.1002/onco.13724

67. Singh V, Sheikh A, Abourehab MAS, Kesharwani P. Dostarlimab as a miracle drug: rising hope against cancer treatment. *Biosensors (Basel)* (2022) 12(8):1–14. doi: 10.3390/bios12080617

68. Xie YH, Chen YX, Fang JY. Comprehensive review of targeted therapy for colorectal cancer. *Signal Transduct Target Ther* (2020) 5(1):22. doi: 10.1038/s41392-020-0116-z

69. Rotte A. Combination of CTLA-4 and PD-1 blockers for treatment of cancer. *J Exp Clin Cancer Res* (2019) 38(1):255. doi: 10.1186/s13046-019-1259-z

70. Savoia P, Astrua C, Fava P. Ipilimumab (Anti-Ctla-4 mab) in the treatment of metastatic melanoma: effectiveness and toxicity management. *Hum Vaccin Immunother* (2016) 12(5):1092–101. doi: 10.1080/21645515.2015.1129478

71. Kooshkaki O, Derakhshani A, Hosseinkhani N, Torabi M, Safaei S, Brunetti O, et al. Combination of ipilimumab and nivolumab in cancers: from clinical practice to ongoing clinical trials. *Int J Mol Sci* (2020) 21(12):1–28. doi: 10.3390/ijms21124427

72. Wookey V, Grothey A. Update on the role of pembrolizumab in patients with unresectable or metastatic colorectal cancer. *Therap Adv Gastroenterol* (2021) 14:17562848211024460. doi: 10.1177/17562848211024460

73. Berg D. Managing the side effects of chemotherapy for colorectal cancer. *Semin Oncol* (1998) 25(5 Suppl 11):53–9.

74. Golshani G, Zhang Y. Advances in immunotherapy for colorectal cancer: a review. *Therap Adv Gastroenterol* (2020) 13:1756284820917527. doi: 10.1177/1756284820917527

75. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

76. Hashem S, Ali TA, Akhtar S, Nisar S, Sageena G, Ali S, et al. Targeting cancer signaling pathways by natural products: exploring promising anti-cancer agents. *BioMed Pharmacother* (2022) 150:113054. doi: 10.1016/j.biopha.2022.113054

77. Choudhari AS, Mandave PC, Deshpande M, Ranjekar P, Prakash O. Phytochemicals in cancer treatment: from preclinical studies to clinical practice. *Front Pharmacol* (2019) 10:1614. doi: 10.3389/fphar.2019.01614

78. Rahman MA, Hannan MA, Dash R, Rahman MH, Islam R, Uddin MJ, et al. Phytochemicals as a complement to cancer chemotherapy: pharmacological

modulation of the autophagy-apoptosis pathway. *Front Pharmacol* (2021) 12:639628. doi: 10.3389/fphar.2021.639628

79. Greenwell M, Rahman PK. Medicinal plants: their use in anticancer treatment. *Int J Pharm Sci Res* (2015) 6(10):4103–12. doi: 10.13040/ijpsr.0975-8232.6(10).4103-12

80. Seca AML, Pinto DCGA. Biological potential and medical use of secondary metabolites. *Medicines (Basel)* (2019) 6(2):1–6. doi: 10.3390/medicines6020066

81. Dhyani P, Quispe C, Sharma E, Bahukhandi A, Sati P, Attri DC, et al. Anticancer potential of alkaloids: a key emphasis to colchicine, vinblastine, vincristine, vindesine, vinorelbine and vincamine. *Cancer Cell Int* (2022) 22(1):206. doi: 10.1186/s12935-022-02624-9

82. Huang M, Lu JJ, Ding J. Natural products in cancer therapy: past, present and future. *Nat Prod Bioprospect* (2021) 11(1):5–13. doi: 10.1007/s13659-020-00293-7

83. Xu Y, Fang F, Miriyala S, Crooks PA, Oberley TD, Chaiswing L, et al. KEAP1 is a redox sensitive target that arbitrates the opposing radiosensitive effects of parthenolide in normal and cancer cells. *Cancer Res* (2013) 73(14):4406–17. doi: 10.1158/0008-5472.Can-12-4297

84. Khan H, Alam W, Alsharif KF, Aschner M, Pervez S, Saso L. Alkaloids and colon cancer: molecular mechanisms and therapeutic implications for cell cycle arrest. *Molecules* (2022) 27(3):1–26. doi: 10.3390/molecules27030920

85. Heinrich M, Mah J, Amirkia V. Alkaloids used as medicines: structural phytochemistry meets biodiversity-an update and forward look. *Molecules* (2021) 26 (7):1–26. doi: 10.3390/molecules26071836

86. Gong X, Chen Z, Han Q, Chen C, Jing L, Liu Y, et al. Sanguinarine triggers intrinsic apoptosis to suppress colorectal cancer growth through disassociation between STRAP and MELK. *BMC Cancer* (2018) 18(1):578. doi: 10.1186/s12885-018-4463-x

87. Matkar SS, Wrischnik LA, Hellmann-Blumberg U. Sanguinarine causes DNA damage and p53-independent cell death in human colon cancer cell lines. *Chem Biol Interact* (2008) 172(1):63–71. doi: 10.1016/j.cbi.2007.12.006

88. Yin HQ, Kim YH, Moon CK, Lee BH. Reactive oxygen species-mediated induction of apoptosis by a plant alkaloid 6-methoxydihydrosanguinarine in HepG2 cells. *Biochem Pharmacol* (2005) 70(2):242–8. doi: 10.1016/j.bcp.2005.04.020

89. Dai W, Mu L, Cui Y, Li Y, Chen P, Xie H, et al. Berberine promotes apoptosis of colorectal cancer *via* regulation of the long non-coding RNA (lncRNA) cancer susceptibility candidate 2 (CASC2)/AU-binding factor 1 (AUF1)/B-cell CLL/Lymphoma 2 (Bcl-2) axis. *Med Sci Monit* (2019) 25:730–8. doi: 10.12659/msm.912082

90. Wang L, Liu L, Shi Y, Cao H, Chaturvedi R, Calcutt MW, et al. Berberine induces caspase-independent cell death in colon tumor cells through activation of apoptosis-inducing factor. *PloS One* (2012) 7(5):e36418. doi: 10.1371/journal.pone.0036418

91. Park JJ, Seo SM, Kim EJ, Lee YJ, Ko YG, Ha J, et al. Berberine inhibits human colon cancer cell migration *via* AMP-activated protein kinase-mediated downregulation of integrin β1 signaling. *Biochem Biophys Res Commun* (2012) 426 (4):461–7. doi: 10.1016/j.bbrc.2012.08.091

92. Hsu WH, Hsieh YS, Kuo HC, Teng CY, Huang HI, Wang CJ, et al. Berberine induces apoptosis in SW620 human colonic carcinoma cells through generation of reactive oxygen species and activation of JNK/p38 MAPK and FasL. *Arch Toxicol* (2007) 81(10):719–28. doi: 10.1007/s00204-006-0169-y

93. Li W, Hua B, Saud SM, Lin H, Hou W, Matter MS, et al. Berberine regulates AMP-activated protein kinase signaling pathways and inhibits colon tumorigenesis in mice. *Mol Carcinog* (2015) 54(10):1096–109. doi: 10.1002/mc.22179

94. Liu X, Ji Q, Ye N, Sui H, Zhou L, Zhu H, et al. Berberine inhibits invasion and metastasis of colorectal cancer cells *via* COX-2/PGE2 mediated JAK2/STAT3 signaling pathway. *PloS One* (2015) 10(5):e0123478. doi: 10.1371/journal.pone.0123478

95. Wu K, Yang Q, Mu Y, Zhou L, Liu Y, Zhou Q, et al. Berberine inhibits the proliferation of colon cancer cells by inactivating wnt/β-catenin signaling. *Int J Oncol* (2012) 41(1):292–8. doi: 10.3892/ijo.2012.1423

96. Li D, Zhang Y, Liu K, Zhao Y, Xu B, Xu L, et al. Berberine inhibits colitis-associated tumorigenesis *via* suppressing inflammatory responses and the consequent EGFR signaling-involved tumor cell growth. *Lab Invest* (2017) 97(11):1343–53. doi: 10.1038/labinvest.2017.71

97. Samadi P, Sarvarian P, Gholipour E, Asenjan KS, Aghebati-Maleki L, Motavalli R, et al. Berberine: a novel therapeutic strategy for cancer. *IUBMB Life* (2020) 72 (10):2065–79. doi: 10.1002/iub.2350

98. Zhao Y, Roy S, Wang C, Goel A. A combined treatment with berberine and andrographis exhibits enhanced anti-cancer activity through suppression of DNA replication in colorectal cancer. *Pharm (Basel)* (2022) 15(3):1–17. doi: 10.3390/ph15030262

99. Guan X, Zheng X, Vong CT, Zhao J, Xiao J, Wang Y, et al. Combined effects of berberine and evodiamine on colorectal cancer cells and cardiomyocytes *in vitro*. *Eur J Pharmacol* (2020) 875:173031. doi: 10.1016/j.ejphar.2020.173031

100. Rattanawong A, Payon V, Limpanasittikul W, Boonkrai C, Mutirangura A, Wonganan P. Cepharanthine exhibits a potent anticancer activity in p53-mutated colorectal cancer cells through upregulation of p21Waf1/Cip1. *Oncol Rep* (2018) 39 (1):227–38. doi: 10.3892/or.2017.6084

101. Han B, Jiang P, Li Z, Yu Y, Huang T, Ye X, et al. Coptisine-induced apoptosis in human colon cancer cells (HCT-116) is mediated by PI3K/Akt and mitochondrial-associated apoptotic pathway. *Phytomedicine* (2018) 48:152–60. doi: 10.1016/j.phymed.2017.12.027

102. Huang T, Xiao Y, Yi L, Li L, Wang M, Tian C, et al. Coptisine from rhizoma coptidis suppresses HCT-116 cells-related tumor growth *in vitro* and *in vivo*. *Sci Rep* (2017) 7:38524. doi: 10.1038/srep38524

103. Cao Q, Hong S, Li Y, Chen H, Shen Y, Shao K, et al. Coptisine suppresses tumor growth and progression by down-regulating MFG-E8 in colorectal cancer. *RSC Adv* (2018) 8(54):30937–45. doi: 10.1039/c8ra05806g

104. Manogaran P, Beeraka NM, Huang CY, Vijaya Padma V. Neferine and isoliensinine enhance 'intracellular uptake of cisplatin' and induce 'ROS-mediated apoptosis' in colorectal cancer cells - a comparative study. *Food Chem Toxicol* (2019) 132:110652. doi: 10.1016/j.fct.2019.110652

105. Manogaran P, Somasundaram B, Viswanadha VP. Reversal of cisplatin resistance by neferine/isoliensinine and their combinatorial regimens with cisplatin-induced apoptosis in cisplatin-resistant colon cancer stem cells (CSCs). *J Biochem Mol Toxicol* (2022) 36(3):e22967. doi: 10.1002/jbt.22967

106. Liu X, Zhang Y, Wu S, Xu M, Shen Y, Yu M, et al. Palmatine induces G2/M phase arrest and mitochondrial-associated pathway apoptosis in colon cancer cells by targeting AURKA. *Biochem Pharmacol* (2020) 175:113933. doi: 10.1016/j.bcp.2020.113933

107. Meng LH, Zhang H, Hayward L, Takemura H, Shao RG, Pommier Y. Tetrandrine induces early G1 arrest in human colon carcinoma cells by down-regulating the activity and inducing the degradation of G1-s-specific cyclin-dependent kinases and by inducing p53 and p21Cip1. *Cancer Res* (2004) 64 (24):9086–92. doi: 10.1158/0008-5472.Can-04-0313

108. Qin R, Shen H, Cao Y, Fang Y, Li H, Chen Q, et al. Tetrandrine induces mitochondria-mediated apoptosis in human gastric cancer BGC-823 cells. *PloS One* (2013) 8(10):e76486. doi: 10.1371/journal.pone.0076486

109. Li X, Zhen D, Lu X, Xu H, Shao Y, Xue Q, et al. Enhanced cytotoxicity and activation of ROS-dependent c-jun NH2-terminal kinase and caspase-3 by low doses of tetrandrine-loaded nanoparticles in lovo cells–a possible Trojan strategy against cancer. *Eur J Pharm Biopharm* (2010) 75(3):334–40. doi: 10.1016/j.ejpb.2010.04.016

110. Santos LS, Silva VR, Menezes LRA, Soares MBP, Costa EV, Bezerra DP. Xylopine induces oxidative stress and causes G(2)/M phase arrest, triggering caspase-mediated apoptosis by p53-independent pathway in HCT116 cells. *Oxid Med Cell Longev* (2017) 2017:7126872. doi: 10.1155/2017/7126872

111. Lorence A, Medina-Bolivar F, Nessler CL. Camptothecin and 10-hydroxycamptothecin from camptotheca acuminata hairy roots. *Plant Cell Rep* (2004) 22(6):437–41. doi: 10.1007/s00299-003-0708-4

112. Ha SW, Kim YJ, Kim W, Lee CS. Antitumor effects of camptothecin combined with conventional anticancer drugs on the cervical and uterine squamous cell carcinoma cell line SiHa. *Korean J Physiol Pharmacol* (2009) 13(2):115–21. doi: 10.4196/kjpp.2009.13.2.115

113. Park K, Abebe W, Fermin C, Reddy G, Habtemariam T, Chung J, et al. Hypoxia inhibition of camptothecin-induced apoptosis by bax loss. *Biologia* (2012) 67:616–21. doi: 10.2478/s11756-012-0037-6

114. Liskova V, Kajsik M, Chovancova B, Roller L, Krizanova O. Camptothecin, triptolide, and apoptosis inducer kit have differential effects on mitochondria in colorectal carcinoma cells. *FEBS Open Bio* (2022) 12(5):913–24. doi: 10.1002/2211-5463.13401

115. Wenzel U, Nickel A, Kuntz S, Daniel H. Ascorbic acid suppresses drug-induced apoptosis in human colon cancer cells by scavenging mitochondrial superoxide anions. *Carcinogenesis* (2004) 25(5):703–12. doi: 10.1093/carcin/bgh079

116. Guo Z, Wang Z, Liang R, Tian H, Chen X, Chen M. Reactive oxygen species activated by mitochondria-specific camptothecin prodrug for enhanced chemotherapy. *Bosn J Basic Med Sci* (2022) 22(6):934–48. doi: 10.17305/bjbms.2022.7194

117. Fujita K, Kubota Y, Ishida H, Sasaki Y. Irinotecan, a key chemotherapeutic drug for metastatic colorectal cancer. *World J Gastroenterol* (2015) 21(43):12234–48. doi: 10.3748/wjg.v21.i43.12234

118. Britten CD, Hilsenbeck SG, Eckhardt SG, Marty J, Mangold G, MacDonald JR, et al. Enhanced antitumor activity of 6-hydroxymethylacylfulvene in combination with irinotecan and 5-fluorouracil in the HT29 human colon tumor xenograft model. *Cancer Res* (1999) 59(5):1049–53.

119. Raymond E, Louvet C, Tournigand C, Coudray AM, Faivre S, De Gramont A, et al. Pemetrexed disodium combined with oxaliplatin, SN38, or 5-fluorouracil, based on the quantitation of drug interactions in human HT29 colon cancer cells. *Int J Oncol* (2002) 21(2):361–7. doi: 10.3892/ijo.21.2.361

120. Allegrini G, Goulette FA, Darnowski JW, Calabresi P. Thrombospondin-1 plus irinotecan: a novel antiangiogenic-chemotherapeutic combination that inhibits the growth of advanced human colon tumor xenografts in mice. *Cancer Chemother Pharmacol* (2004) 53(3):261–6. doi: 10.1007/s00280-003-0712-y

121. Di Bartolomeo M, Ciarlo A, Bertolini A, Barni S, Verusio C, Aitini E, et al. Capecitabine, oxaliplatin and irinotecan in combination, with bevacizumab (COI-b regimen) as first-line treatment of patients with advanced colorectal cancer. an Italian trials of medical oncology phase II study. *Eur J Cancer* (2015) 51(4):473–81. doi: 10.1016/j.ejca.2014.12.020

122. Cai Y, Deng R, Hu H, Zhang J, Ling J, Wu Z, et al. [Analysis on safety and preliminary efficacy of dose-modified regimen of 5-fluorouracil plus oxaliplatin and irinotecan (FOLFOXIRI) in advanced colorectal cancer]. *Zhonghua Wei Chang Wai Ke Za Zhi* (2018) 21(9):1045–50.

123. Reyes-Escogido Mde L, Gonzalez-Mondragon EG, Vazquez-Tzompantzi E. Chemical and pharmacological aspects of capsaicin. *Molecules* (2011) 16(2):1253–70. doi: 10.3390/molecules16021253

124. Yang KM, Pyo JO, Kim GY, Yu R, Han IS, Ju SA, et al. Capsaicin induces apoptosis by generating reactive oxygen species and disrupting mitochondrial transmembrane potential in human colon cancer cell lines. *Cell Mol Biol Lett* (2009) 14(3):497–510. doi: 10.2478/s11658-009-0016-2

125. Kim CS, Park WH, Park JY, Kang JH, Kim MO, Kawada T, et al. Capsaicin, a spicy component of hot pepper, induces apoptosis by activation of the peroxisome proliferator-activated receptor gamma in HT-29 human colon cancer cells. *J Med Food* (2004) 7(3):267–73. doi: 10.1089/jmf.2004.7.267

126. Kim YM, Hwang JT, Kwak DW, Lee YK, Park OJ. Involvement of AMPK signaling cascade in capsaicin-induced apoptosis of HT-29 colon cancer cells. *Ann N Y Acad Sci* (2007) 1095:496–503. doi: 10.1196/annals.1397.053

127. Lu HF, Chen YL, Yang JS, Yang YY, Liu JY, Hsu SC, et al. Antitumor activity of capsaicin on human colon cancer cells *in vitro* and colo 205 tumor xenografts *in vivo*. *J Agric Food Chem* (2010) 58(24):12999–3005. doi: 10.1021/jf103335w

128. Clark R, Lee J, Lee SH. Synergistic anticancer activity of capsaicin and 3,3'-diindolylmethane in human colorectal cancer. *J Agric Food Chem* (2015) 63(17):4297–304. doi: 10.1021/jf506098s

129. Kim MY, Trudel LJ, Wogan GN. Apoptosis induced by capsaicin and resveratrol in colon carcinoma cells requires nitric oxide production and caspase activation. *Anticancer Res* (2009) 29(10):3733–40.

130. Waziri PM, Abdullah R, Yeap SK, Omar AR, Kassim NK, Malami I, et al. Clausenidin induces caspase-dependent apoptosis in colon cancer. *BMC Complement Altern Med* (2016) 16:256. doi: 10.1186/s12906-016-1247-1

131. Song N, Ma J, Hu W, Guo Y, Hui L, Aamer M, et al. Lappaconitine hydrochloride inhibits proliferation and induces apoptosis in human colon cancer HCT-116 cells *via* mitochondrial and MAPK pathway. *Acta Histochem* (2021) 123 (5):151736. doi: 10.1016/j.acthis.2021.151736

132. Yaffe PB, Power Coombs MR, Doucette CD, Walsh M, Hoskin DW. Piperine, an alkaloid from black pepper, inhibits growth of human colon cancer cells *via* G1 arrest and apoptosis triggered by endoplasmic reticulum stress. *Mol Carcinog* (2015) 54 (10):1070–85. doi: 10.1002/mc.22176

133. DA Silva Machado F, Munari FM, Scariot FJ, Echeverrigaray S, Aguzzoli C, Pich CT, et al. Piperlongumine induces apoptosis in colorectal cancer HCT 116 cells independent of bax, p21 and p53 status. *Anticancer Res* (2018) 38(11):6231–6. doi: 10.21873/anticanres.12978

134. Chen W, Lian W, Yuan Y, Li M. The synergistic effects of oxaliplatin and piperlongumine on colorectal cancer are mediated by oxidative stress. *Cell Death Dis* (2019) 10(8):600. doi: 10.1038/s41419-019-1824-6

135. Kim GD. Harmine hydrochloride triggers G2/M cell cycle arrest and apoptosis in HCT116 cells through ERK and PI3K/AKT/mTOR signaling pathways. *Prev Nutr Food Sci* (2021) 26(4):445–52. doi: 10.3746/pnf.2021.26.4.445

136. Liu J, Li Q, Liu Z, Lin L, Zhang X, Cao M, et al. Harmine induces cell cycle arrest and mitochondrial pathway-mediated cellular apoptosis in SW620 cells *via* inhibition of the akt and ERK signaling pathways. *Oncol Rep* (2016) 35(6):3363–70. doi: 10.3892/or.2016.4695

137. Liang L, Wu J, Luo J, Wang L, Chen ZX, Han CL, et al. Oxymatrine reverses 5-fluorouracil resistance by inhibition of colon cancer cell epithelial-mesenchymal transition and NF-κB signaling *in vitro*. *Oncol Lett* (2020) 19(1):519–26. doi: 10.3892/ol.2019.11090

138. Pan D, Zhang W, Zhang N, Xu Y, Chen Y, Peng J, et al. Oxymatrine synergistically enhances doxorubicin anticancer effects in colorectal cancer. *Front Pharmacol* (2021) 12:673432. doi: 10.3389/fphar.2021.673432

139. Kumar A, Singh B, Sharma PR, Bharate SB, Saxena AK, Mondhe DM. A novel microtubule depolymerizing colchicine analogue triggers apoptosis and autophagy in HCT-116 colon cancer cells. *Cell Biochem Funct* (2016) 34(2):69–81. doi: 10.1002/cbf.3166

140. Huang Z, Xu Y, Peng W. Colchicine induces apoptosis in HT–29 human colon cancer cells *via* the AKT and c-jun n-terminal kinase signaling pathways. *Mol Med Rep* (2015) 12(4):5939–44. doi: 10.3892/mmr.2015.4222

141. Seufferlein T, Ettrich TJ, Menzler S, Messmann H, Kleber G, Zipprich A, et al. Green tea extract to prevent colorectal adenomas, results of a randomized, placebo-controlled clinical trial. *Am J Gastroenterol* (2022) 117(6):884–94. doi: 10.14309/ajg.0000000000001706

142. Singh AP, Singh R, Verma SS, Rai V, Kaschula CH, Maiti P, et al. Health benefits of resveratrol: evidence from clinical studies. *Med Res Rev* (2019) 39(5):1851–91. doi: 10.1002/med.21565

143. Wu XY, Zhai J, Huan XK, Xu WW, Tian J, Farhood B. A systematic review of the therapeutic potential of resveratrol during colorectal cancer chemotherapy. *Mini Rev Med Chem* (2022) 23(10):1137–52. doi: 10.2174/1389557522666220907145153

144. Sharma RA, Euden SA, Platton SL, Cooke DN, Shafayat A, Hewitt HR, et al. Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. *Clin Cancer Res* (2004) 10(20):6847–54. doi: 10.1158/1078-0432.Ccr-04-0744

10.3389/fendo.2023.1201198

Glossary

AIF	Apoptosis-inducing factor
AKT	Protein kinase B
APAF1	Apoptotic peptidase activating factor 1
APC	Adenomatous polyposis coli
ATF2	Activating transcription factor 2
AUF1	U-rich element RNA-binding factor 1
AURKA	Aurora kinase A
BAG	Bcl-2-associated athanogene
BAX	Bcl-2-associated X protein
BRAF	Proto-oncogene
CAD	Caspase-activated DNase
CAMP	Cyclic adenosine monophosphate
CHOP	C/EBP homologous protein
C-MYC	Cellular myelocytomatosis oncogene
DAG	Diacylglycerol
DIABLO	Direct IAP-binding protein with low pI
DNA	Deoxyribonucleic acid
E2F1	E2F transcription factor 1
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ELK-1	ETS-domain transcription factor
EMT	Epithelial–mesenchymal transition
ERK1/2	Extracellular signal-regulated kinases
ETS	Erythroblast transformation specific
FADD	Fas-associated death domain
FASL	Fas ligand
FOXO3	Forkhead box O3
G2	Growth 2
GSK-3	Glycogen synthase kinase-3
HIF-1α	Hypoxia-inducible factor 1-alpha
HTRA2 (OMI)	Nuclear-encoded mitochondrial serine protease
IGF-1	Insulin-like growth factor 1
IGFBP-3	Insulin-like growth factor (IGF) binding protein-3
IL	Interleukin
JNK	c-Jun N-terminal kinase
KDR	Kinase insert domain receptor
KI-67	Non-histone nuclear protein
α-KGDH	Alpha-ketoglutarate dehydrogenase
lncRNA CASC2	Long non-coding RNA cancer susceptibility candidate 2
MAPK	Mitogen-activated protein kinase
MEK	Mitogen-activated extracellular signal-regulated kinase
MELK	Maternal embryonic leucine zipper kinase
MFG-E8	Milk Fat Globule EGF And Factor V/VIII Domain Containing
MPTP	Mitochondrial permeability transition pore
mTOR	Mammalian target of rapamycin
NF-kB	Nuclear factor-kappa B
NRF2	Nuclear factor erythroid 2-related factor 2
p21Waf1	Cyclin-dependent kinase inhibitor 1 or CDK-interacting protein 1
P38	p38 mitogen-activated protein
P53	Tumor suppressor protein
PDK1	Phosphoinositide-dependent kinase-1
PI3K	Phosphatidylinositol 3-kinases
PIP2	Phosphatidylinositol 4, 5-bisphosphate
PIP3	Phosphatidylinositol (3, 4, 5)-trisphosphate
PLCγ1	Phospholipase C gamma 1
PUMA	p53 upregulated modulator of apoptosis
RAF	Rapidly accelerated fibrosarcoma
RAS	Rat sarcoma
RIP	Receptor-interacting protein kinases
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SMAC	Second mitochondria-derived activator of caspase
SOS1	Son of sevenless homolog 1
STAT3	Signal transducer and activator of transcription 3
STRAP	Serine-threonine kinase receptor-associated protein
TAK1	Transforming growth factor-β (TGF-β)-activated kinase 1
TGF-β	Transforming growth factor beta
TNFR1	Tumor necrosis factor receptor 1
TNFRSF21	Tumor necrosis factor receptor superfamily member 21
TNF-α	Tumor necrosis factor-alpha
TRADD	Tumor necrosis factor receptor type 1-associated death domain protein
TRAIL-R1	TNF-related apoptosis-inducing ligand receptor 1
TRAMP	Tumor necrosis factor (TNF) receptor family
VEGF	Vascular endothelial growth factor receptor
VEGFR-1	Vascular endothelial growth factor receptor 1
XIAP	X-linked inhibitor of apoptosis protein
WNT	Wingless-related integration site.

frontiers | Frontiers in Endocrinology

TYPE Systematic Review
PUBLISHED 28 August 2023
DOI 10.3389/fendo.2023.1184745

OPEN ACCESS

EDITED BY
Dipak Kumar Sahoo,
Iowa State University, United States

REVIEWED BY
Sunil Kumar Sahoo,
Department of Higher Education, Odisha, India
Sutapa Mukherjee,
Visva-Bharati University, India
Ashish Patel,
Hemchandracharya North Gujarat University, India

*CORRESPONDENCE
Negar Azarpira
negarazarpira@gmail.com

†These authors share senior authorship

RECEIVED 12 March 2023
ACCEPTED 10 August 2023
PUBLISHED 28 August 2023

CITATION
Dehdari Ebrahimi N, Sadeghi A, Shojaei-Zarghani S, Shahlaee MA, Taherifard E, Rahimian Z, Eghlidos Z, Azarpira N and Safarpour AR (2023) Protective effects of exogenous melatonin therapy against oxidative stress to male reproductive tissue caused by anti-cancer chemical and radiation therapy: a systematic review and meta-analysis of animal studies. *Front. Endocrinol.* 14:1184745. doi: 10.3389/fendo.2023.1184745

Protective effects of exogenous melatonin therapy against oxidative stress to male reproductive tissue caused by anti-cancer chemical and radiation therapy: a systematic review and meta-analysis of animal studies

Niloofar Dehdari Ebrahimi[1], Alireza Sadeghi[1,2†], Sara Shojaei-Zarghani[2,3], Mohammad Amin Shahlaee[1], Erfan Taherifard[4], Zahra Rahimian[5], Zahra Eghlidos[5], Negar Azarpira[1*] and Ali Reza Safarpour[2†]

[1]Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, [2]Gastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, [3]Colorectal Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, [4]MPH Department, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran, [5]Department of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Background: Male testicular dysfunction is a considerable complication of anti-cancer therapies, including chemotherapy and radiotherapy, partly due to the increased oxidative stress caused by these treatments. Melatonin is an effective antioxidant agent that protects testicles against physical and toxic chemical stressors in animal models. This study aims to systematically review the melatonin's protective effects against anti-cancer stressors on rodential testicular tissue.

Materials and Method: An extensive search was conducted in Web of Science, Scopus, and PubMed for animal studies investigating exogenous melatonin's protective effects on rodent testicles exposed to anti-cancer chemicals and radiotherapeutic agents. Using the DerSimonian and Laird random-effect model, standardized mean differences and 95% confidence intervals were estimated from the pooled data. The protocol was prospectively registered in the International Prospective Register of Systematic Reviews (PROSPERO: CRD42022355293).

Results: The meta-analysis included 38 studies from 43 studies that were eligible for the review. Rats and mice were exposed to radiotherapy (ionizing radiations such as gamma- and roentgen radiation and radioactive iodine) or chemotherapy (methotrexate, paclitaxel, busulfan, cisplatin, doxorubicin, vinblastine, bleomycin, cyclophosphamide, etoposide, Taxol, procarbazine, docetaxel, and chlorambucil). According to our meta-analysis, all outcomes were significantly improved by melatonin therapy, including sperm quantity and quality (count, motility, viability,

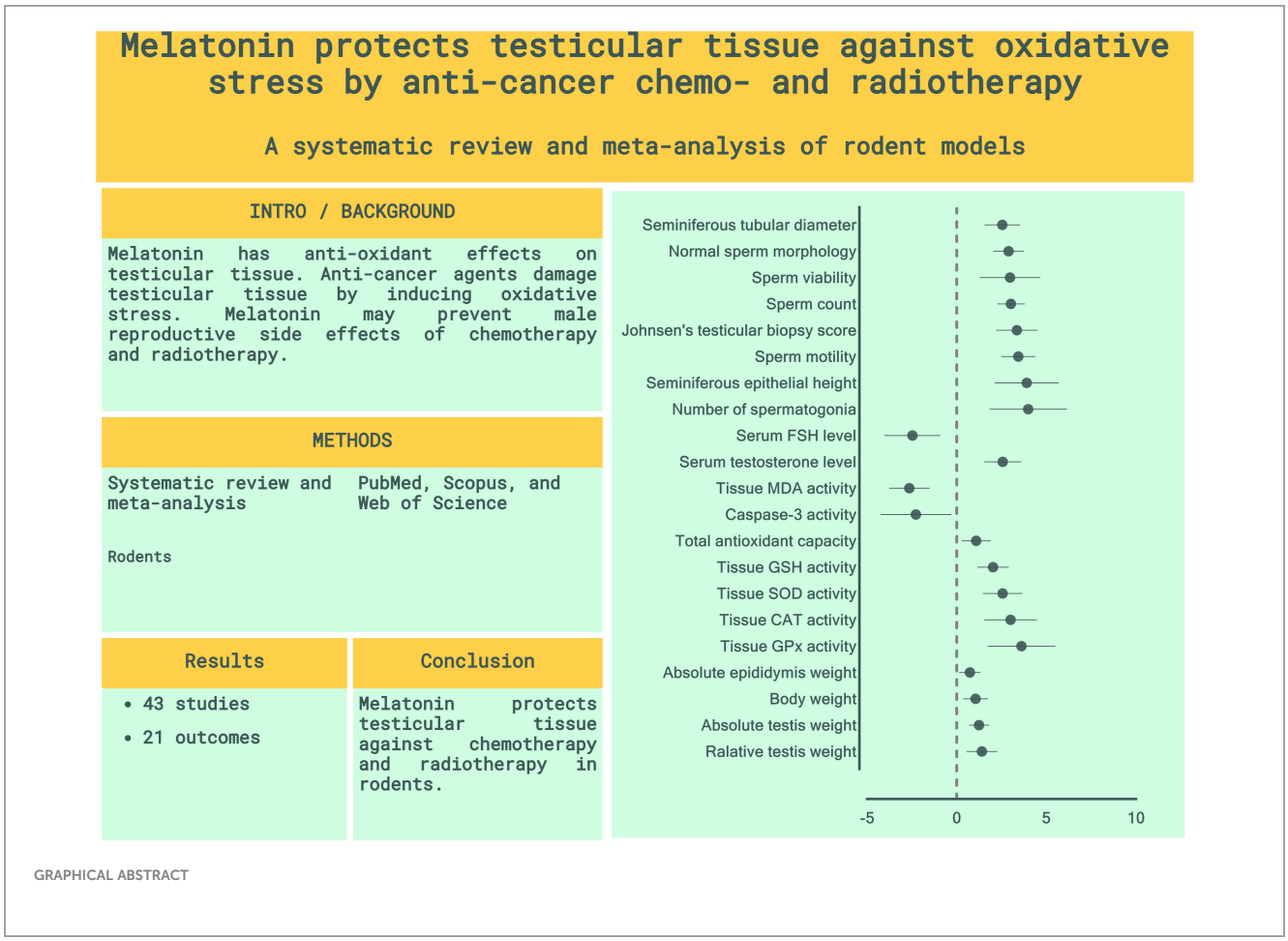
normal morphology, number of spermatogonia, Johnsen's testicular biopsy score, seminiferous tubular diameter, and seminiferous epithelial height), serum level of reproductive hormones (Follicle-Stimulating Hormone and testosterone), tissue markers of oxidative stress (testicular tissue malondialdehyde, superoxide dismutase, glutathione peroxidase, catalase, glutathione, caspase-3, and total antioxidant capacity), and weight-related characteristics (absolute body, epididymis, testis, and relative testis to body weights). Most SYRCLE domains exhibited a high risk of bias in the included studies. Also, significant heterogeneity and small-study effects were detected.

Conclusion: In male rodents, melatonin therapy was related to improved testicular histopathology, reproductive hormones, testis and body weights, and reduced levels of oxidative markers in testicular tissues of male rodents. Future meticulous studies are recommended to provide a robust scientific backbone for human applications.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022355293, identifier CRD42022355293.

KEYWORDS

rodents, melatonin, male reproduction, testicular tissue, cancer, radiotherapy, chemotherapy



GRAPHICAL ABSTRACT

1 Introduction

Cancer is the second dominant cause of death globally. In 2020, 19.3 million new patients with cancer were diagnosed, and 10 million deaths associated with cancer were detected worldwide (1). Radio- and chemotherapy are among the most common treatments for malignancies. These strategies are considered double-edged swords, which exert unwanted side effects on healthy tissues, including the male reproductive system. Radiotherapy and chemotherapy could cause male testicular dysfunction, partly by increasing testicular oxidative stress and subsequently inducing lipid peroxidation, DNA damage, mitochondrial dysfunction, and apoptosis (2, 3). These therapeutic methods could also trigger endoplasmic reticulum (ER) stress and inflammation in the testes, leading to cell death and potentially impairing male fertility (2, 4, 5). Differentiating spermatogonia cells are more sensitive than spermatocytes, spermatids, and Leydig cells, which produce testosterone, to the mentioned cytotoxic effects (6, 7). Radio- and chemotherapy are known to cause several reproductive impairments in males, including but not limited to a decrease in sperm count (oligozoospermia), absence of sperm in the ejaculate (azoospermia), morphological abnormalities in spermatozoa (teratozoospermia), low sperm motility (asthenozoospermia), and reduced sperm viability. These effects may persist for an extended period, possibly lifelong (3, 8). Furthermore, undergoing cancer treatments can lead to reduced testosterone levels, as well as compensatory damage to the hypothalamic-pituitary-gonadal axis and Sertoli cells (2, 9). With dramatically increased survival rates, especially in patients of younger ages, reducing the side effects of anti-cancer therapies and preserving fertility can improve their quality of life.

Melatonin is secreted naturally by the pineal gland and is known for its functions in circadian rhythms. Additionally, research is being conducted to evaluate its effects on various diseases, including cancer, cardiovascular disease, and metabolic disorders (10). Also, melatonin membrane receptors (MT1 and MT2) are detectable in several testicular cells, including Sertoli cells, Leydig cells, and germ cells (11), which suggest fundamental roles in the optimal reproductive function in the physiologic conditions (11–13). Decreased serum melatonin levels and downregulation of its receptors are reported following chemotherapy treatments (14–16). The administration of melatonin has been suggested as a potential protective measure against the adverse effects of radiotherapy and chemotherapy on multiple organs, including the brain, heart, kidney, liver, and intestine. This protective effect is thought to be mediated by various mechanisms, such as anti-inflammatory, antioxidant, anti-nitrosative, anti-apoptotic, immune regulatory, and antioxidant defense system-related gene expression regulatory properties (17, 18).

Several studies have investigated melatonin's protective properties against radiotherapy and chemotherapy-induced injuries on the male reproductive system (19–23). However, no meta-analysis study has reported the net effects and discussed the underlying mechanism. Therefore, we aimed to assess the impact of melatonin on radiotherapy- and chemotherapy-induced male reproductive dysfunction and shed light on the underlying mechanisms.

2 Materials and methods

This systematic review and meta-analysis was designed based on the Preferred Reporting Items for Systematic Reviews and Meta-analyses guideline (PRISMA) (24). Prospective protocol registration was done at the International Prospective Register of Systematic Reviews (PROSPERO: CRD42022355293).

2.1 Data sources and search

A comprehensive search strategy was developed using "melatonin" and "reproductive indices" and related terms. Three online databases (Web of Science, Scopus, and PubMed) were searched for studies published since January 1st, 1970, until September 9th, 2022. Moreover, to include additional studies, a manual backward and forward citation search was conducted for all included studies. The search strategy and syntax details are exhibited in Supplementary Material 1.

2.2 Study selection

The duplicate records were removed and uploaded to the Rayyan web-based tool for systematic review management (25). Three reviewers (NDE, ET, and MAS) screened the records independently by titles and abstracts. Then, full texts were retrieved for each study for screening by eligibility criteria. Disagreements were resolved through discussion.

Studies were considered eligible to include if they met the following criteria: (a) controlled animal studies, (b) included male rodents who were exposed to anti-cancer chemo- or radiotherapy agents, (c) in at least one intervention arm, melatonin was administered, (d) one or more positive control arms (with or without placebo), (e) The major characteristics of testicular tissue have been reported (sperm analyses, biochemical, and histopathologic). Studies were excluded if they had (a) ex-vivo and *in-vitro* designs, (b) non-rodent subjects, (c) stressors other than conventional anti-cancer chemo- and radiotherapy, and (d) a combination of melatonin and other drugs was administered. Furthermore, human trials, letters, and reviews were excluded from this review. We did not apply any restrictions based on the language or date of publication.

2.3 Data extraction and assessment of the risk of bias

Data extraction was performed into an Excel spreadsheet by four reviewers (NDE, NE, ZR, and MAS). The differences were resolved by discussion. Based on the results of each study, the following outcomes were extracted (if available): (a) study characteristics (first author, country, and publication year), (b) subject characteristics (sample size, age, and species), (c) chemical or radiation agent and their dosages, route of administration, and

duration of exposure, (d) melatonin's dosage, duration of therapy, administration route, and timing of administration relative to stressor, (e) sperm-related characteristics (count, motility, viability, normal morphology, number of spermatogonia, seminiferous epithelial height, Johnsen's testicular biopsy score (JTBS), and seminiferous tubular diameter), (f) serum reproductive hormone levels (Follicle-Stimulating Hormone (FSH) and testosterone), (g) tissue oxidative stress markers (glutathione (GSH), Catalase (CAT), testicular tissue Superoxide dismutase (SOD), Malondialdehyde (MDA), glutathione peroxidase (GPx), Caspase-3, and Total Antioxidant Capacity (TAC)), and (h) weight-related characteristics (absolute body, testis, epididymis, and relative testis to body weights).

Based on the Systematic Review Centre for Laboratory Animal Experiments (SYRCLE) tool for animal intervention studies, the risk of bias was assessed independently by two reviewers (AS and NDE) (26).

2.4 Data synthesis and statistical analysis

Data were analyzed using Stata MP Version 16 (StataCorp, College Station, TX, USA), and a p-value <0.05 was considered statistically significant. Based on the DerSimonian-Laird method, a random effect model was utilized to pool the effect sizes using Standardized Mean Difference (SMD) for meta-analyses. Also, a 95% confidence interval (CI) was reported for each effect size. The residual heterogeneity between studies was evaluated using the Cochran's Q statistic, I-squared, and p-value. I-squared was interpreted as "perhaps not important", "moderate heterogeneity", "substantial heterogeneity", and "considerable heterogeneity" when values were 0-40%, 30-60%, 50-90%, and 75-100%, respectively (27). Multiple intervention arms were combined using Cochrane's formula to avoid overcalculations in the studies with shared control groups (27). To identify potential sources of heterogeneity, subgroup analyses were applied only in cases of three or more available studies per subgroup. Also, to obtain missing data, reviewers tried to reach the authors via email and waited for at least one month for responses. Studies were removed from the analyses if their missing data were crucial. Also, when minimum, median, quartiles, and maximum were the only available statistics, mean and standard deviation were estimated using previously published statistical methods (28, 29). Furthermore, funnel plots were developed for outcomes with more than ten studies (27). Visual inspection for asymmetry and Egger's regression test for small-study effects were done to detect publication bias (30).

3 Results

3.1 Search results

A total of 10,039 and 5 records were obtained from the systematic database and manual citation searching, respectively. The title and abstract of 9,028 unique documents were screened after omitting 1,016 duplicate records. 97 articles were checked for eligibility, and a final 43 articles were included in the systematic review. Among the included studies, 5 (21, 31–34) were only included in the narrative evidence synthesis, and 38 were used in the meta-analyses. The PRISMA flow diagram is presented in Figure 1.

3.2 Study characteristics

Included studies were published between 2003 and 2023 in English (n=36) (14, 19–22, 31–66) and Persian (n=2) (67, 68). The studies were published from Iran (n=11) (20, 40, 45, 53–55, 59, 61, 65, 67, 68), Turkey (n=9) (21, 31, 36–39, 43, 47, 64), Egypt (n=9) (33–35, 41, 42, 44, 46, 48, 60), China (n=7) (14, 19, 32, 49, 62, 63, 66), India (n=4) (22, 50, 52, 57), Thailand (n=1) (58), Nigeria (n=1) (56), and South Korea (n=1) (51). Studies employed rats (n=25) (20–22, 31, 34–37, 39, 41–44, 46–48, 51, 56–58, 61, 62, 64, 65, 68) and mice (n=18) (14, 19, 32, 33, 38, 40, 45, 49, 50, 52–55, 59, 60, 63, 66, 67) as subjects. To induce stress, the included studies employed ionizing radiations (n=9) (21, 39, 46, 48–50, 59, 60, 64) and chemical agents (n=34) (14, 19, 20, 22, 31–38, 40–45, 47, 51–58, 61–63, 65–68). For chemical therapy, methotrexate (58, 62), paclitaxel (63), busulfan (19, 32–34, 45, 53–55, 65, 67, 68), cisplatin (14, 20, 22, 37, 40–44, 47), doxorubicin (51, 57, 66), vinblastine (22), bleomycin (20, 22), cyclophosphamide (31, 47, 52, 61), etoposide (20), Taxol (35), procarbazine (36), docetaxel (38), and chlorambucil (56) were employed. Melatonin was administered intraperitoneal (IP, n=32) (14, 19–22, 31–41, 43, 45–47, 49, 50, 53–55, 58–61, 63–68) and oral (n=8) (42, 44, 48, 51, 52, 56, 57, 62). Detailed study characteristics, including stressor and melatonin dosages, duration of exposure to each one, and number and age of rodents, are provided in Table 1 and Supplementary Material 2.

3.3 Outcomes

The pooled SMDs were statistically significant for all of the 21 outcomes. The outcomes were classified into four categories: (a) sperm-related parameters, (b) reproductive hormones, (c) markers of oxidative stress and apoptosis in testicular tissue, and (d) body and testicular weights. The pooled outcomes included absolute epididymis, testis, and body weights, testis to body relative weight, caspase-3 activity, tissue CAT, GPX, MDA, SOD, and GSH activity, TAC, serum FSH and testosterone levels, JTBS, normal sperm morphology, number of spermatogonia, seminiferous epithelial height, seminiferous tubular diameter, sperm count, motility, and viability. The overall pooled effect sizes for each outcome are summarized in the Figure 2. Detailed forest plots of the overall pooled effects sizes for each outcome are presented in Figures 3–6.

3.3.1 Sperm-related parameters

The pooled SMDs for each sperm-related parameter were: JTBS (SMD = 3.36, 95% CI: 2.21 to 4.51, p-value <0.01), normal sperm morphology (SMD = 2.9, 95% CI: 2.04 to 3.76, p-value <0.01), number of spermatogonia (SMD = 3.99, 95% CI: 1.83 to 6.16, p-value <0.01), seminiferous epithelial height (SMD = 3.91, 95% CI: 2.12 to 5.7, p-value <0.01), seminiferous tubular diameter (SMD =

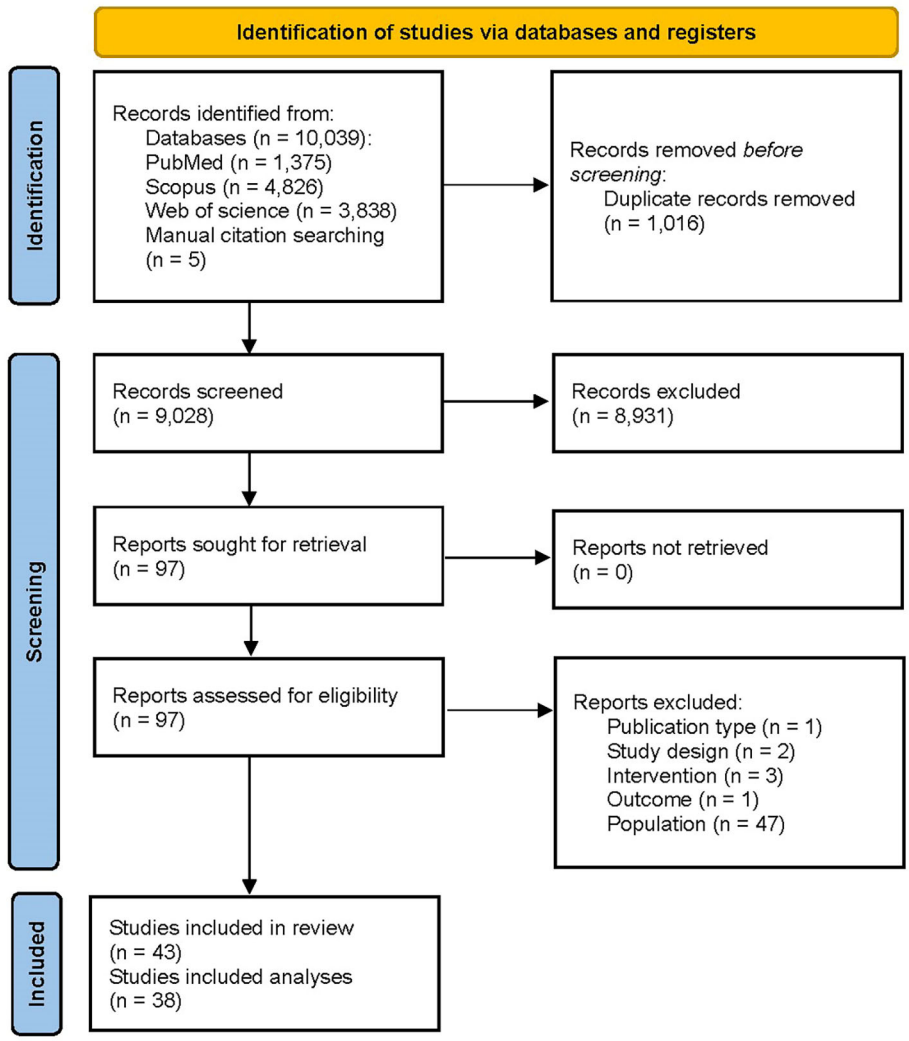


FIGURE 1
PRISMA flow diagram illustrating the process of selection of the studies.

2.55, 95% CI: 1.56 to 3.54, p-value <0.01), sperm count (SMD = 3.03, 95% CI: 2.26 to 3.79, p-value <0.01), motility (SMD = 3.44, 95% CI: 2.5 to 4.39, p-value <0.01), and viability (SMD = 2.98, 95% CI: 1.29 to 4.68, p-value <0.01). Between-study heterogeneity was substantial to considerable for sperm-related parameters with JTBS (I^2 = 75.88% and p-value for Q test <0.01), normal sperm morphology (I^2 = 78.48% and p-value for Q test <0.01), number of spermatogonia (I^2 = 86.16% and p-value for Q test <0.01), seminiferous epithelial height (I^2 = 90.1% and p-value for Q test <0.01), seminiferous tubular diameter (I^2 = 80.84% and p-value for Q test <0.01), sperm count (I^2 = 82.04% and p-value for Q test <0.01), motility (I^2 = 83.15% and p-value for Q test <0.01), and viability (I^2 = 88.72% and p-value for Q test <0.01).

3.3.2 Reproductive hormones

The combined SMDs for serum FSH and testosterone levels were (SMD = -2.47, 95% CI: -4.03 to -0.9, p-value <0.01) and (SMD = 2.57, 95% CI: 1.54 to 3.6, p-value <0.01), respectively. Between-study heterogeneity was considerable for serum reproductive hormone levels with FSH (I^2 = 88.9% and p-value for Q test <0.01) and testosterone (I^2 = 89.17% and p-value for Q test <0.01).

3.3.3 Testicular tissue's oxidative markers

For each oxidative marker, the pooled SMDs were as follows: caspase-3 (SMD = -2.28, 95% CI: -4.25 to -0.32, p-value = 0.02), tissue CAT (SMD = -2.28, 95% CI: -4.25 to -0.32, p-value = 0.02), GPX (SMD = 3.62, 95% CI: 1.73 to 5.5, p-value <0.01), MDA (SMD = -2.64, 95% CI: -3.76 to -1.52, p-value <0.01), SOD (SMD = 2.56, 95% CI: 1.46 to 3.67, p-value <0.01), and GSH (SMD = 2.03, 95% CI: 1.15 to 2.91, p-value <0.01) activity, and TAC (SMD = 1.09, 95% CI: 0.28 to 1.9, p-value = 0.01). Between-study heterogeneity was considerable for oxidative markers of testicular tissue with caspase-3 (I^2 = 85.43% and p-value for Q test <0.01), tissue CAT (I^2 = 87.3% and p-value for Q test <0.01), GPX (I^2 = 85.61% and p-value for Q test <0.01), MDA (I^2 = 90.79% and p-value for Q test <0.01), SOD (I^2 = 87.3% and p-value for Q test <0.01), and GSH (I^2 = 80.83% and

10.3389/fendo.2023.1184745

TABLE 1 Basic characteristics of the included studies.

First author [year]	Country	Rodent	Age of subjects	Number of subjects (intervention/ control)	Model of intervention	Type of OS	SYRCLE score
Wang [2018] (62)	China	Rats	N/M	8/8	Preventive	Chemical agent (Methotrexate)	2
Wang [2022] (63)	China	Mice	8 weeks	10/10	Therapeutic	Chemical agent (Paclitaxel)	3
Yalcınkaya [2009] (poster) (64)	Turkey	Rats	10-12 weeks	10/10	Therapeutic	Radiation (Gamma radiation)	N/A
Zangoie [2019] (65)	Iran	Rats	N/M	6/6	Therapeutic	Chemical agent (Busulfan)	2
Zhang [2022] (14)	China	Mice	8 weeks	8/8	Therapeutic	Chemical agent (Cisplatin)	4
Zi [2022] (66)	China	Mice	6-8 weeks	5/5	Preventive	Chemical agent (Doxorubicin)	3
Hussein [2006] (46)	Egypt	Rats	3 months	20/20	Preventive	Radiation (Roentgen radiation)	2
Khan [2015] (49)	China	Mice	8-9 weeks	3/3	Preventive	Radiation (Gamma radiation)	2
Kushwaha [2021] (50)	India	Mice	8-10 weeks	6/6	Preventive	Radiation (Gamma radiation)	2
Lee [2012] (51)	South Korea	Rats	8 weeks	6/6	Preventive	Chemical agent (Doxorubicin)	3
Madhu [2015] (22)	India	Rats	N/M	8/8	Preventive	Chemical agent (Cisplatin + Vinblastine + Bleomycin)	4
Manda [2003] (52)	India	Mice	6-8weeks	10/10	Preventive	Chemical agent (Cyclophosphamide)	3
Mirhoseini [2014] (53)	Iran	Mice	6-7 weeks	7/7	Therapeutic	Chemical agent (Busulfan)	3
Taheri Moghadam [2021] (54)	Iran	Mice	4-6 weeks	6/6	Preventive	Chemical agent (Busulfan)	3
Moradi [2021] (20)	Iran	Rats	13-15 weeks	5/5	Preventive	Chemical agent (Bleomycin + Etoposide + Cisplatin)	3
Cebi Sen [2018] (39)	Turkey	Rats	N/M	12/12	Preventive	Radiation (Radioactive iodine)	3
Patil [2009] (57)	India	Rats	N/M	6/6	Preventive	Chemical agent (Doxorubicin)	2
Aboelwafa [2022] (35)	Egypt	Rats	16-18 weeks	5/5	Therapeutic	Chemical agent (Taxol)	4
Alp [2014] (36)	Turkey	Rats	N/M	6/8	Preventive	Chemical agent (Procarbazine)	2
Atessahin [2006] (37)	Turkey	Rats	8 weeks	6/6	Preventive	Chemical agent (Cisplatin)	3
Baş [2019] (38)	Turkey	Mice	6-8 weeks	8/8	Therapeutic	Chemical agent (Docetaxel)	3
Chabra [2014] (40)	Iran	Mice	N/M	5/5	Preventive	Chemical agent (Cisplatin)	2
Cui [2017] (19)	China	Mice	8 weeks	3/3	Therapeutic	Chemical agent (Busulfan)	3
Edrees [2012] (41)	Egypt	Rats	N/M	5/5	Preventive	Chemical agent (Cisplatin)	2
Kamal El-Dein [2020] (48)	Egypt	Rats	N/M	6/6	Therapeutic	Radiation (Gamma radiation)	3
El-Shafaei [2018] (42)	Egypt	Rats	N/M	10/10	Therapeutic	Chemical agent (Cisplatin)	3
Yilmaz [2019] (43)	Turkey	Rats	3-5 months	8/8	Preventive	Chemical agent (Cisplatin)	3
Filobbos [2020] (44)	Egypt	Rats	12 weeks	10/10	Preventive	Chemical agent (Cisplatin)	3
Mohamad Ghasemi [2010] (i) (45)	Iran	Mice	N/M	6/6	Therapeutic	Chemical agent (Busulfan)	3

(Continued)

TABLE 1 Continued

First author [year]	Country	Rodent	Age of subjects	Number of subjects (intervention/ control)	Model of intervention	Type of OS	SYRCLE score
Ilbey [2008] (47)	Turkey	Rats	6 weeks	6/6	Preventive	Chemical agent (Cisplatin and Cyclophosphamide)	3
Mohamad Ghasemi [2010] (ii) (55)	Iran	Mice	6-8weeks	8/8	Therapeutic	Chemical agent (Busulfan)	2
Ferdosi Khosroshahi [2013] (Farsi) (68)	Iran	Rats	N/M	10/10	Therapeutic	Chemical agent (Busulfan)	2
Mohammd Ghasemi [2009] (Farsi) (67)	Iran	Mice	6-8 weeks	8/8	Therapeutic	Chemical agent (Busulfan)	2
Olayaki [2019] (56)	Nigeria	Rats	N/M	10/10	Therapeutic	Chemical agent (Chlorambucil)	2
Tawfik [2006] (60)	Egypt	Mice	7-9 weeks	6/6	Preventive	Radiation (Gamma radiation)	3
Sukhorum [2020] (58)	Thailand	Rats	N/M	8/8	Preventive	Chemical agent (Methotrexate)	2
Tajabadi [2020] (59)	Iran	Mice	6-8 weeks	5/5	Therapeutic	Radiation (Gamma radiation)	2
Torabi [2017] (61)	Iran	Rats	6-8 weeks	7/7	Preventive	Chemical agent (Cyclophosphamide)	3
Take [2009] (21)	Turkey	Rats	6-7 weeks	32/32	Preventive	Ionizing irradiation	2
Zhang [2019] (32)	China	Mice	3 weeks	20/20	Preventive	Chemical agent (Busulfan)	3
Abou-El-Naga [2021] (33)	Egypt	Mice	N/M	5/5	Therapeutic	Chemical agent (Busulfan)	3
Abd-El-Aziz [2012] (34)	Egypt	Rats	N/M	10/7	Therapeutic	Chemical agent (Busulfan)	3
Simsec [2008] (31)	Turkey	Rats	5-6 weeks	5/5	Preventive	Chemical agent (Cyclophosphamide)	2

OS, oxidative stress; N/M, not mentioned; N/A, not applicable; SYRCLE, Systematic Review Centre for Laboratory Animal Experimentation.

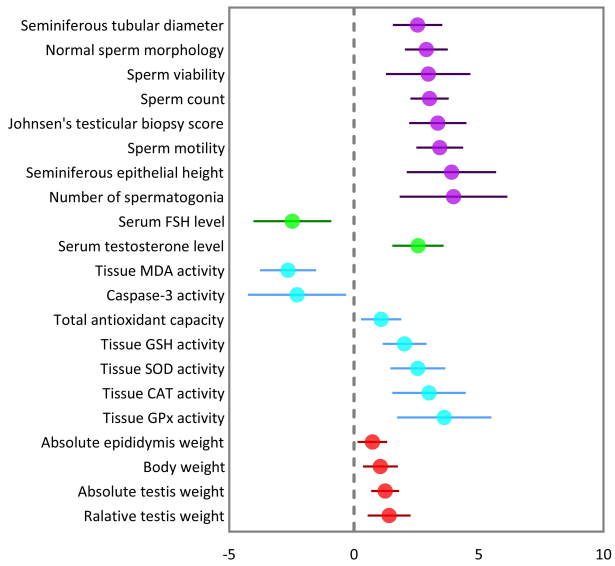


FIGURE 2
Summary of overall pooled effect sizes for each outcome. Sperm-related parameters are indicated in purple, reproductive hormones in green, oxidative markers of testicular tissue in cyan, and body and testicular weights in red.

p-value for Q test <0.01) activity, and TAC (I^2 = 55.14% and p-value for Q test 0.06).

3.3.4 Body and testicular weights

The pooled SMDs were absolute epididymis (SMD = 0.74, 95% CI: 0.15 to 1.33, p-value = 0.01), testis (SMD = 1.25, 95% CI: 0.69 to 1.81, p-value <0.01), and body weights (SMD = 1.06, 95% CI: 0.36 to 1.76, p-value <0.01), and testis to body relative weight (SMD = 1.41, 95% CI: 0.55 to 2.26, p-value <0.01). Body and testicular weights showed moderate to substantial heterogeneity between studies with absolute epididymis (I^2 = 49.45% and p-value for Q test 0.06), testis (I^2 = 78.68% and p-value for Q test <0.01), and body weights (I^2 = 79.33% and p-value for Q test <0.01), and testis to body relative weight (I^2 = 74.82% and p-value for Q test <0.01).

3.4 Subgroup analyses

The subgroup analyses were conducted on rodent species (mice versus rats), timing of intervention (preventive versus therapeutic, respectively, indicating melatonin therapy was started before and after the induction of stress), route of administration of melatonin, and type of stressor (chemical

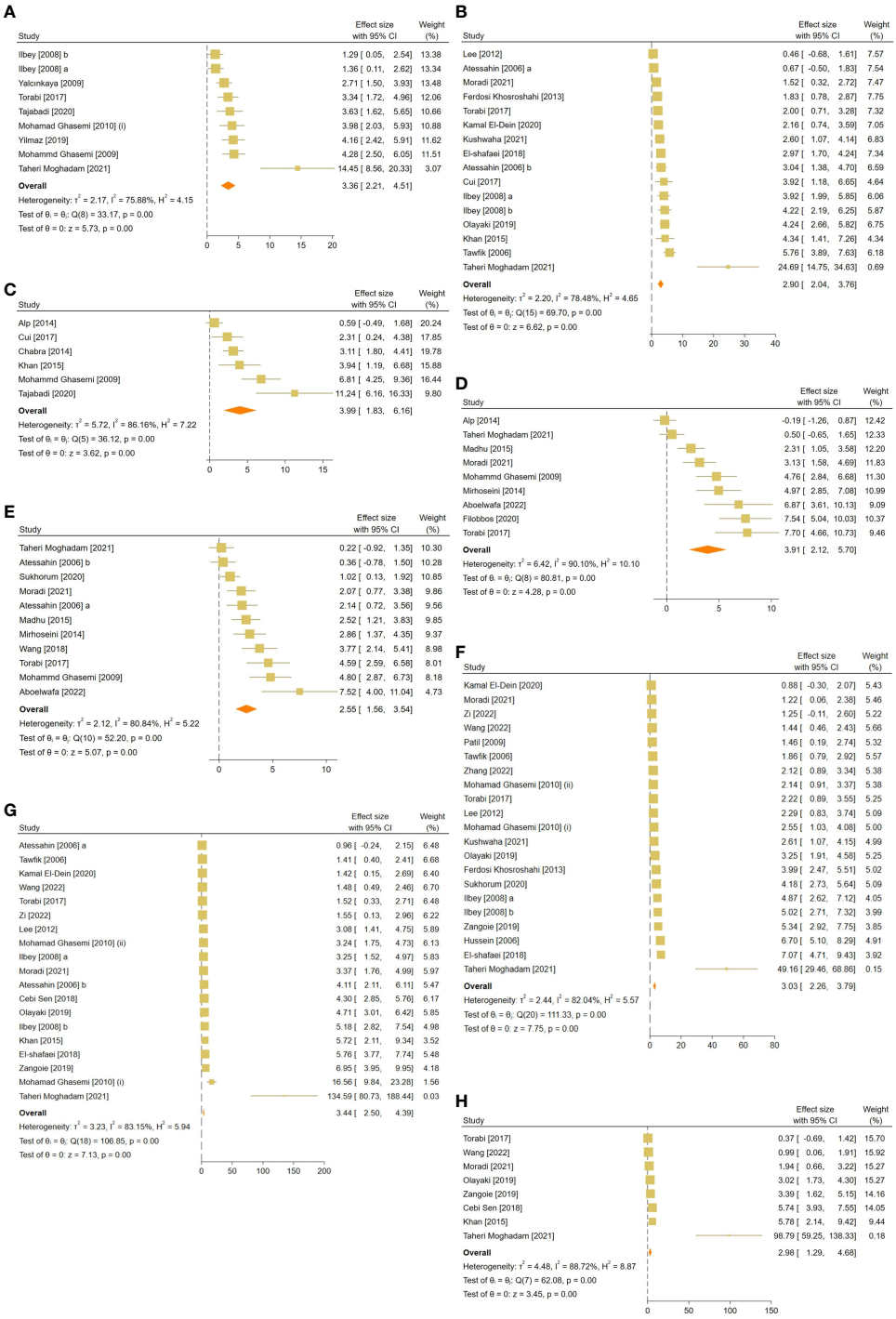


FIGURE 3
Forest plots for the overall pooled effects sizes of sperm-related parameters including **(A)** JTBS, **(B)** normal sperm morphology, **(C)** number of spermatogonia, **(D)** seminiferous epithelial height, **(E)** seminiferous tubular diameter, **(F)** sperm count, **(G)** sperm motility, and **(H)** sperm viability. JTBS, Johnsen's testicular biopsy score.

versus radiation). Subgroup analyses failed to indicate the source of heterogeneity. However, significant between-group differences were observed between the relative timing of intervention for serum FSH level and rodent species for JTBS and normal sperm morphology and count. The forest plots for subgroup analyses are provided in the Supplementary Material 3.

3.5 Sensitivity analyses and risk of bias assessment

The results' robustness was assessed using the leave-one-out method. After removing each study from the analyses, the pooled effect sizes did not significantly change. The forest plots for

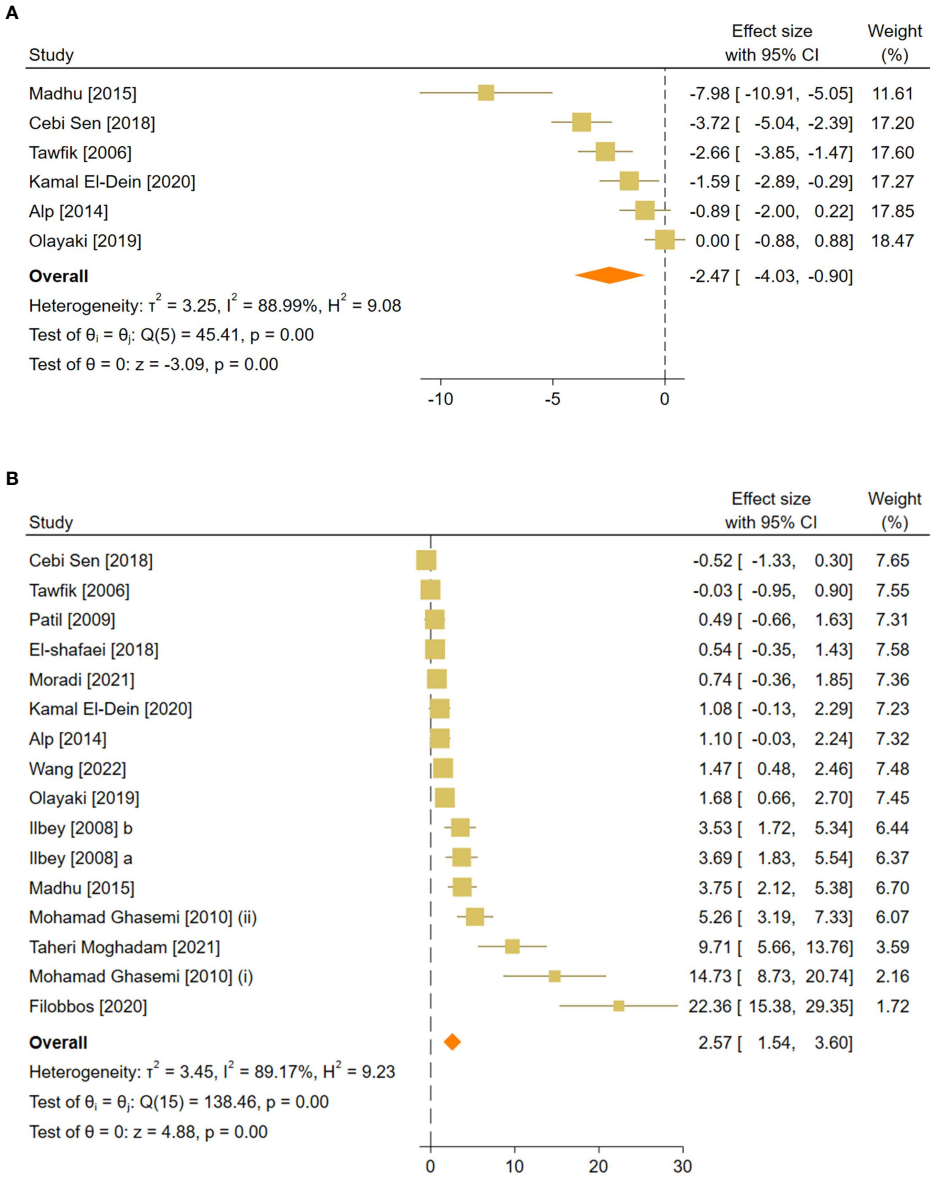


FIGURE 4
Forest plots for the overall pooled effects sizes of reproductive hormones, including **(A)** serum FSH and **(B)** testosterone level. FSH, Follicle-Stimulating Hormone.

sensitivity analyses for each outcome are provided in the Supplementary Material 4.

A risk of bias assessment was conducted using the SYRCLE tool for evaluating included studies. A study would receive a score of 1 if regarded as low risk in each domain. Based on the included studies, the scores ranged from 2 to 4. According to the evaluations of the studies, the results regarding sequence generation, random housing, allocation concealment, random outcome assessment, and blinding were all deemed unclear. No other sources of bias were detected for studies. The risk of bias assessment was impossible for one of the included studies since it was a poster (64). Detailed quality assessment results are presented in Supplementary Materials 5 and Figure 7.

3.6 Publication bias

Funnel plots were created for the following outcomes: absolute testis weight, body weight, normal sperm morphology, seminiferous tubular diameter, serum testosterone level, sperm count, sperm motility, tissue GSH, MDA, and SOD. Evaluations for publication bias showed a significant small-study effect across the outcomes. Nevertheless, it is essential to interpret the results of the small-study effects tests with caution since they may be affected by other factors. For example, in the presence of between-study heterogeneity (the case of this study), the symmetry of funnel plots can be affected (30, 69). The funnel plots and Egger's test results for small-effect studies are provided in the Supplementary Material 6.

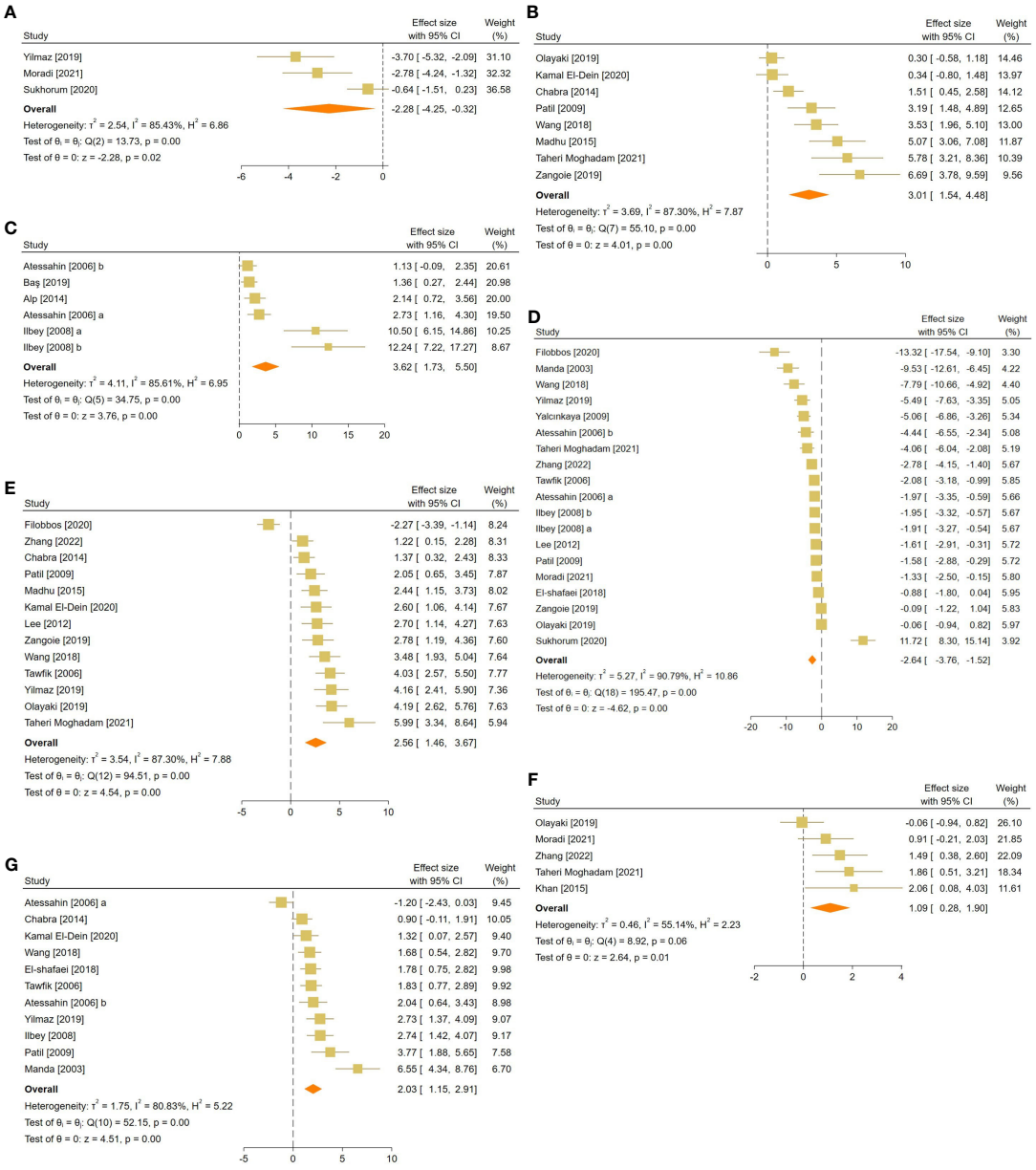


FIGURE 5
Forest plots for the overall pooled effects sizes of testicular tissue's oxidative markers, including **(A)** caspase-3, **(B)** tissue catalase, **(C)** glutathione peroxidase, **(D)** malondialdehyde, **(E)** superoxide dismutase activity, **(F)** total antioxidant capacity, and **(G)** glutathione activity.

4 Discussion

We demonstrated that melatonin could have beneficial effects against testicular abnormalities induced by radiotherapy and chemotherapy. Furthermore, we found that melatonin had a significantly greater impact on seminiferous tubular diameter, GPx, and FSH levels in preventive models rather than in therapeutic models. The strength of the melatonin's effects on JTBS, sperm counts, and morphology also depended on the animal type. We also detected the model of intervention and rodent species as the sources of heterogeneity in different analyses.

4.1 Sperm quantity and quality

In the current meta-analysis, melatonin restored testicular injuries caused by radiotherapy and chemotherapy, which was indicated by increased spermatogonia and sperm count, normal morphology, motility, and viability, testis and epididymal weight, and seminiferous tubular height and diameter. These results agree with our previous meta-analyses, which revealed the beneficial impact of melatonin on testicular injuries induced by metabolic disorders, physical and toxic chemical triggers in animal models (70–72). Radio- and chemotherapy can cause disturbances in

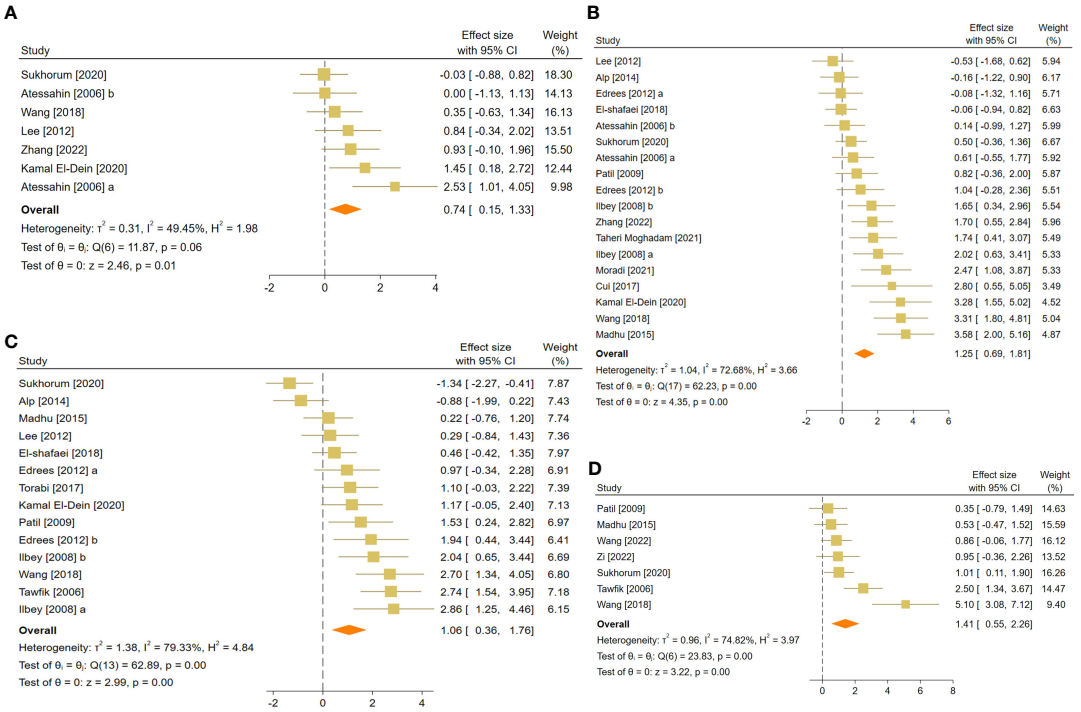


FIGURE 6
Forest plots for the overall pooled effects sizes of body and testicular weights, including **(A)** absolute epididymis weight, **(B)** absolute testis weight, **(C)** body weight, and **(D)** testis to body relative weight.

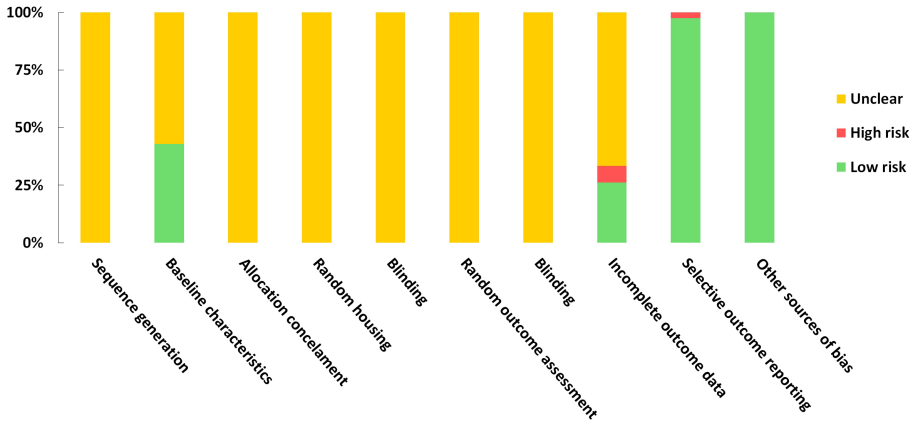


FIGURE 7
Risk of bias graph on judgements about each risk of bias item presented as proportions across all the included studies.

spermatogenesis through different mechanisms. These treatments may exert their effect by damaging DNA (DNA cross-link, breakage, alkylation, and intercalation) and induction of apoptosis, lipid peroxidation, increased oxidative stress, inflammation, hormonal imbalance, and mitochondrial damage, which result in abnormal sperm characteristics (6). Melatonin, as a potent antioxidant with anti-inflammatory and anti-apoptotic properties, can cross the cell membrane and penetrate the nucleus (73). As a direct free radical scavenger, melatonin can protect DNA against the destructive effects of Reactive oxygen species (ROS) induced by chemotherapy and radiotherapy (74). Melatonin's ability to counteract the harmful effects of anti-cancer treatments can improve sperm morphology, motility, count, and viability. Zhang et al. reported that melatonin alleviates the cytotoxicity and anti-mitotic effects of busulfan, an alkylating chemotherapy agent, in the cultured spermatogonial progenitor cells. They found

that the blockage of MT1 and MT2 in these cells antagonizes the observed effects of melatonin (32). In another *in-vitro* study, melatonin reversed the morphological changes caused by busulfan in the type A spermatogonial stem cells (19).

4.2 Reproductive hormone levels

Testosterone is produced by the Leydig cells located in the testis's interstitial space, and the luteinizing hormone induces its secretion. Testosterone is required for normal spermatogenesis, and its serum concentration is positively associated with normal sperm morphology and higher live birth rates (75). Sertoli cells, located in the seminiferous tubules with critical roles in spermatogenesis and androgen synthesis, are also targeted by FSH (76). Melatonin administration elevated animal testosterone and reduced FSH levels in this meta-analysis. According to our recent meta-analysis, melatonin increases testosterone levels but does not affect FSH in rodents with toxin-induced testicular injuries (70). The existing body of research suggests that melatonin can inhibit the biosynthesis of FSH by decreasing the secretion of gonadotropin-releasing hormone (GnRH). Since melatonin administration has been observed to diminish the number of pituitary GnRH receptors, it is plausible that the observed reductions in plasma FSH concentrations may stem from inhibiting the pubertal increase in GnRH secretion (77).

Previous studies have yielded inconsistent findings regarding the impact of melatonin on testosterone levels (70–72). In this regard, da Costa et al. have reported that melatonin supplementation in pubescent rats may lead to a decline in testosterone levels in adulthood, potentially due to its influence on the estrogenic capacity of Leydig cells. Nonetheless, they also demonstrated that melatonin could exert a protective effect against the decrease in testosterone levels caused by the deleterious effects of diabetes, suggesting this protective effect may stem from melatonin's ability to upregulate androgen receptor genes (78). Our results suggest melatonin's protective effects against decreased testosterone levels induced by anti-cancer treatments. The blockages of MT1 and MT2 in the Leydig cell membrane downregulated steroidogenic genes (79). Melatonin can increase the expression of steroidogenic genes by binding to its nuclear receptors, including retinoic acid receptor-related orphan receptor α (RORα) (13). Furthermore, elevated melatonin levels improve testosterone synthesis by decreasing Leydig cells' apoptosis (13), which may explain melatonin's protective effect in our study. Nonetheless, there is contradictory evidence. Melatonin did not affect testosterone levels in animals with physical damage to the testes (71) and healthy human males (80, 81). Therefore, there is a need for more studies to determine melatonin's effects on reproductive hormones and male infertility induced by oxidative stress.

4.3 Oxidative stress

Oxidative stress is among the causative factors for male infertility (82). In this regard, our results demonstrated melatonin's beneficial effects on testicular enzymatic and non-enzymatic antioxidants in this study. By stimulating the activities of key antioxidant enzymes such as CAT, GSH-Px, SOD, and GSH while concurrently reducing the activity of MDA, a marker of lipid peroxidation, melatonin protects the testicular tissue against oxidative damage-induced radiation and chemotherapy. Previously, we detected similar efficacy of melatonin in metabolic disorders, physical- and chemical-induced testicular injuries (70–72). Furthermore, melatonin decreased microwave and radiofrequency electromagnetic radiation-induced oxidative stress (83). Literature suggests that melatonin increases antioxidant enzyme expression and activity during physiological and pathological conditions. These enzymes play a crucial role in mitigating the deleterious effects of free radicals by converting them into less reactive or non-toxic molecules, thus serving as a vital defense mechanism against oxidative stress. These enzymes can be recursively altered by free radicals, compromising their efficacy. In this context, melatonin acts as a potent scavenger of free radicals and can directly neutralize their destructive effects. Therefore, melatonin exerts a dual influence on the antioxidant system, both directly and indirectly, by regulating the activity of antioxidant enzymes and mitigating their damage by free radicals (84–86). In a recent study, Zhang et al. observed that the administration of cisplatin to mice results in apoptosis of Leydig cells by the downregulation of the SIRT1/Nrf2 signaling pathway, which plays a crucial role in anti-inflammatory response, anti-oxidative stress, and cell protection. However, the authors also suggest that melatonin can counteract the harmful effects of cisplatin by stimulating the SIRT1/Nrf2 pathway through its interaction with MT1/MT2 receptors (14, 87). Furthermore, melatonin, as a potent scavenger of reactive oxygen and nitrogen species, could also alleviate free radical formation by improving the electron transport chain efficiency of the inner mitochondrial membrane; by doing so, melatonin can reduce electron leakage, which is a significant source of free radical formation (88).

4.4 ER stress and apoptosis

In this study, we observed melatonin's beneficial effects on reducing caspase-3 activity, which is a crucial mediator of apoptosis. This result aligns with our previous studies indicating melatonin protection against the apoptotic effects of metabolic disorders, physical injuries, environmental pollutants, and heavy metals on testes (70–72). Melatonin could alleviate testicular B-cell lymphoma-2 (Bcl-2)-associated X pro-apoptotic protein (BAX) and upregulate Bcl-2 anti-apoptotic protein following chemotherapy (20, 89). Radio- and chemotherapy could also trigger ER stress through different signaling pathways (including inositol-dependent protein 1 α (IRE1α), PRKR-like ER kinase (PERK)-eukaryotic translation initiating factor 2α (eIF2α), and MAPK), leading to cell death and potentially impairing male fertility (90).

Melatonin has been demonstrated to mitigate ER stress and inhibit intrinsic apoptotic pathways in anti-cancer treatment-

induced ER stress (17, 19). In this regard, melatonin counteracted busulfan-induced ER stress and its downstream apoptotic proteins, including P53, caspases, and CCAAT enhancer binding protein (C/EBP) homologous protein (CHOP), in mouse testes and spermatogonial stem cells (19). Melatonin may reverse radiotherapy and chemotherapy-induced ER stress by suppressing the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway (17). Eliminating ER stress by melatonin could improve blood-testis barrier impairment and, thereby, spermatogenesis abnormalities following busulfan treatment (91).

4.5 Inflammation

Pro-inflammatory cytokines play a key role in maintaining the normal physiological functions of testicular cells by acting as growth and differentiation factors (92). However, their increased levels during acute and chronic genitourinary tract inflammation are linked to oxidative stress and male infertility (93). Melatonin supplementation is reported to reduce testicular inflammation in infertile men (94). It may also reverse the radiotherapy- and chemotherapy-induced male reproductive toxicities by attenuating the testicular levels of inflammatory cytokines, including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) (2, 5, 17, 20, 62, 95). These effects may be attributed to melatonin's inhibition of the p38 mitogen-activated protein kinase (MAPK) signaling pathway and, subsequently, toll-like receptor 4 (TLR-4) and nuclear factor kappa B (NF-κB) in the testes (96). The activated TLRs are associated with low sperm motility, sperm apoptosis, and male infertility (97). Yet, more studies should be performed to evaluate other affected cytokines and cascades by exogenous melatonin.

4.6 Limitations

Our study had several limitations. First, our data was extracted from animal studies, and it is unclear whether such effects could be translated to humans. Furthermore, most available animal studies evaluating the effects of melatonin therapy on male infertility used rodent models, making the conclusions hard to generalize to other animals. Second, there was high methodological and statistical heterogeneity between the included studies. Third, our meta-analysis is also limited by the low quality of the eligible studies and a high level of publication bias. Also, a dose-response meta-analysis was not feasible due to insufficient data and differences in the route of administration. Finally, none of the included studies have reported and evaluated possible adverse outcomes.

5 Conclusion

In the current meta-analysis of animal studies, we conclude melatonin's protective influence on the side effects of radiotherapy and chemotherapy on testicular tissue. Improving testicular function and morphology, ameliorating hormone levels, and alleviating oxidative stress and apoptosis are some proposed mechanisms for the observed effects of melatonin. However, more meticulous animal studies should be performed to clarify other potential underlying mechanisms. Future studies are recommended to evaluate melatonin dose responses to provide doses with anti-infertility effects.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

The study was conceptualized by NA and ND and designed by AS, ND, SS-Z, and ARS. ND, AS, and ET searched the databases. ZE, ZR, ND, and MS extracted the data. MS and ND performed the quality assessment. AS performed meta-analyses. AS visualized the data and designed the graphical abstract. Drafts of the manuscript were provided by ND, AS, and SS-Z. NA, ARS, ND, and ET supervised the study. All authors made substantial contributions to the article and endorsed the final version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1184745/full#supplementary-material

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin* (2021) 71(3):209–49. doi: 10.3322/caac.21660

2. Ghafouri-Fard S, Shoorei H, Abak A, Seify M, Mohaqiq M, Keshmir F, et al. Effects of chemotherapeutic agents on male germ cells and possible ameliorating impact of antioxidants. *Biomedicine Pharmacother.* (2021) 142:112040. doi: 10.1016/j.biopha.2021.112040

3. Roychoudhury S, Das A, Panner Selvam MK, Chakraborty S, Slama P, Sikka SC, et al. Recent publication trends in radiotherapy and male infertility over two decades: a scientometric analysis. *Front Cell Dev Biol* (2022) 889. doi: 10.3389/fcell.2022.877079

4. Cetinkaya-Un B, Un B, Akpolat M, Andic F, Yazir Y. Human amnion membrane-derived mesenchymal stem cells and conditioned medium can ameliorate X-irradiation-induced testicular injury by reducing endoplasmic reticulum stress and apoptosis. *Reprod Sci* (2022) 29(3):1–11. doi: 10.1007/s43032-021-00753-6

5. Qu N, Itoh M, Sakabe K. Effects of chemotherapy and radiotherapy on spermatogenesis: The role of testicular immunology. *Int J Mol Sci* (2019) 20(4):957. doi: 10.3390/ijms20040957

6. Meistrich ML. Effects of chemotherapy and radiotherapy on spermatogenesis in humans. *Fertility sterility.* (2013) 100(5):1180–6. doi: 10.1016/j.fertnstert.2013.08.010

7. Brydøy M, Fosså SD, Dahl O, Bjøro T. Gonadal dysfunction and fertility problems in cancer survivors. *Acta Oncologica* (2007) 46(4):480–9. doi: 10.1080/02841860601166958

8. Okada K, Fujisawa M. Recovery of spermatogenesis following cancer treatment with cytotoxic chemotherapy and radiotherapy. *World J men's Health* (2019) 37(2):166–74. doi: 10.5534/wjmh.180043

9. Farhood B, Mortezaee K, Haghi-Aminjan H, Khanlarkhani N, Salehi E, Nashtaei MS, et al. A systematic review of radiation-induced testicular toxicities following radiotherapy for prostate cancer. *J Cell Physiol* (2019) 234(9):14828–37. doi: 10.1002/jcp.28283

10. Lai JC, Tandon P, Bernal W, Tapper EB, Ekong U, Dasarathy S, et al. Malnutrition, frailty, and sarcopenia in patients with cirrhosis: 2021 practice guidance by the american association for the study of liver diseases. *Hepatol (Baltimore Md)* (2021) 74(3):1611–44. doi: 10.1002/hep.32049

11. Kozioł K, Broda D, Romerowicz-Misielak M, Nowak S, Koziorowski M. Melatonin concentration in peripheral blood and melatonin receptors (MT1 and MT2) in the testis and epididymis of male roe deer during active spermatogenesis. *Theriogenology* (2020) 149:25–37. doi: 10.1016/j.theriogenology.2020.03.025

12. Lampiao F, Du Plessis SS. New developments of the effect of melatonin on reproduction. *World J Obstetrics Gynecol* (2013) 2(2):8–15. doi: 10.5317/wjog.v2.i2.8

13. Yang M, Guan S, Tao J, Zhu K, Lv D, Wang J, et al. Melatonin promotes male reproductive performance and increases testosterone synthesis in mamMalian Leydig cells. *Biol Reprod* (2021) 104(6):1322–36. doi: 10.1093/biolre/ioab046

14. Zhang J, Fang Y, Tang D, Xu X, Zhu X, Wu S, et al. Activation of MT1/MT2 to protect testes and leydig cells against cisplatin-induced oxidative stress through the SIRT1/Nrf2 signaling pathway. *Cells.* (2022) 11(10):1690. doi: 10.3390/cells11101690

15. Li W, Kwok CC-H, Chan DC-W, Ho AW-Y, Ho C-S, Zhang J, et al. Disruption of sleep, sleep-wake activity rhythm, and nocturnal melatonin production in breast cancer patients undergoing adjuvant chemotherapy: prospective cohort study. *Sleep Med* (2019) 55:14–21. doi: 10.1016/j.sleep.2018.11.022

16. de Castro TB, Bordin-Junior NA, de Almeida EA, de Campos Zuccari DAP. Evaluation of melatonin and AFMK levels in women with breast cancer. *Endocrine.* (2018) 62:242–9. doi: 10.1007/s12020-018-1624-2

17. Ma Z, Xu L, Liu D, Zhang X, Di S, Li W, et al. Utilizing melatonin to alleviate side effects of chemotherapy: a potentially good partner for treating cancer with ageing. *Oxid Med Cell Longevity* (2020) 2020. doi: 10.1155/2020/6841581

18. Najafi M, Shirazi A, Motevaseli E, Geraily G, Norouzi F, Heidari M, et al. The melatonin immunomodulatory actions in radiotherapy. *Biophys Rev* (2017) 9:139–48. doi: 10.1007/s12551-017-0256-8

19. Cui Y, Ren L, Li B, Fang J, Zhai Y, He X, et al. Melatonin relieves busulfan-induced spermaTogonial stem cell apoptosis of mouse testis by inhibiting endoplasmic reticulum stress. *Cell Physiol Biochem* (2017) 44(6):2407–21. doi: 10.1159/000486165

20. Moradi M, Goodarzi N, Faramarzi A, Cheraghi H, Hashemian AH, Jalili C. Melatonin protects rats testes against bleomycin, etoposide, and cisplatin-induced toxicity via mitigating nitro-oxidative stress and apoptosis. *Biomedicine Pharmacotherapy.* (2021) 138:111481. doi: 10.1016/j.biopha.2021.111481

21. Take G, Erdogan D, Helvacioglu F, Göktas G, Ozbey G, Uluoglu C, et al. Effect of melatonin and time of administration on irradiation-induced damage to rat testes. *Braz J Med Biol Res* (2009) 42(7):621–8. doi: 10.1590/S0100-879X2009000700006

22. Madhu P, Reddy KP, Reddy PS. Role of melatonin in mitigating chemotherapy-induced testicular dysfunction in Wistar rats. *Drug Chem Toxicol* (2016) 39(2):137–46. doi: 10.3109/01480545.2015.1055359

23. Haghi-Aminjan H, Asghari MH, Farhood B, Rahimifard M, Hashemi Goradel N, Abdollahi M. The role of melatonin on chemotherapy-induced reproductive toxicity. *J Pharm Pharmacol* (2018) 70(3):291–306. doi: 10.1111/jphp.12855

24. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* (2021) 372:n71. doi: 10.1136/bmj.n71

25. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan—a web and mobile app for systematic reviews. *Systematic Rev* (2016) 5(1):210. doi: 10.1186/s13643-016-0384-4

26. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res methodology.* (2014) 14:43. doi: 10.1186/1471-2288-14-43

27. Abbasnezhad A, Choghakhori R, Kashkooli S, Alipour M, Asbaghi O, Mohammadi R. Effect of L-carnitine on liver enzymes and biochemical factors in hepatic encephalopathy: A systematic review and meta-analysis. *J Gastroenterol Hepatol.* (2019) 34(12):2062–70. doi: 10.1111/jgh.14765

28. Luo D, Wan X, Liu J, Tong T. Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range. *Stat Methods Med Res* (2018) 27(6):1785–805. doi: 10.1177/0962280216669183

29. Shi J, Luo D, Weng H, Zeng XT, Lin L, Chu H, et al. Optimally estimating the sample standard deviation from the five-number summary. *Res Synth Methods* (2020) 11(5):641–54. doi: 10.1002/jrsm.1429

30. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *Bmj* (1997) 315(7109):629–34. doi: 10.1136/bmj.315.7109.629

31. Simsek A, Otunctemur A, Özcan L, Cilli M, Polat E, Somay A, et al. Preventive effects of melatonin in cisplatin and cyclophosphamide associated testes damage. *Eur Urol Suppl* (2008) 7:93–. doi: 10.1016/S1569-9056(08)60093-7

32. Zhang X, Xia Q, Wei R, Song H, Mi J, Lin Z, et al. Melatonin protects spermaTogonia from the stress of chemotherapy and oxidation via eliminating reactive oxidative species. *Free Radic Biol Med* (2019) 137:74–86. doi: 10.1016/j.freeradbiomed.2019.04.009

33. Abou-El-Naga A-M, Mousa S-A, Althobaiti F, Fayad E, Fahim E-S. Ameliorative effects of melatonin and zinc oxide nanoparticles treatment against adverse effects of busulfan induced infertility in male albino mice. *BIOCELL* (2022) 46(2):535–45. doi: 10.32604/biocell.2022.017739

34. Abd El Aziz DH, Metwally HG. The effect of stem cell therapy versus melatonin on the changes induced by busulfan in the testes of adult rat: histological and immunohistochemical studies. *Egyptian J Histol* (2013) 36(1):175–84. doi: 10.1097/01.EHX.0000425579.77855.ea

35. Aboelwafa HR, Ramadan RA, El-Kott AF, Abdelhamid FM. The protective effect of melatonin supplementation against taxol-induced testicular cytotoxicity in adult rats. *Braz J Med Biol Res* (2022) 55:e11614. doi: 10.1590/1414-431x2021e11614

36. Alp BF, Kesik V, Malkoç E, Yiğit N, Saldır M, Babacan O, et al. The effect of melatonin on procarbazine induced testicular toxicity on rats. *Syst Biol Reprod Med* (2014) 60(6):323–8. doi: 10.3109/19396368.2014.930212

37. Ateşşahin A, Sahna E, Türk G, Ceribaşi AO, Yilmaz S, Yüce A, et al. Chemoprotective effect of melatonin against cisplatin-induced testicular toxicity in rats. *J Pineal Res* (2006) 41(1):21–7. doi: 10.1111/j.1600-079X.2006.00327.x

38. Baş E, Nazıroğlu M. Treatment with melatonin and selenium attenuates docetaxel-induced apoptosis and oxidative injury in kidney and testes of mice. *Andrologia.* (2019) 51(8):e13320. doi: 10.1111/and.13320

39. Cebi Sen C, Yumusak N, Atilgan HI, Sadic M, Koca G, Korkmaz M. The protective effect of melatonin on sperm quality in rat after radioiodine treatment. *Andrologia.* (2018) 50(4):e12962. doi: 10.1111/and.12962

40. Chabra A, Shokrzadeh M, Naghshvar F, Salehi F, Ahmadi A. Melatonin ameliorates oxidative stress and reproductive toxicity induced by cyclophosphamide in male mice. *Hum Exp Toxicol* (2014) 33(2):185–95. doi: 10.1177/0960327113489052

41. Edrees Z, kader H, Embaby A, hameed E. The effect of melatonin on the testes of rats treated with cyclophosphamide: Histological and immunohistochemical study. *Egyptian J Histol* (2012) 35:822–32. doi: 10.1097/01.EHX.0000419785.84206.d2

42. El-Shafaei A, Abdelmaksoud R, Elshorbagy A, Zahran N, Elabd R. Protective effect of melatonin versus montelukast in cisplatin-induced seminiferous tubule damage in rats. *Andrologia.* (2018) 50(9):e13077. doi: 10.1111/and.13077

43. Eren H, Mercantepe T, Tumkaya L, Mercantepe F, Dil E, Horsanali MO, et al. Evaluation of the protective effects of amifostine and melatonin against cisplatin induced testis injury via oxidative stress and apoptosis in rats. *Exp Mol Pathol* (2020) 112:104324. doi: 10.1016/j.yexmp.2019.104324

44. Filobbos S, Amin N, Yacoub M, Abd El_Hakim KR. Possible protective effect of melatonin on cisplatin-induced testicular toxicity in adult albino rats. A histological and immunohistochemical study. *Egyptian J Histol* (2020) 43(3):891–901. doi: 10.21608/EJH.2020.21561.1221

45. Ghasemi FM, Faghani M, Khajehjahromi S, Bahadori M, Nasiri EE, Hemadi M. Effect of melatonin on proliferative activity and apoptosis in spermatogenic cells in mouse under chemotherapy. *J Reprod Contraception* (2010) 21(2):79–94. doi: 10.1016/S1001-7844(10)60016-8

46. Hussein MR, Abu-Dief EE, Abou El-Ghait AT, Adly MA, Abdelraheem MH. Melatonin and roentgen irradiation of the testis. *Fertil Steril.* (2006) 86(3):750–2. doi: 10.1016/j.fertnstert.2006.02.094

47. Ilbey YO, Ozbek E, Simsek A, Otunctemur A, Cekmen M, Somay A. Potential chemoprotective effect of melatonin in cyclophosphamide- and cisplatin-induced testicular damage in rats. *Fertil Steril* (2009) 92(3):1124–32. doi: 10.1016/j.fertnstert.2008.07.1758

48. Kamal El-Dein EMKE-D, Anees LM. Ameliorative role of melatonin against cypermethrin or gamma irradiation induced testicular damage in male rats. *Int J Radiat Res* (2020) 18(4):765–76. doi: 10.18869/acadpub.ijrr.18.4.765

49. Khan S, Adhikari JS, Rizvi MA, Chaudhury NK. Radioprotective potential of melatonin against 60Co γ-ray-induced testicular injury in male C57BL/6 mice. *J Biomed Science.* (2015) 22(1):61. doi: 10.1186/s12929-015-0156-9

50. Kushwaha R, Nishad DK, Bhatnagar A, Khar RK. Melatonin-caffeine combination modulates gamma radiation-induced sperm malformations in C57BL/6 male mice at sublethal dose of gamma radiation. *J Pharm Bioallied Sci* (2021) 13 (2):268–75. doi: 10.4103/jpbs.JPBS_303_20

51. Lee K-M, Lee I-C, Kim S-H, Moon C, Park S-H, Shin D-H, et al. Melatonin attenuates doxorubicin-induced testicular toxicity in rats. *Andrologia* (2012) 44 (s1):796–803. doi: 10.1111/j.1439-0272.2011.01269.x

52. Manda K, Bhatia AL. Prophylactic action of melatonin against cyclophosphamide-induced oxidative stress in mice. *Cell Biol Toxicol* (2003) 19 (6):367–72. doi: 10.1023/B:CBTO.0000013342.17370.16

53. Mirhoseini M, Saki G, Hemadi M, Khodadadi A, Mohammadi Asl J. Melatonin and testicular damage in busulfan treated mice. *Iran Red Crescent Med J* (2014) 16(2): e14463. doi: 10.5812/ircmj.14463

54. Moghadam MT, Dadfar R, Khorsandi L. The effects of ozone and melatonin on busulfan-induced testicular damage in mice. *JBRA Assist Reprod* (2021) 25(2):176–84. doi: 10.5935/1518-0557.20200081

55. Mohammad Ghasemi F, FaghaniLangroudi M, Falah Karkan M. The protective effect of melatonin on sperm parameters, epididymis and seminal vesicle morphology in adult mouse treated with busulfan. *Anatomical Sci J* (2010) 8(30).

56. Olayaki LA, Adeyemi WJ, Adeyemi E, Osawaru O, Busura I, Jimoh S. Melatonin enhanced the restoration of biochemical profile in chlorambucil treated-rats: examination of after-withdrawal effects of the drug. *J Afr Assoc Physiol Sci* (2020) 7 (2):80–7.

57. Patil L, Balaraman R. Effect of melatonin on doxorubicin induced testicular damage in rats. *Int J PharmTech Res CODEN(USA): IJPRIF ISSN.* (2023) 1:974–4304.

58. Sukhorum W, Umka Welbat J, Krutsri S, Iamsaard Comma S. Protective effect of melatonin against methotrexate-induced testicular damage in the rat model: An experimental study. *Int J Reprod Biomed* (2020) 18(5):327–38. doi: 10.18502/ijrm.v13i5.7153

59. Tajabadi E, Javadi A, Azar NA, Najafi M, Shirazi A, Shabeeb D, et al. Radioprotective effect of a combination of melatonin and metformin on mice spermatogenesis: A histological study. *Int J Reprod Biomed* (2020) 18(12):1073–80. doi: 10.18502/ijrm.v18i12.8029

60. Tawfik SS, Mansour HH, El-Shamy E, Sallam MH. Radioprotective effect and follow-up of melatonin as antifertility drug in male adult mice submitted to whole-body γ Irradiation. *Egyptian J Radiat Sci App* (2006) 19(2):331–51.

61. Torabi F, Malekzadeh Shafaroudi M, Rezaei N. Combined protective effect of zinc oxide nanoparticles and melatonin on cyclophosphamide-induced toxicity in testicular histology and sperm parameters in adult Wistar rats. *Int J Reprod Biomed* (2017) 15(7):403–12. doi: 10.29252/ijrm.15.7.403

62. Wang Y, Zhao TT, Zhao HY, Wang H. Melatonin protects methotrexate-induced testicular injury in rats. *Eur Rev Med Pharmacol Sci* (2018) 22(21):7517–25. doi: 10.26355/eurrev_201811_16293

63. Wang Z, Teng Z, Wang Z, Song Z, Zhu P, Li N, et al. Melatonin ameliorates paclitaxel-induced mice spermatogenesis and fertility defects. *J Cell Mol Med* (2022) 26 (4):1219–28. doi: 10.1111/jcmm.17177

64. Yalçınkaya F, Gökçe A, Guven O, Davarcı M, Cikim G, Yekeler H, et al. N88 Protective effect of vitamine E and melatonin against radiation induced damage in testis of rat. *Eur Urol Suppl* (2009) 8:599–. doi: 10.1016/S1569-9056(09)74862-6

65. Zangoie R, Eshraghi H, Shirian S, Kadivar A, Nazari H, Aali E. Melatonin synergistically enhances protective effect of atorvastatin against busulfan-induced spermatogenesis injuries in a rat model. *Comp Clin Pathol* (2020) 29(1):161–6. doi: 10.1007/s00580-019-03040-8

66. Zi T, Liu Y, Zhang Y, Wang Z, Wang Z, Zhan S, et al. Protective effect of melatonin on alleviating early oxidative stress induced by DOX in mice spermatogenesis and sperm quality maintaining. *Reprod Biol Endocrinol* (2022) 20 (1):105. doi: 10.1186/s12958-022-00977-4

67. Mohamadghasemi F, Faghani M, Khajehjahromi S. The protective effects of melatonin on the histological changes of testis in busulfan-treated adult mice. *J Reprod Infertil* (2010) 11(2):67.

68. Ferdosi Khosroshahi A, Bakhtiari M, Soleimani Rad J, Koroji M, Roshangar L, Janzadeh A, et al. Study of the effect of exogenous melatonin on sperm fertility in busulfan induced oligospermic of pinealectomeized rat. *Razi J Med Sci* (2013) 20(110):77–86.

69. Sterne JAC, Egger M. Regression methods to detect publication and other bias in meta-analysis. *Publ Bias Meta-Analysis* (2005) 99–110. doi: 10.1002/0470870168.ch6

70. Dehdari Ebrahimi N, Parsa S, Nozari F, Shahlaee MA, Maktabi A, Sayadi M, et al. Protective effects of melatonin against the toxic effects of environmental pollutants and heavy metals on testicular tissue: A systematic review and meta-analysis of animal studies. *Front Endocrinol* (2023) 14. doi: 10.3389/fendo.2023.1119553

71. Dehdari Ebrahimi N, Shojaei-Zarghani S, Taherifard E, Dastghaib S, Parsa S, Mohammadi N, et al. Protective effects of melatonin against physical injuries to testicular tissue: A systematic review and meta-analysis of animal models. *Front Endocrinol* (2023) 14. doi: 10.3389/fendo.2023.1119553

72. Dehdari Ebrahimi N, Sadeghi A, Ala M, Ebrahimi F, Pakbaz S, Azarpira N. Protective effects of melatonin against oxidative stress induced by metabolic disorders in the male reproductive system: A systematic review and meta-analysis of rodent models. *Front Endocrinol* (2023) 14. doi: 10.3389/fendo.2023.1202560

73. Reiter RJ, Tan DX, Mayo JC, Sainz RM, Leon J, Czarnocki Z. Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans. *Acta Biochim Polonica.* (2003) 50(4):1129–46. doi: 10.18388/abp.2003_3637

74. Ressmeyer AR, Mayo JC, Zelosko V, Sáinz RM, Tan DX, Poeggeler B, et al. Antioxidant properties of the melatonin metabolite N1-acetyl-5-methoxykynuramine (AMK): scavenging of free radicals and prevention of protein destruction. *Redox Rep Commun Free Radical Res* (2003) 8(4):205–13. doi: 10.1179/135100003225002709

75. Trussell J, Coward RM, Santoro N, Stetter C, Kunselman A, Diamond MP, et al. Association between testosterone, semen parameters, and live birth in men with unexplained infertility in an intrauterine insemination population. *Fertility sterility.* (2019) 111(6):1129–34. doi: 10.1016/j.fertnstert.2019.01.034

76. Oduwole OO, Peltoketo H, Huhtaniemi IT. Role of follicle-stimulating hormone in spermatogenesis. *Front endocrinology.* (2018) 9:763. doi: 10.3389/fendo.2018.00763

77. Lang U, Aubert ML, Rivest RW, Vinas-Bradtke JC, Sizonenko PC. Daily afternoon administration of melatonin does not irreversibly inhibit sexual maturation in the male rat. *Endocrinology* (1984) 115(6):2303–10. doi: 10.1210/endo-115-6-2303

78. da Costa CF, Gobbo MG, Taboga SR, Pinto-Fochi ME, Góes RM. Melatonin intake since weaning ameliorates steroidogenic function and sperm motility of streptozotocin-induced diabetic rats. *Andrology* (2016) 4(3):526–41. doi: 10.1111/andr.12158

79. Gao Y, Wu X, Zhao S, Zhang Y, Ma H, Yang Z, et al. Melatonin receptor depletion suppressed hCG-induced testosterone expression in mouse Leydig cells. *Cell Mol Biol letters.* (2019) 24(1):1–14. doi: 10.1186/s11658-019-0147-z

80. Luboshitzky R, Levi M, Shen-Orr Z, Blumenfeld Z, Herer P, Lavie P. Long-term melatonin administration does not alter pituitary-gonadal hormone secretion in normal men. *Hum reproduction.* (2000) 15(1):60–5. doi: 10.1093/humrep/15.1.60

81. Zizzo J, Reddy R, Kulkarni N, Blachman-Braun R, Ramasamy R. Impact of low-dose melatonin supplementation on testosterone levels in US adult males. *Urology* (2022) 169:92–5. doi: 10.1016/j.urology.2022.07.048

82. Mannucci A, Argento FR, Fini E, Coccia ME, Taddei N, Becatti M, et al. The impact of oxidative stress in male infertility. *Front Mol Biosci* (2022) 8:1344. doi: 10.3389/fmolb.2021.799294

83. Shokri M, Shamsaei ME, Malekshah AK, Amiri FT. The protective effect of melatonin on radiofrequency electromagnetic fields of mobile phone-induced testicular damage in an experimental mouse model. *Andrologia* (2020) 52(11):e13834. doi: 10.1111/and.13834

84. Aysun H, Burcu B. (2018), Ch. 3.

85. Chainy GBN, Sahoo DK. Hormones and oxidative stress: an overview. *Free Radical Res* (2020) 54(1):1–26. doi: 10.1080/10715762.2019.1702656

86. Sahoo DK, Chainy GBN. Hormone-linked redox status and its modulation by antioxidants. *Vitam Horm.* (2023) 121:197–246. doi: 10.1016/bs.vh.2022.10.007

87. Chung J-Y, Chen H, Zirkin B. Sirt1 and Nrf2: regulation of Leydig cell oxidant/antioxidant intracellular environment and steroid formation. *Biol Reprod* (2021) 105 (5):1307–16. doi: 10.1093/biolre/ioab150

88. Leon J, Acuña-Castroviejo D, Sainz RM, Mayo JC, Tan DX, Reiter RJ. Melatonin and mitochondrial function. *Life Sci* (2004) 75(7):765–90. doi: 10.1016/j.lfs.2004.03.003

89. Zi T, Liu Y, Zhang Y, Wang Z, Wang Z, Zhan S, et al. Protective effect of melatonin on alleviating early oxidative stress induced by DOX in mice spermatogenesis and sperm quality maintaining. *Reprod Biol Endocrinol* (2022) 20 (1):1–11. doi: 10.1186/s12958-022-00977-4

90. Karna KK, Shin YS, Choi BR, Kim HK, Park JK. The role of endoplasmic reticulum stress response in male reproductive physiology and pathology: A review. *World J Mens Health* (2020) 38(4):484–94. doi: 10.5534/wjmh.190038

91. Zhao J, Wang M, Wang Y, Xu J, Ma C, Tang Y, et al. Endoplasmic reticulum stress promotes blood-testis barrier impairment in mice with busulfan-induced oligospermia through PERK-eIF2α signaling pathway. *Toxicology* (2022) 473:153193. doi: 10.1016/j.tox.2022.153193

92. Hales DB, Diemer T, Hales KH. Role of cytokines in testicular function. *Endocrine.* (1999) 10:201–17. doi: 10.1007/BF02738619

93. Azenabor A, Ekun AO, Akinloye O. Impact of inflammation on male reproductive tract. *J Reprod Infertil* (2015) 16(3):123.

94. Riviere E, Rossi SP, Tavalieri YE, de Toro MMM, Ponzio R, Puigdomenech E, et al. Melatonin daily oral supplementation attenuates inflammation and oxidative stress in testes of men with altered spermatogenesis of unknown aetiology. *Mol Cell Endocrinology* (2020) 515:110889. doi: 10.1016/j.mce.2020.110889

95. Akaras N, Bal T, Atilay H, Selli J, Halici M. Protective effects of agomelatine on testicular damage caused by bortezomib. *Biotech Histochem* (2017) 92(8):552–9. doi: 10.1080/10520295.2017.1350748

96. Deng S-L, Zhang B-L, Reiter RJ, Liu Y-X. Melatonin ameliorates inflammation and oxidative stress by suppressing the p38MAPK signaling pathway in LPS-induced sheep orchitis. *Antioxidants* (2020) 9(12):1277. doi: 10.3390/antiox9121277

97. Fujita Y, Mihara T, Okazaki T, Shitanaka M, Kushino R, Ikeda C, et al. Toll-like receptors (TLR) 2 and 4 on human sperm recognize bacterial endotoxins and mediate apoptosis. *Hum Reprod* (2011) 26(10):2799–806. doi: 10.1093/humrep/der234

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 07 November 2023
DOI 10.3389/fendo.2023.1244300

OPEN ACCESS

EDITED BY
Dipak Kumar Sahoo,
Iowa State University, United States

REVIEWED BY
Suvranil Ghosh,
University of Texas Southwestern Medical Center, United States
Virendra Kumar Yadav,
Hemchandracharya North Gujarat University, India

*CORRESPONDENCE
Adriana Kolesarova
adriana.kolesarova@uniag.sk

RECEIVED 22 June 2023
ACCEPTED 20 October 2023
PUBLISHED 07 November 2023

CITATION
Mihal M, Roychoudhury S, Sirotkin AV and Kolesarova A (2023) Sea buckthorn, its bioactive constituents, and mechanism of action: potential application in female reproduction. *Front. Endocrinol.* 14:1244300. doi: 10.3389/fendo.2023.1244300

Sea buckthorn, its bioactive constituents, and mechanism of action: potential application in female reproduction

Michal Mihal[1], Shubhadeep Roychoudhury[2], Alexander V. Sirotkin[3] and Adriana Kolesarova[1,4]*

[1]Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovakia, [2]Department of Life Science and Bioinformatics, Assam University, Silchar, India, [3]Department of Zoology and Anthropology, Faculty of Natural Sciences, Constantine the Philosopher University in Nitra, Nitra, Slovakia, [4]AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, Nitra, Slovakia

Sea buckthorn (*Hippophae rhamnoides* L.) is a flowering shrub, and its berries have been utilized for decades as a raw ingredient in cuisines and herbal remedies. This evidence-based study focuses on its key bioactive constituents, and mechanism of protective effects with a focus on female reproductive processes. Parts of the plant contain phenols, carotenoids (lycopene, carotene, lutein, and zeaxanthin), flavonoids (isorhamnetin, quercetin, glycosides, and kaempferol), tocopherols, sterols, polyunsaturated fatty acids, minerals, vitamins, omega 3, 6, 9 and rare omega 7 fatty acids etc. Key polyphenolic flavonoids such as isorhamnetin and quercetin are believed to be mainly responsible behind its health benefits (against cardiovascular diseases, metabolic syndrome, obesity etc.) through properties including anti-cancer, antioxidant, and anti-inflammatory activities. These sea buckthorn constituents appear to mediate healthy ovarian cell proliferation, death, and hormone release, as well as decrease ovarian cancer possibly through apoptosis, and hormonal (estrogen) release. Thus, sea buckthorn and its bioactive ingredients may have potential in the management of gynecological problems such as uterine inflammation, endometriosis, and easing symptoms of vulvovaginal atrophy in postmenopausal women (by targeting inflammatory cytokines and vascular endothelial growth factor – VEGF). Apigenin, myricetin, and luteolin have also been recommended as prospective ovarian cancer preventative and adjuvant therapy options as they can inhibit ovarian cancerogenesis by triggering apoptosis and halting the cell cycle in ovarian tumors. Furthermore, its oil (containing carotenoid, sterol, and hypericin) has been speculated as an alternative to estrogen replacement therapy for postmenopausal women particularly to improve vaginal epithelial integrity. However, it is uncertain whether steroid hormone receptors, reactive oxygen species (ROS), and inflammatory regulators are actually behind sea buckhorn's actions. Sea

buckthorn, and its compounds' health promoting potential warrants further validation not just *in vitro* and in animal research, but also in clinical trials to identify and/or standardize optimal methods of delivery of biologically active molecules.

KEYWORDS

***Hippophae rhamnoides*, isorhamnetin, quercetin, reproduction, female, proliferation, apoptosis, cancer**

1 Introduction

Nowadays, population diseases are becoming more and more widespread. Many factors are responsible for this phenomenon, be it stress, free radical production or lifestyle. The maintenance of the body's redox status has been credited in large part to hormones (1). For example, estradiol has been proven to have a greater impact on the oxidant-antioxidant balance in numerous tissues. Although progesterone lacks the typical chemical structure of an antioxidant, it appears to lessen oxidative damage when present at high amounts (2, 3). Despite these findings, it is important to supplement dietary polyphenols and phytonutrients commonly found in plants. This evidence-based study focuses on the bioactive constituents, and mechanism(s) of protective effects of sea buckthorn (*Hippophae rhamnoides* L.) with a focus on female reproductive processes. Sea buckthorn is a flowering shrub belonging to the family *Elaeagnaceae*. It is native to cold temperate regions of Eurasia (4). This economically and ecologically important medicinal plant is a winter hardy, dioecious, wind-pollinated multipurpose shrub bearing yellow or orange berries with nitrogen-fixing ability. It grows widely in cold regions of the Indian Himalayas, China, Russia, and many other North American and European countries. Due to its enormous potential as a bioresource for land restoration, preventing soil erosion, and its variety of uses, it is frequently referred to as "cold desert gold" (5). Because of its usage in pharmaceutical and cosmetic compounds, as a source of energy, soil enhancer, and as rich nutritional content, sea buckthorn has a high economic worth (6). Almost all parts of the plant may be used as food, firewood, traditional medicine, and a fence. This plant includes many chemical compounds with a range of biological and medicinal effects (7). Sea buckthorn has been used for centuries as a medicinal and nutritional supplement across Asia and Europe (8). Its berries have been utilized for decades in many parts of the world as a raw ingredient in cuisines and herbal remedies. Berries' therapeutic and/or nutritional properties make them an affordable source of raw material for the pharmaceutical industry, which benefits mankind (7). As herbal dietary supplements are used more often in many nations, it is crucial to regulate food items that include these ingredients. However, there is little information on the plant and its extracts' safety assessment (8). Several medicinal benefits of this plant have been well documented, including antioxidant, antitumor, hepatoprotective, or immunomodulating activities (9, 10). Medicinal plants have widely been acknowledged to be the basis for active principles for both therapeutic and preventive measures. Recently, a range of pharmaceuticals have been reported for their antioxidant and anticancer potentials and regulation of hormonal levels to the advantage of management of several disease conditions. Alkaloids, phenols, and acetogenins isolated from graviola (*Annona muricata*) have not only shown promise as possible cancer-fighting agents but also in modulation of cellular proliferation and necrosis. This plant's extract has been reported to downregulate anti-apoptotic genes involved in the pro-cancer metabolic pathways and decreasing the expression of proteins involved in cell invasion and metastasis while upregulating proapoptotic genes and genes involved in the destruction of cancer cells (11). Plant-derived polyphenols including resveratrol, curcumin, quercetin, green tea flavonoids, caffeic acid phenethyl ester, luteolin, xanthohumol, genistein, alpinetin, proanthocyanidins, anthocyanins, silymarin as well as phenolic substances such as thymol, alkaloids like berberine, storage polysaccharides like tamarind xyloglucan, and antioxidant hormones (e.g. melatonin) have been reported to target cellular signaling pathways to reduce intestinal inflammation occurring with inflammatory bowel disorder (1). Plant-based inhibitors of dipeptidyl peptidase-IV (an enzyme that triggers the catalysis of insulinotropic hormones by abating endogenous insulin levels and elevating glucose levels in blood plasma) such as alkaloids, phenolic acids, flavonoids, quercetin, and coumarin have recently been proposed as anti-diabetic by virtue of their hypoglycemic and antioxidative properties (12).

However, a summary of sea buckthorn's physiological and therapeutic effects on female reproductive systems and/or diseases is still lacking. The latest research on sea buckthorn's components, characteristics, physiological effects, and therapeutic uses is reviewed together with their methods of action at multiple regulatory levels, with a focus on female reproductive systems.

The aim of this study was to review the progress in the research on sea buckthorn [regardless of the Latin name, *Hippophae rhamnoides* (including all subspecies)] and its potential application in female reproduction made from 2015 to 2023. Publications about the biological activity of sea buckthorn extracts and their constituents and the mechanism(s) of action have also been described. However, the number of available articles on pharmacological properties of different extracts or natural products from this plant is very large, hence the concerned section of the article only highlights some

important aspects of research made during the last five years. The literature search was performed using Google Scholar, PubMed, and Scopus search engines, with a time limit from 2015 to 2023. Keywords "sea buckthorn" or 'rhamnoides' were combined with 'flavonoids', 'isorhamnetin', "phenolic compounds", 'quercetin', 'ovarian tumor', 'female reproduction', "anti-inflammatory activity", "anticancer activity", "antiviral activity" etc. Finally, 84 original articles from this period were included in this review.

2 Major bioactive constituents

Together with leaves, sea buckthorn berries are rich in a variety of vitamins and other physiologically active components, including up to 106 nutraceutical and 74 bioactive chemicals (13) or even up to 190 bioactive compounds (4). Different parts of the plant contain phenols, carotenoids (lycopene, carotene, lutein, and zeaxanthin), flavonoids (isorhamnetin, quercetin, glycosides, and kaempferol), tocopherols, sterols (4, 13–15), polyunsaturated fatty acids, minerals, vitamins, omega 3, 6, 9 and rare omega 7 fatty acids (4), and dietary fibers (16). The oil derived from sea buckthorn seed is the only natural oil that contains a 1:1 ratio of omega 3 and omega 6 fatty acids (linolenic and linoleic acids) and has β-sitosterol as primary phytosterol (16). Berries are an excellent supply of vital polyunsaturated fatty acids, sugars, and tocopherols, while leaves are a good source of polyphenols (17, 18). *H. rhamnoides L. subsp. yunnanensis* (Yunnanensis), *H. rhamnoides L. subsp. mongolica* (Mongolica), *H. rhamnoides L. subsp. turkestanica* (Turkestanica) *and H. rhamnoides L. subsp. sinensis* are four different subspecies of sea buckthorn that have had their phytochemical compositions studied. *H. rhamnoides L. subsp. yunnanensis* has the largest cellular antioxidant and antiproliferative characteristics, whereas *sinensis* subspecies has the highest total phenolic content and related total antioxidant activity (19). Total flavonoid concentration of sea buckthorn is around 23 mg quercetin equivalent/g dried extract, and total polyphenol content is about 46 mg gallic acid equivalent/g dried extract (20). However, the bioactive content of berries is also affected by age, fruit size, climate, geographic location, and extraction process (21). Zheng et al. (22) found a variety of beneficial chemicals in these berries, including oleanolic acid, 19-alpha-hydroxy ursolic acid, succinic acid, ursolic acid, 5-hydroxymethyl-2-furancarbox-aldehyde, octacosanoic acid, palmitic acid, hippophae cerebroside, and 1-O-hexadecanolenin. Recently, a number of phytoprostanes, phytofurans, tocopherols, tocotrienols, carotenoids, and free amino acids have been detected in sea buckthorn berry juice (23). Berries contain high amounts of polysaccharides (18) and dietary fibers (7, 16). Some bioactive phenolic components, including quercetin-3-O-galactoside, quercetin-3-O-glucoside, kaempferol, and isorhamnetin (24, 25), as well as flavonol glycosides (di- and tri-glycosides) (15) have been detected in leaf extracts. Six compounds from sea buckthorn leaf extract have been isolated previously: kaempferol-3-O- β-α-(6"-O-coumaryl) glycoside, 1-feruloyl-β-α-glucopyranoside, isorhamnetin-3-O-glucoside, quercetin-3-O-β-α-glucopyranoside, quercetin-3-O-β-α-glucopyranosyl-7-O-α-l-rhamnopyranoside, and isorhamnetin-3-O-rutinoside (26). Tannin fractions from leaves have been separated, and the main components are hydrolyzable gallo- and ellagi-tannins of the monomeric type: strictinin, isostrictinin, casuarinin, and casuarictin (25). Data show that sea buckthorn is a rich source of several biologically active compounds that may be helpful to health and effective in the prevention and treatment of a variety of illnesses (27). As mentioned above, key sea buckthorn polyphenolic flavonoids include isorhamnetin and quercetin (Figure 1).

3 Physiological and therapeutic actions

In recent years, research has shown that sea buckthorn can help with illness prevention and healing, including viral infections and cancer (15, 28) owing to its antioxidant (29, 30), anti-inflammatory (31), antiviral (32), antimicrobial (24, 30, 33), and antibacterial (34, 35) properties. Cardioprotective, anti-atherogenic, hepatoprotective, hypolipidemic (29, 36, 37), dermatological (4), antiproliferative (20), and anticancer (e.g., colon, liver, lung, cervical, ovarian, and breast cancer cells) effects have been reported, too (6, 15, 38). Proanthocyanidins, curcumin, and resveratrol have been demonstrated to have considerable advantages in cancer chemoprevention and radiotherapy (39).

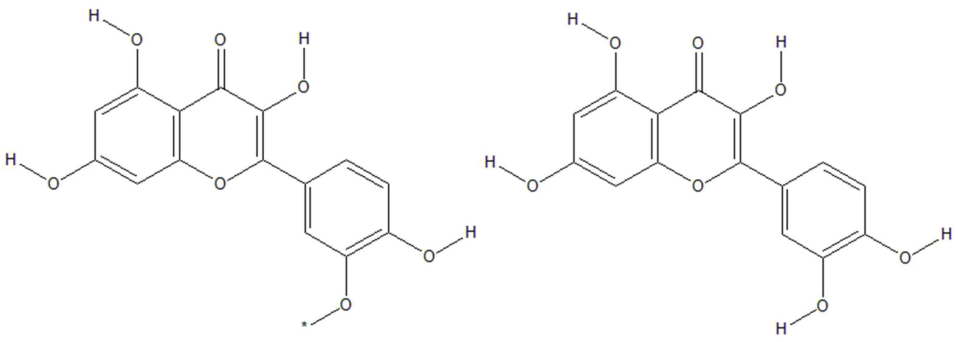


FIGURE 1
Sea buckthorn polyphenolic flavonoids isorhamnetin (left) and quercetin (right).

Similarly, kaempferol has been shown to suppress the growth of breast cancer (40). A higher dietary intake of phenolic substances, particularly flavonoids, and procyanidins, has been linked to a decreased risk of cancer (41). Aurolognans, bioactive constituents of sea buckthorn, have been linked with hepatoprotective, hypolipidemic, and anti-obesity effects (37). Moreover, anti-inflammatory action of this plant could be due to the presence of triterpenes – oleanolic, asiatic, and maslinic acids (42). Table 1 summarizes the physiological and therapeutic activities of sea buckthorn preparations through *in vivo* and *in vitro* experimentations on several experimental models.

3.1 Protective role against cardiovascular diseases, metabolic syndrome, and obesity

The physicochemical and functional features of sea buckthorn berry pomace powder (PP) justify its usage as a fiber-rich dietary

TABLE 1 Physiological and therapeutic actions of sea buckthorn preparations.

Action (s)	Preparation	Experimental model	Results	Reference(s)
Anti-inflammatory	Ethanolic leaf extract	Human keratinocytes cell line HaCaT, human monocytic leukemia cell line THP-1	Inhibition of tumor necrosis factor α (TNF α) and intercellular adhesion molecule 1 expression by casuarinin present in sea buckthorn; decrease in TNF α-induced pro-inflammatory mediators, such as interleukin 6, interleukin 1β, interleukin 8, monocyte chemoattractant protein-1	(31)
	Myricetin (a flavonoid from sea buckthorn)	Rats with high-fat-diet	Reducing inflammation by regulating butyric acid producing intestinal microorganisms and protecting intestinal barrier function	(43)
	Sea buckthorn extract	Rats with high-fat-diet	Promotion of ZO-1 and occludin mRNA expression in intestinal tight junction proteins; repair of intestinal mucosa; anti-inflammatory role in inhibiting the signal pathway of NOD-like receptor	(44)
Antioxidant	Aqueous seed extract	Liposome model system, *Listeria monocytogenes, Yersinia enterocolitica*	Good antioxidant effect in different assay systems (reducing power, DPPH assay and liposome model system).	(33)
	Fermented sea buckthorn juice	H_2O_2-treated C_2C_{12} cells	Increase in intracellular SOD and glutathione peroxidase (GSH-Px) activity; decrease in ROS content, catalase (CAT) activity, and malondialdehyde (MDA) content	(30)
	Aqueous and hydroalcoholic leaf extract	Baby hamster kidney cell line 21 BHK-21, *Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli*	Potent antioxidant activity determined by ABTS, DPPH and FRAP assays; cytoprotective activity against hydrogen peroxide, hypoxanthine-xanthine oxidase induced cell damage; growth inhibition against bacteria.	(24)
Antiviral	Ethanolic leaf extract	Dengue virus type-2 infected human peripheral blood mononuclear cells	Maintaining the cell viability of Dengue-infected cells, decrease in the TNF α and increase in the interferon γ production in Dengue-infected cells.	(32)
	Sea buckthorn leaf extract	MDCK (Madin-Darby Canine Kidney) cells infected with influenza viruses A/Victoria, A/PR, B/Lee and B/Maryland	Extreme anti-influenza activity; flavonols did not interact directly with influenza viral particles, and inhibited initial stage of virus replication only	(15)
	Sea buckthorn DMSO extract	HSV-2 infected Vero cells	Dose dependent inhibitory effect against HSV-2 virus	(45)
Antibacterial	Phenol rich fraction from leaves	*Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*, *Streptococcus pneumoniae* and *Staphylococcus aureus*	Broad spectrum antibacterial effect by growth inhibition of certain medically important bacterial species.	(34)
	Chitosan extracted from sea buckthorn leaves	*Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Listeria monocytogenes*	Enhanced antibacterial properties	(30)
	Sea buckthorn seeds	*Escherichia coli, Staphylococcus aureus, Salmonella, Pseudomonas aeruginosa, Bacillus subtilis*	Potential promotion of food preservation by sea buckthorn seed polyphenols	(46)

(Continued)

TABLE 1 Continued

Action (s)	Preparation	Experimental model	Results	Reference(s)
Anticancer	Ethyl acetate and ethanol:water sea buckthorn extracts	Co-cultured human intestinal epithelial cell line Caco-2, human hepatocyte carcinoma cell line Hep G2	Dose-dependent antiproliferative effect by inhibition of cancer cell proliferation.	(6)
	Copper nanoparticles synthesized from stem extracts of sea buckthorn	HeLa cells	Concentration-dependent reduction in cell viability (as measured by MTT assay), and apoptotic activity (as detected by ROS production)	(47)
	Sea buckthorn leaf extract	Lung cancer cells NCL-H1299, human ovarian cancer cells HeLa and SKOV, and cervix cancer cells Caski	Fascinatingly higher and wider range of cytotoxic activities against lung, ovarian, and cervical cancer cells	(15)
Hepatoprotective	Ethanolic extract of berries	Male mice C57BL/6	Protective effect against acetaminophen (APAP)-induced hepatotoxicity associated with the activation of the Nrf-2/HO-1-SOD-2 signaling pathway; suppression of APAP-induced increase in the ratio of B-cell lymphoma protein 2-associated X.	(13)
	Ethanolic extract of sea buckthorn berries	Pathogen-free Kunming mouse	Sea buckthorn flavonoids significantly reduced the weight, liver fat accumulation, and serum triglyceride level of obese mice induced by high-fat diet and inhibited the chronic inflammatory reaction caused by obesity	(48)
	Sea buckthorn berry seed oil (SBO)	BALB/c mice	Protective effect of SBO against cyclophosphamide -induced liver damage, which reflected its antioxidant properties	(49)

additive (16). PP had strong hydration qualities in addition to having a high protein content (21.09 g/100 g) such as 4.24 g/g and 9.98 mL/g of water-holding capacity and swelling capacity, respectively. The functional potential of the tested PP was determined by its *in vitro* hypoglycemic and hypolipidemic qualities, which were shown to be comparable to and, in some cases, superior to those of other dietary fiber powders made from by-products of the processing of fruits and vegetables. Berry PP had a cholesterol-binding capacity of 21.11 to 23.13 mg/g (16). Traditionally, sea buckthorn berries also serve as a Chinese medicine with multiple bioactivities (18). A recent bioassay-guided investigation applied to seek the hepatoprotective and hypolipidemic ingredients has been able to isolate three new (10 → 10")-biauronlignans (1–3), three new 10-(4"-hydroxy-benzyl)-auronlignans (4–6), three new 10-O-β-D-glucopyranosyl-auronlignans (7–9), and eleven known auronlignan derivatives (10–20). Their structures have been established using lengthy and thorough infrared (IR), ultraviolet/visible (UV/Vis), nuclear magnetic resonance (NMR), and mass spectroscopy (MS) spectrum investigations, and these results have been compared with the published references. While compounds 2, 5, 8, and 12 displayed mild pancreatic lipase activity inhibition and reduced the moderately FFA-induced lipid accumulation in HepG2 liver cells, compounds 1, 4, 7, 11, 15, and 19 demonstrated moderate hepatoprotective activities against the damage in acetaminophen-induced HepG2 cells (37). In addition, structural data of a homogeneous polysaccharide from sea buckthorn (SBP-1-A) has recently been described, and it was discovered that SBP-1-A has a backbone of around 3,4).-β-l-Rhap-(1 → 4)-α-d-GalAp-(1 → with side chains made up of α-l-Araf, β-d-Galp, β-d-Glcp, and α-d-Glcp, of which the arabinose, glucose, and galactose residues have been identified as the primary monosaccharide compositions with a percentage surpassing 92%. Furthermore, the protein-free polysaccharide fraction (SBP-1) obtained after isolation of crude SBP showed an outstanding anti-obesity effect. According to the findings, consuming SBP-1 might increase the expression of peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1α), uncoupling protein 1 (UCP-1), and PR domain containing 16 (PRDM 16) in adipocytes, activate brown adipocytes, and boost thermogenesis, which would prevent fat buildup and weight gain. It is crucial to remember that the type of preparation, its chemical composition, and its concentration all appear to have an impact on how sea buckthorn preparations affect hemostasis. The sea buckthorn preparations seem to be excellent regulators of hemostasis, particularly blood platelet function, due to their high phytochemical contents, notably phenolic components. Additionally, it is still uncertain how much of these preparations should be used for prophylaxis and therapy, and recommendations for using sea buckthorn preparations are frequently based on sparse clinical investigations. Thus, more randomized clinical studies with bigger samples are required, particularly those including healthy volunteers and those with the greatest cardiovascular risk factors. Additionally, the effects of several sea buckthorn components on hemostasis, including fibrinolysis and coagulation systems as well as blood platelet activities, should be studied in these trials. Since there is currently no reliable information about the anti-hemorrhagic effectiveness of sea buckthorn preparations in either people or animals, it is also crucial to investigate the role of various sea

buckthorn products in the prevention and treatment of cardiovascular disorders (50). Recently, phytoprostanes in sea buckthorn juices have been discovered, and their quantities are highly connected with the capacity to reduce inflammation through inhibition of the 15-lipoxygenase enzyme (23). Due to the presence of possible inhibitors of α-amylase, α-glucosidase (tocopherols, tocotrienols, and certain amino acids), and pancreatic lipase (xanthophylls), sea buckthorn juice can be an intriguing anti-diabetic and anti-obesity diet. Juices act more effectively in lowering neurological alterations due to the presence of phytoprostanes, phytofurans, tocopherols, tocotrienols, and amino acids, making them possible anti-aging agents in the prevention of Alzheimer's disease, the most prevalent kind of dementia. Juice from sea buckthorn may be crucial in the body's fight against diseases brought on by free radical assault (23). Sea buckthorn insoluble dietary fiber (IDF) can be modified to increase its *in vitro* hypoglycemic capacity. Examples of these modifications include IDF, milled insoluble dietary fiber, and co-modified insoluble dietary fiber. Ball milling, as well as ball milling coupled with cellulose treatment reportedly enhanced the characteristics of IDF which provide a foundation for the extensive utilization of sea buckthorn resources (27).

3.2 Anti-inflammatory properties

Maslinic acid functions via the nuclear factor kappa light chain enhancer of activated B cells (NF-κB) and erythroid 2-related factor 2 (Nrf2) signaling pathways, whereas oleanolic and asiatic acids act via the NF-κB, mitogen-activated protein kinase (MAPK), and Nrf2 signaling pathways to exert anti-inflammatory effects on macrophages. These three substances can be employed as natural anti-inflammatory dietary supplements because they exhibited specific inhibitory effects on the LPS-induced inflammatory response *in vitro*. However, more research is necessary, including *in vivo* investigations, to encourage the usage of sea buckthorn-derived products (42). The active components of sea buckthorn that are responsible for the biological effects haven't yet been fully identified. The flavonoids quercetin and isorhamnetin, a 3′-O-methylated metabolite of quercetin, are thought to be principally in charge. There is proof that isorhamnetin can prevent cells from proliferating (42), promote apoptosis and mitigate tumor development (51–53), suppress inflammatory processes (33, 42, 54), improve cognitive functions (54), and affect numerous metabolic processes (33). The anti-cancer (29, 55), cardioprotective (56), and anti-obesity (57) effects of quercetin have been reported, too. Presence of several flavonoids including isorhamnetin (54, 58), auronlignan (18), polysaccharides (37, 59), and dietary fibers (16, 27) indicate towards the anti-cholesterol and anti-obesity effects of sea buckthorn. Thus, a number of physiological and pathological processes can be targeted by sea buckthorn and its bioactive compounds. Nevertheless, a majority of the studies on sea buckhorn action were performed for medicinal purposes in pathological conditions, including on cancer cells. Therefore, obtained information could potentially be helpful for management of tumors, but the biological value of the information is limited due to the fact that whether and how the dietary consumption of sea buckwheat could affect healthy organism and its cells. Even the applicability of sea buckthorn for treatment of cancer is yet to be sufficiently demonstrated by clinical trials. Furthermore, sea buckthorn constituents responsible for the effect of the whole plant remains to be identified. The molecules, which might be responsible for sea buckhorn effect have been hypothesized on the basis of their presence in the plant and the similarity of their as well as whole plant effects. But there isn't a single thorough experiment that has compared these impacts. Furthermore, there is no conclusive evidence of a functional connection from the similarities of the effects. Therefore, extensive research is needed to identify the components of sea buckthorn that are responsible for its biological and therapeutic effects.

4 Mechanism of action

4.1 Mechanism of action of sea buckthorn

Although the evidence for each of these processes is weak and the interactions between these mechanisms are poorly understood, extracellular and intracellular modes of action of sea buckthorn and its constituents on cells have been postulated. Nevertheless, it has been proposed that the ability of sea buckthorn to prevent and to treat infections and cancer (15) as mentioned previously can mainly be due to its antioxidant (29), anti-inflammatory (31), antiviral (32), antimicrobial (24, 33), and antibacterial (34, 35) properties. Numerous clinical disorders, including allergies, cancer, and many others, are mostly driven by inflammation. By stimulating Nrf2-dependent pathways, sea buckthorn's anti-inflammatory effects may be mediated (60). An effective anti-inflammatory target, the heme oxygenase-1 (HO-1) axis, is known to be regulated in part by Nrf2. Nrf2 is essential for regulating the production of antioxidant genes, which in turn have anti-inflammatory effects (60). The protective action of sea buckthorn polysaccharide is linked to the activation of the Nrf-2/HO-1-SOD-2 signaling pathway (36). Recent investigations revealed a relationship between the production of additional inflammatory mediators including the NF-κB pathway and macrophage metabolism and the Nrf2/antioxidant response element system (60). It's interesting to note that a sea buckthorn polysaccharide has been shown to protect the liver against acetaminophen (APAP)-induced liver damage in rats. Enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have been able to be reduced by it (36). The antioxidant qualities of sea buckthorn's components have been used to describe a variety of its therapeutic benefits. In the liver, brain, and plasma, for instance, sea buckthorn supplementation elevated glutathione (GSH) and GSH-Px levels and the production of nitric oxide (NO) and the inducible nitric oxide synthase (iNOS), which was linked to lessened liver damage (36) and oxidative and nitrosative stress in liver and brain of rats (61). Antioxidant chemicals, especially phenolic components such as flavonoids kaempferol, isorhamnetin, and quercetin, are responsible for sea buckthorn's antitumor action. These flavonoids defend against oxidative stress, which can cause cancer and genetic alterations in

cells (50, 62). The formation of ROS was reduced along with the reduction in glioma cell viability after sea buckthorn extract treatment, at the very least (26). Masoodi et al. (63) has proposed that sea buckthorn reduces the production of a certain antigen and inhibits cellular growth in prostate cancer cells. Additionally, rat glioma cells' fast multiplication was suppressed by sea buckthorn leaf extract, which is thought to have done so via causing the first stages of cell death. The increased expression of B-cell lymphoma 2/Bcl-2-associated X protein (Bcl-2/Bax) and acetaminophen-induced inhibition of c-Jun N-terminal kinase phosphorylation are further signs of sea buckthorn's impact on cytoplasmic apoptosis (36). The pro-apoptotic Bax gene expression was increased by sea buckthorn extracts, and its localization, accumulation, and translocation in the nuclei were all encouraged (26). However, as demonstrated in human retinoblastoma cells, the quercetin-induced rise in cytochrome c levels together with the activation of caspase-3 and caspase-9 results in apoptosis in cancer cells (64). Additionally, it was shown previously that buckthorn procyanidins might cause cell death in a dose-dependent way (65). It is possible that these procyanidins might block intracellular fatty acid synthase (FAS) activity and cause human breast cancer cells to undergo apoptosis. At least, sea buckthorn procyanidins were discovered to restrict the proliferation of cancer cells, and FAS is a critical enzyme for *de novo* long-chain fatty acid production, which is present in high amounts in cancer cells (65). Several flavonoids (especially isorhamnetin) affecting a number of enzymes regulating fat synthesis and metabolism can be responsible for their hypolipidemic, cholesterol-lowering, and anti-obesity effects (58). In addition, the anti-obesity effects of sea buckthorn polysaccharides could be due to their stimulatory action on brown adipose tissue and thermogenesis inducing its "burning" (37). Finally, sea buckthorn's anti-diabetic and anti-obesity effects can be attributed to the ability of its dietary fibers to reduce glucose production and metabolism via suppression of glucose adsorption, glucose diffusion inhibition, starch digestion inhibition, starch pasting interference, and α-amylase activity (27). Androgen receptors are the next potential mediator of sea buckthorn's actions on tissues that are dependent on hormones. These receptors control the expression of androgen-responsive genes through ligand-dependent transcription factors. The target androgen responsive genes cannot be activated, and prostate cancer growth cannot be stopped if the androgen receptor is somehow kept in the cytoplasm and its shuttling into the nucleus is blocked. Prostate cancer cells' androgen receptors have been shown to be affected by the administration of sea buckthorn leaf extracts, which was correlated with the suppression of genes that respond to androgens, cellular growth, and survival of these cells (63). The beneficial effects of sea buckthorn on spermatogenesis may be due to its effect on androgen receptors. By increasing spermatogonia proliferation, stem cell survival, and lowering sperm abnormalities, sea buckthorn therapy enhanced spermatogenesis and had a protective effect against the negative effects of gamma radiation (66). Available literature demonstrates a number of signaling molecules and mechanisms mediating sea buckthorn's actions on various targets in the organism and/or their pathologies at a cellular level. Some of their mediators could be specific for particular target cells or organs (e.g., steroid hormones for steroid-dependent processes). Other mediators could be more pervasive, such as those that influence cell proliferation, apoptosis, and oxidative processes. Additionally, although they haven't been fully explored yet, functional hierarchy linkages between the mediators of sea buckthorn's effects are feasible. Last but not least, studies of the effects of sea buckthorn and its mediators have primarily used cell cultures. As a result, appropriate *in vivo* research should be used to confirm the findings gained using such models.

4.2 Mechanism of action of selected sea buckthorn constituents – isorhamnetin and quercetin

One of the most potent active components in sea buckthorn berries is isorhamnetin, a 3′-O-methylated metabolite of quercetin that has a wide range of pharmacological effects, including anti-cancer ones. The modulation of PI3K/AKT/PKB, NF-κB, MAPK, and other signaling pathways, as well as the production of associated cytokines and protein kinases involved in controlling cell apoptosis and proliferation, are all part of the mechanisms of action (53, 54). Through the activation of apoptotic genes and apoptosis and the downregulation of oncogenes, isorhamnetin can inhibit the development of cancer. Isorhamnetin has also been demonstrated to inhibit the PI3K-AKT-mTOR pathway (phosphatidylinositol 3-kinase, protein kinase B, and the mammalian target of rapamycin), which in turn inhibits the proliferation of cancer cells by inducing cell cycle arrest at the G2/M phase. Additionally, isorhamnetin can increase the production of cyclin B1 protein while decreasing the phosphorylation levels of AKT, phosph-p70S6 kinase, and phosph-4E-BP1 proteins (52). Additionally, isorhamnetin can enhance liver and kidney functioning by lowering blood levels of urea nitrogen, AST, and ALT. Additionally, by preventing the dimerization of the toll-like receptor 4, isorhamnetin can reduce infection-induced liver and kidney inflammation as well as inflammation-induced cell death (33). Some physiological actions of isorhamnetin could be mediated by an interplay of several intracellular signaling pathways. For example, isorhamnetin can mitigate the adverse effect of obesity on cognitive functions by suppression of neuroinflammation via downregulation of MAPK- and NFkB-dependent pathways (54). Quercetin is another ingredient that may contribute to the benefits of sea buckthorn. Quercetin's ability to influence intracellular signaling pathways that regulate cell proliferation and apoptosis may be the cause of its anti-cancer effects (29, 55, 67). It can cause cell cycle arrest through suppression of its promoters cyclin B1 and MAPK/ERK1/2 and activation of transcription factor p53. It can also prolong DNA repair and promote apoptosis through inhibition of survivin, activation of transforming growth factor-β (TGF-β), PI3K/AKT/mTOR, Wnt/-catenin, NOTCH, sonic hedgehog signaling pathway (SHH), Janus kinas Additionally, quercetin can inhibit tumorigenesis by controlling VEGF and its receptors, which are the factors that stimulate tumor vascularization (55). The presence of quercetin, tannins, and other polyphenolic flavonoids in sea buckthorn extract, as well as their radical scavenging and anti-

inflammatory properties, may be responsible for the protection of spermatozoa (66), and cardiomyocytes (56, 57). Some possible mechanisms of action of sea buckthorn and its bioactive components on cancer cells are summarized in Figure 2. In fine, the literature concerning the mechanisms and/or mediators of action of sea buckhorn compounds isorhamnetin and quercetin indicate an existence of multiple pathways of these molecules on target cells. The key mechanisms of their action (e.g., related to cell proliferation, apoptosis, or oxidative stress) are like mechanisms of sea buckthorn whole plant's effects. Nevertheless, it remains to be investigated whether the effects of the whole plant and its mechanisms

of action could be explained by the presence of only isorhamnetin and quercetin, or other constituents and their mechanisms of action could be involved in mediating sea buckthorn's effects. Furthermore, the data concerning these mediators, which were obtained predominantly by *in vitro* experiments, require verification by corresponding *in vivo* studies.

5 Effect on female reproductive processes

5.1 Ovary cancer preventive effects of sea buckthorn

There is no evidence accessible in scientific databases about the effects of entire sea buckthorn on ovarian functions. Dietary sea buckthorn oil did not influence bovine ovarian folliculogenesis, oocyte quality, or embryo developmental ability (68). Nonetheless, some physiologically active sea buckthorn elements have been shown to influence female reproductive processes. However, the direct impact of quercetin on fundamental ovarian cell processes (proliferation, apoptosis, and hormone release) may vary depending on the species (69). Quercetin also inhibits the development of human metastatic ovarian cancer cells and affects the intrinsic apoptotic mechanism (70–73). Isorhamnetin, another sea buckthorn component, can stimulate ovarian cell proliferation and estrogen release (52, 70), and suppress estrogen-dependent ovarian cancer development (70, 73). Other physiologically active components of sea buckhorn have also been proven to be advantageous to ovarian cancer cells. Apigenin, myricetin, and luteolin have been shown to cause apoptosis, decrease cell proliferation, limit cell invasion, and stop the cell cycle of ovarian cancer. Furthermore, apigenin, myricetin, and luteolin have been recommended as prospective ovarian cancer preventative and adjuvant therapy options (70, 74). Another sea buckthorn ingredient that can inhibit ovarian cancerogenesis is kaempherol. In cultivated ovarian cancer cells, it can at least trigger apoptosis and halt the cell cycle (70, 73, 75–77). Furthermore, kaempherol can inhibit angiogenesis in ovarian tumors (76). As a result, sea buckthorn components may influence ovarian cell proliferation, death, and hormone release. In ovarian cancer cells, on the other hand, sea buckthorn components can cause apoptosis, decrease cell proliferation, and stop the cell cycle. Nonetheless, the relevance of the collected data is restricted by the fact that the claimed effects on ovarian functions are primarily the product of *in vitro* investigations, with majority of these tests being done on ovarian cancer cells rather than healthy cells. There is a need for more information on the entire sea buckthorn's activity on ovarian functions (including dysfunctions and malignant transformation) both *in vitro* and *in vivo*.

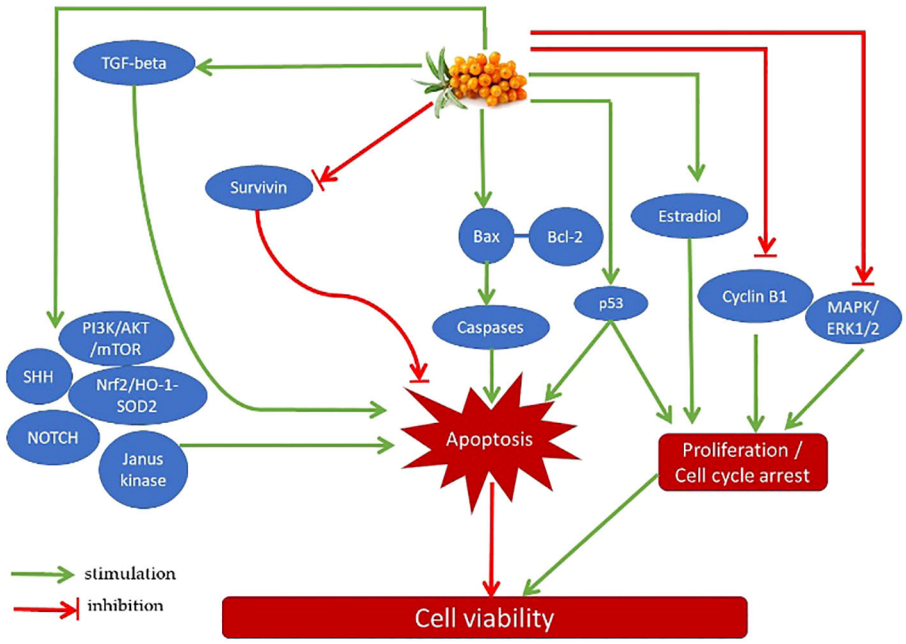


FIGURE 2
Possible mechanisms of action of sea buckthorn and its bioactive components on cancer cells.

5.2 Effect on vagina and uterus

Traditionally, sea buckthorn has been used to treat gynecological diseases such as uterine inflammation and endometriosis. Its oil helps reduce the symptoms of endometriosis and uterine inflammation. These effects might be linked to the carotenoid, sterol, and hypericin content of the plant (55). Consumption of sea buckthorn extract or oil may also be beneficial in the avoidance of vaginal difficulties during menopause, which is associated with vaginal atrophy and the thinning and drying of the vaginal mucosa. Menopausal women who used sea buckthorn oil had better vaginal epithelial integrity and a higher vaginal health score. It has been proposed as an alternative to estrogen replacement therapy for postmenopausal women's vaginal health (78). Furthermore, a novel vaginal gel containing sea buckthorn oil (Meclon Idra Alfasigma) has recently been registered. It appears to be a viable option as a local agent for alleviating symptoms of vulvovaginal atrophy (vaginal dryness, itching, and burning feeling) and enhancing sexual function in postmenopausal women (79). Sea buckthorn also has a high concentration of vitamins C and E. Infertile or subfertile women undergoing controlled ovarian stimulation may benefit from vitamin C and E supplementation in terms of uterine features, endometrial thickness, and endometrial blood flow. Furthermore, the antioxidant and anticoagulant properties of vitamins C and E are assumed to be responsible for the increase in fertility (80). In contrast to its effect on the ovary, sea buckthorn, and its components have been shown to have a therapeutic effect on the management of gynecological problems such as uterine inflammation, endometriosis, and signs of vulvovaginal atrophy in postmenopausal women. We summarized the important effects in Table 2. However, the effect of sea buckthorn on the healthy vagina and uterus has not been thoroughly established. Furthermore, the components of sea buckthorn that affect these organs are mainly unknown. Even the role of vitamins in mediating the benefits of sea buckthorn is more or less theoretical and requires scientific validation.

TABLE 2 Physiological and therapeutic actions of sea buckthorn and its constituencies relating to female reproductive processes.

Therapeutic action(s)	Preparation	Experimental model	Refer-ence(s)
In vivo studies			
Prevention of vaginal complications during menopause	Sea buckthorn extract/oil (Three months, during which subjects consumed 3 g of sea buckthorn or placebo oil daily)	Menopausal women	(78)
Improving sexual function in postmenopausal women	Sea buckthorn oil (Active vaginal gel or placebo was applied for 14 days and then twice a week for 90 consecutive days)	Menopausal women	(79)
Can improve uterine characteristics, endometrial thickness, and endometrial blood flow	Sea buckthorn [2 years combined therapy of clomiphine citrate (from day 2-6 of the cycle) and vitamins E (400mg) and C (500 mg) (ndash;from day 1-14 days of the cycle)]	Infertile and subfertile women	(80)
Cell line studies			
Suppresson of estrogen-dependent ovarian cancer development	Isorhamnetin and quercetin DMSO solution	Human ovarian granulosa-like KGN cells	(70)
Promoting ovarian cell proliferation, estrogen release	Isorhamnetin DMSO solution	Human ovarian granulosa-like KGN cells	(70)
Inducing apoptosis and blocking cell cycle in cultured ovarian cancer cells	Kaempherol DMSO solution	Human breast cancer MCF-7 and MDA-MB-453 cells	(75)
Suppression of angiogenesis in ovarian tumor	Kaempherol DMSO solution	Triple-negative breast cancer cells (TNBC)	(76)

6 Mechanism of action on female reproductive processes

There is inadequate information to support the mechanism of sea buckthorn's impacts on female reproductive systems. Nonetheless, existing evidence allows us to sketch some of the processes and mediators of sea buckthorn or its active ingredients in the female reproductive system. Sea buckthorn oil has been shown to reverse endometriosis in rat. The therapy lowered the levels of inflammatory cytokines (inflammation markers and promoters) and VEGF (angiogenesis markers and promoters) (55). Therefore, cytokines and VEGF could be extracellular mediators of the curative action of sea buckthorn on endometriosis. Imran et al. (76) also hypothesized that kaempferol would reduce tumor development and angiogenesis by lowering VEGF expression via hypoxia-inducible factor 1α (HIF-1α), a physiological activator of VEGF synthesis. Some sea buckthorn flavonoids have also been shown to operate on ovarian cells via intracellular regulators of proliferation and death. For example, quercetin promotes caspase-3 expression, which may result in DNA fragmentation and apoptosis. In addition, quercetin has been demonstrated to reduce the expression of anti-apoptotic proteins while increasing the synthesis of pro-apoptotic proteins in a variety of cancer cell lines, including ovarian cancer (71, 72). Isorhamnetin has the potential to influence ovarian cancer cell proliferation and apoptosis by targeting intracellular PI3K/Akt

signaling pathway promoters of the cell cycle (cyclins) and apoptosis (Bax, Bcl, and cytochrome) (52, 77). Kampherol has been shown to enhance the production of morphological indications of apoptosis (membrane blebbing) and the accumulation of apoptotic intracellular markers and promoters (caspases 3, 8, and 9, as well as Bax) while decreasing the expression of anti-apoptotic Bcl-2. Furthermore, kaempferol caused cell cycle arrest at the G0/G1 checkpoint, as well as inhibition of cyclin B1 and Cdc2 expression (75). Imran et al. (76) suggested that kaempferol can induce apoptosis and cell cycle arrest at the G2/M phase via upregulation of checkpoint kinase 2/cell division cycle 25C/cyclin-dependent kinase 2 (Chk2/Cdc25C/Cdc2), receptors DR5 and DR4, c-Jun N-terminal kinase (JNK), C/EBP homologous protein (CHOP), p38, p21, the extracellular signal-regulated kinase 1/2 (ERK1/2) proteins, caspase-3, -7, -8, Bad, Bax, and p53 proteins. The ability of sea buckthorn (50) and isorhamnetin (52) to affect ROS, the known promoters of apoptosis, indicates that this sea buckthorn flavonoid can impact ovarian cell viability and result in events impacting oxidative stress. Finally, sea buckhorn constituents isorhamnetin (52, 70) and quercetin (69, 81) can affect the production of estrogens and other ovarian hormones, which are considered as key regulators of ovarian functions and female reproduction and fecundity (81). This fact indicates that sea buckthorn might impact reproductive processes through hormonal mechanisms, too. The few relevant published findings suggest that sea buckthorn may be useful in the treatment of endometriosis by influencing extracellular regulators of inflammatory processes such as cytokines and VEGF. However, the intracellular mediators of this therapeutic activity still need to be identified and verified. It is uncertain whether this plant or its active ingredients have an effect on the healthy vagina and uterus. More is known about the mechanisms/mediators of sea buckthorn and its components' impact on ovarian cells. Sea buckthorn flavonoids have been shown to suppress ovarian cancer cells by downregulating VEGF, anti-apoptotic proteins, upregulating pro-apoptotic proteins, suppressing the cell cycle at various checkpoints, p-AKT, and inducing oxidative and endoplasmic reticulum stress and autophagy. Hormones may also play a role in modulating the effects of plant constituents' isorhamnetin and quercetin on female reproductive organs. However, it should be emphasized that the mediators and processes of sea buckthorn or its components are postulated primarily on the basis of indirect evidence - since these regulators have been altered following the administration of the plant or its constituents. More direct experimental data is needed to understand the functional interrelationships between plant compounds and reproductive process regulators. When compared to known mediators of its effects on non-reproductive processes, the number of recognized mediators of sea buckthorn on female reproductive organs is small. More research would very certainly add to the list of mediators of sea buckthorn's physiological and therapeutic effects on female reproductive systems. Although hierarchical interrelationships between numerous mediators of sea buckthorn's activities on the ovary, vagina, and uterus are plausible, they have yet to be thoroughly investigated.

7 Potential for application in reproduction

Sea buckthorn is being studied as a functional food as well as a herbal medication for animal and human health, including the treatment of numerous female reproductive diseases. Although there are some publications on the effects of sea buckthorn chemicals quercetin and isorhamnetin on healthy ovarian cells, it is uncertain if sea buckthorn extract or its constituents could be effective in influencing healthy female reproductive processes. More data, however, is available on the use of sea buckthorn and its components to prevent and/or perhaps treat ovarian cancer. Flavonoids found in sea buckthorn can decrease cancer cell proliferation, cause apoptosis, prevent cell cycle arrest, and slow tumor development. This might point to the possible use of sea buckthorn flavonoids in the prevention and treatment of ovarian cancer.

Furthermore, ovarian cancer is linked to other gynecological diseases such as endometriosis (82). The potential use of sea buckthorn and its active ingredients in the treatment of gynecological problems such as uterine inflammation, endometriosis, and symptoms of vulvovaginal atrophy in postmenopausal women has been proven (82). No toxicity of sea buckthorn berries (50) or sea buckthorn berry oil (8) has been reported including no treatment-related maternal toxicity or embryotoxicity (8). According to the data, sea buckthorn products can be used as functional foods, nutritional supplements, and medicines. However, it cannot be ruled out that extracted and purified sea buckthorn compounds/molecules might be used in place of dietary sea buckthorn or its extract. Although such a substitute may be more expensive, the dose and ingredients may be easier to define, and the biological and/or therapeutic efficiency may be greater and more predictive than the raw plant product. Taken together, the available evidence on sea buckthorn's beneficial effects suggests that it has potential therapeutic applications in phytotherapy of cancer, endometriosis, and/or other reproductive dysfunctions.

8 Conclusions and possible direction of future studies

Sea buckthorn elements appear to alter healthy ovarian cell proliferation, death, and hormone release, as well as decrease ovarian cancer (by triggering ovarian cancer cell apoptosis and autophagy, decreasing cell growth, invasion, and halting the cell cycle). Furthermore, sea buckthorn and its bioactive ingredients may be effective in the treatment of gynecological problems such as uterine inflammation, endometriosis, and easing symptoms of vulvovaginal atrophy in postmenopausal women by targeting inflammatory cytokines and VEGF, as previously indicated. Nonetheless, many elements of sea buckhorn activity and application remain unknown to science. Inadequate research has been conducted on the effects of sea buckhorn extract on female

reproductive processes and the roles of major individual elements. There is no information concerning the possible functional interrelationship among various plant constituents in the regulation of reproductive and non-reproductive processes, for example.

The mediators of sea buckthorn action have also been studied insufficiently, whilst the role and hierarchical interrelationships between signaling molecules mediating sea buckthorn actions remain rather speculative so far. They are based mainly on similar interrelationships between mediators of other substances. For example, it is possible that plant flavonoids with antioxidant properties could block ROS, prevent oxidative stress, and resulting inflammatory processes, mutagenesis, apoptosis, and arrest cell cycle (81, 83). It is possible that this is a case of sea buckhorn isoflavones, too. Nevertheless, such mechanisms might be proposed based on indirect indications only – the action of flavones on some indices of oxidative, inflammatory processes, apoptosis, or proliferation. Furthermore, plant flavonoids usually have phytoestrogenic properties – the ability to affect the receptors of steroid hormones, which in turn are the important regulators of cell proliferation, apoptosis, and cancerogenesis (81, 84). Sea buckthorn components/molecules can affect steroid hormones and steroid hormones-dependent processes, as discussed earlier. However, it is uncertain whether steroid hormone receptors, ROS, inflammatory regulators, and so on actually cause sea buckhorn function. Although research on this plant has concentrated on its medicinal potential and use, the function of sea buckhorn extract and some of its important components on a healthy female reproductive system is still mostly unknown. It is also necessary to identify and/or standardize optimal methods of delivering biologically active molecules of sea buckhorn. This plant's and its compounds' medicinal potential should be validated not just *in vitro* and in animal research, but also in clinical trials. The findings of the few reported studies listed above may just be the beginning steps toward understanding the biology and therapeutic potential of this interesting plant and its active ingredients, which will require further confirmatory research.

Author contributions

Conceptualization: MM, SR, AK. Writing – original draft preparation: MM. Writing – review and editing: SR, AK. Supervision: AK.

Funding

This study was supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic projects APVV-18-0312, VEGA 1/0266/20, and KEGA 033SPU-4/2021.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sahoo DK, Chainy GBN. Chapter Eight - Hormone-linked redox status and its modulation by antioxidants. In: Litwack G, editor. *Vitamins and hormones*, vol. 121. Cambridge, Massachusetts, USA: Academic Press (2023). p. 197–246. Available at: https://www.sciencedirect.com/science/article/pii/S0083672922000826.

2. Chainy GBN, Sahoo DK. Hormones and oxidative stress: an overview. *Free Radic Res* (2020) 54(1):1–26. doi: 10.1080/10715762.2019.1702656

3. Hernández-Rabaza V, López-Pedrajas R, Almansa I. Progesterone, lipoic acid, and sulforaphane as promising antioxidants for retinal diseases: A review. *Antioxidants* (2019) 8(3):53. doi: 10.3390/antiox8030053

4. Pundir S, Garg P, Dviwedi A, Ali A, Kapoor VK, Kapoor D, et al. Ethnomedicinal uses, phytochemistry and dermatological effects of Hippophae rhamnoides L.: A review. *J Ethnopharmacol* (2021) 266:113434. doi: 10.1016/j.jep.2020.113434

5. Kalia RK, Singh R, Rai MK, Mishra GP, Singh SR, Dhawan AK. Biotechnological interventions in sea buckthorn (Hippophae L.): current status and future prospects. *Trees* (2011) 25(4):559–75. doi: 10.1007/s00468-011-0543-0

6. Grey C, Widén C, Adlercreutz P, Rumpunen K, Duan RD. Antiproliferative effects of sea buckthorn (Hippophae rhamnoides L.) extracts on human colon and liver cancer cell lines. *Food Chem* (2010) 120(4):1004–10. doi: 10.1016/j.foodchem.2009.11.039

7. Ozturk M, Hakeem KR, Ashraf M, Ahmad MSA. *Global perspectives on underutilized crops*. Cham: Springer International Publishing (2018). Available at: http://link.springer.com/10.1007/978-3-319-77776-4.

8. Wen P, Zhao P, Qin G, Tang S, Li B, Zhang J, et al. Genotoxicity and teratogenicity of seabuckthorn (*Hippophae rhamnoides* L.) berry oil. *Drug Chem Toxicol* (2020) 43(4):391–7. doi: 10.1080/01480545.2018.1497047

9. Ting HC, Hsu YW, Tsai CF, Lu FJ, Chou MC, Chen WK. The *in vitro* and *in vivo* antioxidant properties of seabuckthorn (Hippophae rhamnoides L.) seed oil. *Food Chem* (2011) 125(2):652–9. doi: 10.1016/j.foodchem.2010.09.057

10. Gunenc A, Khoury C, Legault C, Mirrashed H, Rijke J, Hosseinian F. Seabuckthorn as a novel prebiotic source improves probiotic viability in yogurt. *LWT - Food Sci Technol* (2016) 66:490–5. doi: 10.1016/j.lwt.2015.10.061

11. Ilango S, Sahoo DK, Paital B, Kathirvel K, Gabriel JI, Subramaniam K, et al. A review on annona muricata and its anticancer activity. *Cancers* (2022) 14(18):4539. doi: 10.3390/cancers14184539

12. Chhabria S, Mathur S, Vadakan S, Sahoo DK, Mishra P, Paital B. A review on phytochemical and pharmacological facets of tropical ethnomedicinal plants as reformed DPP-IV inhibitors to regulate incretin activity. *Front Endocrinol* (2022) 13. doi: 10.3389/fendo.2022.1027237

13. Wang K, Xu Z, Liao X. Bioactive compounds, health benefits and functional food products of sea buckthorn: a review. *Crit Rev Food Sci Nutr* (2022) 62(24):6761–82. doi: 10.1080/10408398.2021.1905605

14. Sajfrtová M, Ličková I, Wimmerová M, Sovová H, Wimmer Z. β-sitosterol: supercritical carbon dioxide extraction from sea buckthorn (Hippophae rhamnoides L.) seeds. *Int J Mol Sci* (2010) 11(4):1842–50. doi: 10.3390/ijms11041842

15. Enkhtaivan G, Maria John KM, Pandurangan M, Hur JH, Leutou AS, Kim DH. Extreme effects of Seabuckthorn extracts on influenza viruses and human cancer cells and correlation between flavonol glycosides and biological activities of extracts. *Saudi J Biol Sci* (2017) 24(7):1646–56. doi: 10.1016/j.sjbs.2016.01.004

16. Jurevičiūtė I, Keršienė M, Bašinskienė L, Leskauskaitė D, Jasutienė I. Characterization of berry pomace powders as dietary fiber-rich food ingredients with functional properties. *Foods* (2022) 11(5):716. doi: 10.3390/foods11050716

17. Yang B, Kallio HP. Fatty acid composition of lipids in sea buckthorn (*Hippophaë rhamnoides* L.) berries of different origins. *J Agric Food Chem* (2001) 49(4):1939–47. doi: 10.1021/jf001059s

18. Ma X, Yang W, Kallio H, Yang B. Health promoting properties and sensory characteristics of phytochemicals in berries and leaves of sea buckthorn (*Hippophaë rhamnoides*). *Crit Rev Food Sci Nutr* (2022) 62(14):3798–816. doi: 10.1080/10408398.2020.1869921

19. Guo R, Guo X, Li T, Fu X, Liu RH. Comparative assessment of phytochemical profiles, antioxidant and antiproliferative activities of Sea buckthorn (Hippophaë rhamnoides L.) berries. *Food Chem* (2017) 221:997–1003. doi: 10.1016/j.foodchem.2016.11.063

20. Zhamanbayeva GT, Aralbayeva AN, Murzakhmetova MK, Tuleukhanov ST, Danilenko M. Cooperative antiproliferative and differentiation-enhancing activity of medicinal plant extracts in acute myeloid leukemia cells. *BioMed Pharmacother* (2016) 82:80–9. doi: 10.1016/j.biopha.2016.04.062

21. Leskinen HM, Suomela JP, Yang B, Kallio HP. Regioisomer compositions of vaccenic and oleic acid containing triacylglycerols in sea buckthorn (Hippophaë rhamnoides) pulp oils: influence of origin and weather conditions. *J Agric Food Chem* (2010) 58(1):537–45. doi: 10.1021/jf902679v

22. Zheng WH, Bai HY, Han S, Bao F, Zhang KX, Sun LL, et al. Analysis on the constituents of branches, berries, and leaves of *hippophae rhamnoides* L. by UHPLC-ESI-QTOF-MS and their anti-inflammatory activities. *Nat Prod Commun* (2019) 14 (8):1–10. doi: 10.1177/1934578X19871404

23. Tkacz K, Gil-Izquierdo Á, Medina S, Turkiewicz IP, Domínguez-Perles R, Nowicka P, et al. Phytoprostanes, phytofurans, tocopherols, tocotrienols, carotenoids and free amino acids and biological potential of sea buckthorn juices. *J Sci Food Agric* (2022) 102(1):185–97. doi: 10.1002/jsfa.11345

24. Upadhyay NK, Yogendra Kumar MS, Gupta A. Antioxidant, cytoprotective and antibacterial effects of Sea buckthorn (Hippophae rhamnoides L.) leaves. *Food Chem Toxicol* (2010) 48(12):3443–8. doi: 10.1016/j.fct.2010.09.019

25. Wang Z, Zhao F, Wei P, Chai X, Hou G, Meng Q. Phytochemistry, health benefits, and food applications of sea buckthorn (Hippophae rhamnoides L.): A comprehensive review. *Front Nutr* (2022) 9:1036295. doi: 10.3389/fnut.2022.1036295

26. Kim SJ, Hwang E, Yi SS, Song KD, Lee HK, Heo TH, et al. Sea buckthorn leaf extract inhibits glioma cell growth by reducing reactive oxygen species and promoting apoptosis. *Appl Biochem Biotechnol* (2017) 182(4):1663–74. doi: 10.1007/s12010-017-2425-4

27. Zhu Y, Ji X, Yuen M, Yuen T, Yuen H, Wang M, et al. Effects of ball milling combined with cellulase treatment on physicochemical properties and *in vitro* hypoglycemic ability of sea buckthorn seed meal insoluble dietary fiber. *Front Nutr* (2022) 8:820672. doi: 10.3389/fnut.2021.820672

28. Dadhwal P, Dhingra HK, Dwivedi V, Alarifi S, Kalasariya H, Yadav VK, et al. (sea buckthorn) mediated green synthesis of copper nanoparticles and their application in anticancer activity. *Front Mol Biosci* (2023) 10:1246728. doi: 10.3389/fmolb.2023.1246728

29. Kashyap P, Riar CS, Sea Buckthorn JN. *Antioxidants in fruits: properties and health benefits*. Nayik GA, Gull A, editors. Singapore: Springer Singapore (2020) p. 201–25. doi: 10.1007/978-981-15-7285-2_11

30. Liu X, Lv M, Maimaitiyiming R, Chen K, Tuerhong N, Yang J, et al. Development of fermented sea buckthorn (Hippophae rhamnoides L.) juice and investigation of its antioxidant and antimicrobial activity. *Front Nutr* (2023) 10:1120748. doi: 10.3389/fnut.2023.1120748

31. Kwon DJ, Bae YS, Ju SM, Goh AR, Choi SY, Park J. Casuarinin suppresses TNF-α-induced ICAM-1 expression via blockade of NF-κB activation in HaCaT cells. *Biochem Biophys Res Commun* (2011) 409(4):780–5. doi: 10.1016/j.bbrc.2011.05.088

32. Jain M, Ganju L, Katiyal A, Padwad Y, Mishra KP, Chanda S, et al. Effect of Hippophae rhamnoides leaf extract against Dengue virus infection in human blood-derived macrophages. *Phytomedicine* (2008) 15(10):793–9. doi: 10.1016/j.phymed.2008.04.017

33. Chauhan AS, Negi PS, Ramteke RS. Antioxidant and antibacterial activities of aqueous extract of Seabuckthorn (Hippophae rhamnoides) seeds. *Fitoterapia* (2007) 78 (7):590–2. doi: 10.1016/j.fitote.2007.06.004

34. Yogendra Kumar MS, Tirpude RJ, Maheshwari DT, Bansal A, Misra K. Antioxidant and antimicrobial properties of phenolic rich fraction of Seabuckthorn (Hippophae rhamnoides L.) leaves *in vitro*. *Food Chem* (2013) 141(4):3443–50. doi: 10.1016/j.foodchem.2013.06.057

35. Widén C, Renvert S, Persson GR. Antibacterial activity of berry juices, an *in vitro* study. *Acta Odontol Scand* (2015) 73(7):539–43. doi: 10.3109/00016357.2014.887773

36. Wang X, Liu J, Zhang X, Zhao S, Zou K, Xie J, et al. Seabuckthorn berry polysaccharide extracts protect against acetaminophen induced hepatotoxicity in mice via activating the Nrf-2/HO-1-SOD-2 signaling pathway. *Phytomedicine* (2018) 38:90–7. doi: 10.1016/j.phymed.2017.11.007

37. Ma Z, Sun Q, Chang L, Peng J, Zhang M, Ding X, et al. A natural anti-obesity reagent derived from sea buckthorn polysaccharides: Structure characterization and anti-obesity evaluation *in vivo*. *Food Chem* (2022) 375:131884. doi: 10.1016/j.foodchem.2021.131884

38. Ali I, Zahra N ul A RR, Sharif H MM, Bhatti HA. A New Potent Anti-cancer Corosolic Ester Identified from the Super Miracle Plant Hippophae rhamnoides (Sea buckthorn). *Biochem Mod Appl* (2019) 2:24–9. doi: 10.33805/2638-7735.119

39. Ko JH, Sethi G, Um JY, Shanmugam MK, Arfuso F, Kumar AP, et al. The role of resveratrol in cancer therapy. *Int J Mol Sci* (2017) 18(12):2589. doi: 10.3390/ijms18122589

40. Yang S, Si L, Jia Y, Jian W, Yu Q, Wang M, et al. Kaempferol exerts anti-proliferative effects on human ovarian cancer cells by inducing apoptosis, G0/G1 cell cycle arrest and modulation of MEK/ERK and STAT3 pathways. *J BUON* (2019) 24 (3):975–81.

41. Kristo A, Klimis-Zacas D, Sikalidis A. Protective role of dietary berries in cancer. *Antioxidants* (2016) 5(4):37. doi: 10.3390/antiox5040037

42. Han Y, Yuan C, Zhou X, Han Y, He Y, Ouyang J, et al. Anti-inflammatory activity of three triterpene from hippophae rhamnoides L. @ in lipopolysaccharide-stimulated RAW264.7 cells. *Int J Mol Sci* (2021) 22(21):12009. doi: 10.3390/ijms222112009

43. Sun WL, Li XY, Dou HY, Wang XD, Li JD, Shen L, et al. Myricetin supplementation decreases hepatic lipid synthesis and inflammation by modulating gut microbiota. *Cell Rep* (2021) 36(9):109641. doi: 10.1016/j.celrep.2021.109641

44. He N, Wang Q, Huang H, Chen J, Wu G, Zhu M, et al. A comprehensive review on extraction, structure, detection, bioactivity, and metabolism of flavonoids from sea buckthorn (*Hippophae rhamnoides* L.). *J Food Biochem* (2023) 2023:e4839124. doi: 10.1155/2023/4839124

45. Rédei D, Kúsz N, Rafai T, Bogdanov A, Burián K, Csorba A, et al. 14-Noreudesmanes and a phenylpropane heterodimer from sea buckthorn berry inhibit Herpes simplex type 2 virus replication. *Tetrahedron* (2019) 75(10):1364–70. doi: 10.1016/j.tet.2019.01.050

46. Huang H, Li Y, Gui F, Yang P, Zhang J, Li W, et al. Optimizing the purification process of polyphenols of sea buckthorn seed and its potential freshness effect. *LWT* (2023) 173:114380. doi: 10.1016/j.lwt.2022.114380

47. Dadhwal P, Dhingra HK, Dwivedi V, Alarifi S, Kalasariya H, Yadav VK, et al. Frontiers | Hippophae rhamnoides L. (sea buckthorn) mediated green synthesis of copper nanoparticles and their application in anticancer activity. *Front Mol Biosci* (2023) 10:1246728 doi: 10.3389/fmolb.2023.1246728

48. Zhao H, Kong L, Shao M, Liu J, Sun C, Li C, et al. Protective effect of flavonoids extract of Hippophae rhamnoides L. @ on alcoholic fatty liver disease through regulating intestinal flora and inhibiting TAK1/p38MAPK/p65NF-κB pathway. *J Ethnopharmacol* (2022) 292:115225. doi: 10.1016/j.jep.2022.115225

49. Saeed GN, Ahsin S, Sarwar M. HEPATOPROTECTIVE EFFECT OF SEA BUCKTHORN BERRY SEED OIL IN CYCLOPHOSPHAMIDE-INDUCED HEPATIC TOXICITY IN BALB/c MICE. *Pak J Physiol* (2023) 19(2):20–4.

50. Olas B. Berry phenolic antioxidants – implications for human health? *Front Pharmacol* (2018) 9:78. doi: 10.3389/fphar.2018.00078

51. Li C, Yang X, Chen C, Cai S, Hu J. Isorhamnetin suppresses colon cancer cell growth through the PI3K-Akt-mTOR pathway. *Mol Med Rep* (2014) 9(3):935–40. doi: 10.3892/mmr.2014.1886

52. Li X, Chen H, Zhang Z, Xu D, Duan J, Li X, et al. Isorhamnetin promotes estrogen biosynthesis and proliferation in porcine granulosa cells via the PI3K/akt signaling pathway. *J Agric Food Chem* (2021) 69(23):6535–42. doi: 10.1021/acs.jafc.1c01543

53. Qin Y, Jiang W, Li A, Gao M, Liu H, Gao Y, et al. The combination of paraformaldehyde and glutaraldehyde is a potential fixative for mitochondria. *Biomolecules* (2021) 11(5):711. doi: 10.3390/biom11050711

54. Mulati A, Zhang X, Zhao T, Ren B, Wang L, Liu X, et al. Isorhamnetin attenuates high-fat and high-fructose diet induced cognitive impairments and neuroinflammation by mediating MAPK and NFκB signaling pathways. *Food Funct* (2021) 12(19):9261–72. doi: 10.1039/D0FO03165H

55. Farooqi AA, Jabeen S, Attar R, Yaylim I, Xu B. Quercetin-mediated regulation of signal transduction cascades and microRNAs: Natural weapon against cancer. *J Cell Biochem* (2018) 119(12):9664–74. doi: 10.1002/jcb.27488

56. Ferenczyova K, Kalocayova B, Bartekova M. Potential implications of quercetin and its derivatives in cardioprotection. *Int J Mol Sci* (2020) 21(5):1585. doi: 10.3390/ijms21051585

57. Carrasco-Pozo C, Cires MJ, Gotteland M. Quercetin and epigallocatechin gallate in the prevention and treatment of obesity: from molecular to clinical studies. *J Med Food* (2019) 22(8):753–70. doi: 10.1089/jmf.2018.0193

58. Xiao PT, Liu SY, Kuang YJ, Jiang ZM, Lin Y, Xie ZS, et al. Network pharmacology analysis and experimental validation to explore the mechanism of sea buckthorn flavonoids on hyperlipidemia. *J Ethnopharmacol* (2021) 264:113380. doi: 10.1016/j.jep.2020.113380

59. Lan Y, Sun Q, Ma Z, Peng J, Zhang M, Wang C, et al. Seabuckthorn polysaccharide ameliorates high-fat diet-induced obesity by gut microbiota-SCFAs-liver axis. *Food Funct* (2022) 13(5):2925–37. doi: 10.1039/D1FO03147C

60. Saha S, Buttari B, Panieri E, Profumo E, Saso L. An overview of nrf2 signaling pathway and its role in inflammation. *Molecules* (2020) 25(22):5474. doi: 10.3390/molecules25225474

61. Cavak N, Gumustas MK, Cekic SD. The protective role of hippophae rhamnoides L. @ on rat brain and liver tissues exposed to cold plus immobilization stress model. *Turk Neurosurg* (2022) 32(4):587–94. doi: 10.5137/1019-5149.JTN.23766-18.3

62. Olas B, Skalski B, Ulanowska K. The anticancer activity of sea buckthorn [Elaeagnus rhamnoides (L.) A. Nelson]. *Front Pharmacol* (2018) 9:232. doi: 10.3389/fphar.2018.00232

63. Masoodi KZ, Wani W, Dar ZA, Mansoor S, Anam-ul-Haq S, Farooq I, et al. Sea buckthorn (Hippophae rhamnoides L.) inhibits cellular proliferation, wound healing and decreases expression of prostate specific antigen in prostate cancer cells *in vitro*. *J Funct Foods* (2020) 73:104102. doi: 10.1016/j.jff.2020.104102

64. Liu H, Zhou M. Antitumor effect of Quercetin on Y79 retinoblastoma cells via activation of JNK and p38 MAPK pathways. *BMC Complement Altern Med* (2017) 17 (1):531. doi: 10.1186/s12906-017-2023-6

65. Wang Y, Nie F, Ouyang J, Wang X, Ma X. Inhibitory effects of sea buckthorn procyanidins on fatty acid synthase and MDA-MB-231 cells. *Tumor Biol* (2014) 35 (10):9563–9. doi: 10.1007/s13277-014-2233-1

66. Goel HC, Samanta N, Kannan K, Kumar IP, Bala M. Protection of spermatogenesis in mice against gamma ray induced damage by Hippophae rhamnoides. *Andrologia* (2006) 38(6):199–207. doi: 10.1111/j.1439-0272.2006.00740.x

67. Sharma A, Kashyap D, Sak K, Tuli HS, Sharma AK. Therapeutic charm of quercetin and its derivatives: a review of research and patents. *Pharm Pat Anal* (2018) 7 (1):15–32. doi: 10.4155/ppa-2017-0030

68. Plante-Dubé M, Picard C, Gilbert I, Robert C, Fievez V, Vlaeminck B, et al. Effects of a dietary supplement enriched in palmitoleic acid on fatty acid composition of follicular fluid, granulosa cell metabolism, and oocyte developmental capacity in early lactation dairy cows. *J Dairy Sci* (2021) 104(3):3693–706. doi: 10.3168/jds.2020-19191

69. Sirotkin AV, Štochmaľová A, Alexa R, Kádasi A, Bauer M, Grossmann R, et al. Quercetin directly inhibits basal ovarian cell functions and their response to the stimulatory action of FSH. *Eur J Pharmacol* (2019) 860:172560. doi: 10.1016/j.ejphar.2019.172560

70. Lu Df, Yang Lj, Wang F, Zhang Gl. Inhibitory effect of luteolin on estrogen biosynthesis in human ovarian granulosa cells by suppression of aromatase (CYP19). *J Agric Food Chem* (2012) 60(34):8411–8. doi: 10.1021/jf3022817

71. Bhat FA, Sharmila G, Balakrishnan S, Singh PR, Srinivasan N, Arunakaran J. Epidermal growth factor-induced prostate cancer (PC3) cell survival and proliferation is inhibited by quercetin, a plant flavonoid through apoptotic machinery. *BioMed Prev Nutr* (2014) 4(4):459–68. doi: 10.1016/j.bionut.2014.07.003

72. Teekaraman D, Elayapillai SP, Viswanathan MP, Jagadeesan A. Quercetin inhibits human metastatic ovarian cancer cell growth and modulates components of the intrinsic apoptotic pathway in PA-1 cell line. *Chem Biol Interact* (2019) 300:91–100. doi: 10.1016/j.cbi.2019.01.008

73. Li M, Zhang W, Yang L, Wang H, Wang Y, Huang K, et al. The mechanism of xiaoyao san in the treatment of ovarian cancer by network pharmacology and the effect of stigmasterol on the PI3K/akt pathway. *Dis Markers* (2021) 2021:1–10. doi: 10.1155/2021/4304507

74. Tavsan Z, Kayali HA. Flavonoids showed anticancer effects on the ovarian cancer cells: Involvement of reactive oxygen species, apoptosis, cell cycle and invasion. *BioMed Pharmacother* (2019) 116:109004. doi: 10.1016/j.biopha.2019.109004

75. Kim T, Choi E. Equol induced apoptosis via cell cycle arrest in human breast cancer MDA-MB-453 but not MCF-7 cells. *Mol Med Rep* (2008) 1(2):239–44. doi: 10.3892/mmr.1.2.239

76. Imran M, Salehi B, Sharifi-Rad J, Aslam Gondal T, Saeed F, Imran A, et al. Kaempferol: a key emphasis to its anticancer potential. *Molecules* (2019) 24(12):2277. doi: 10.3390/molecules24122277

77. El-Kott AF, Shati AA, Al-Kahtani MA, Alharbi SA. Kaempferol induces cell death in A2780 ovarian cancer cells and increases their sensitivity to cisplatin by activation of cytotoxic endoplasmic reticulum-mediated autophagy and inhibition of protein kinase B. 66. *Folia Biol (Praha)* (2020) 66(1):36–46. doi: 10.14712/fb2020066010036

78. Larmo PS, Yang B, Hyssälä J, Kallio HP, Erkkola R. Effects of sea buckthorn oil intake on vaginal atrophy in postmenopausal women: a randomized, double-blind, placebo-controlled study. *Maturitas* (2014) 79(3):316–21. doi: 10.1016/j.maturitas.2014.07.010

79. De Seta F, Caruso S, Di Lorenzo G, Romano F, Mirandola M, Nappi RE. Efficacy and safety of a new vaginal gel for the treatment of symptoms associated with vulvovaginal atrophy in postmenopausal women: A double-blind randomized placebo-controlled study. *Maturitas* (2021) 147:34–40. doi: 10.1016/j.maturitas.2021.03.002

80. Nasir SA. Improved endometrial thickness and vascularity following vitamins E and C administration in infertile women undergoing controlled ovarian stimulation. *Pharmaceuticals (Basel)* (2021) 14(4):373. doi: 10.3390/ph14040373

81. Sirotkin AV, Alwasel SH, Harrath AH. The influence of plant isoflavones daidzein and equol on female reproductive processes. *Pharmaceuticals* (2021) 14 (4):373. doi: 10.3390/ph14040373

82. Kvaskoff M, Mahamat-Saleh Y, Farland LV, Shigesi N, Terry KL, Harris HR, et al. Endometriosis and cancer: a systematic review and meta-analysis. *Hum Reprod Update* (2021) 27(2):393–420. doi: 10.1093/humupd/dmaa045

83. Scuto M, Ontario ML, Salinaro AT, Caligiuri I, Rampulla F, Zimbone V, et al. Redox modulation by plant polyphenols targeting vitagenes for chemoprevention and therapy: Relevance to novel anti-cancer interventions and mini-brain organoid technology. *Free Radic Biol Med* (2022) 179:59–75. doi: 10.1016/j.freeradbiomed.2021.12.267

84. Sirotkin AV, Harrath AH. Phytoestrogens and their effects. *Eur J Pharmacol* (2014) 741:230–6. doi: 10.1016/j.ejphar.2014.07.057

10.3389/fendo.2023.1244300

Glossary

ALT	alanine aminotransferase
APAP	acetaminophen-induced apoptosis
AST	aspartate aminotransferase
Bcl-2/Bax	B-cell lymphoma 2/Bcl-2-associated X protein
CAT	catalase
FAS	fatty acid synthase
GSH	glutathione
GSH-Px	glutathione peroxidase
HFD	high fat diet
IDF	insoluble dietary fiber
iNOS	inducible nitric oxide synthase
IR	infrared
LDL-C	lipoprotein-cholesterol
MAPK	mitogen-activated protein kinase
MDA	malondialdehyde
MS	mass spectroscopy
NF-κB	nuclear factor kappa light chain enhancer of activated B cells
NMR	nuclear magnetic resonance
NO	nitric oxide
Nrf2	erythroid 2–related factor 2
p-Akt	phosphoinositide 3-kinase
PGC1α	peroxisome proliferator-activated receptor gamma coactivator 1
PI3K-Akt-mTOR	phosphatidylinositol 3-kinase – protein kinase B - the mammalian target of rapamycin
PP	pomace powder
PRDM 16	PR domain-containing 16
ROS	reactive oxygen species
SBSO	sea buckthorn oil
SHH	sonic hedgehog signaling pathway
SOD	superoxide dismutase
STAT	signal transducer and activator of transcription
TC	total cholesterol
TG	triglyceride
TGF β	transforming growth factor-beta
TNF α	tumor necrosis factor alpha
UCP-1	uncoupling protein 1
UV/Vis	ultraviolet/visible
VEGF	vascular endothelial growth factor

frontiers | Frontiers in Endocrinology

TYPE Original Research
PUBLISHED 08 November 2023
DOI 10.3389/fendo.2023.1277155

OPEN ACCESS

EDITED BY
Luna Samanta,
Ravenshaw University, India

REVIEWED BY
Sanjeeb Kumar Mandal,
Chaitanya Bharathi Institute of Technology, India
Nithar Ranjan Madhu,
West Bengal State University, India

*CORRESPONDENCE
Adriana Kolesarova
adriana.kolesarova@uniag.sk

RECEIVED 14 August 2023
ACCEPTED 20 October 2023
PUBLISHED 08 November 2023

CITATION
Kolesarova A, Baldovska S, Kohut L, Vasicek J, Ivanisova E, Arvay J, Duracka M and Roychoudhury S (2023) Modulatory effect of pomegranate peel extract on key regulators of ovarian cellular processes *in vitro*.
Front. Endocrinol. 14:1277155.
doi: 10.3389/fendo.2023.1277155

Modulatory effect of pomegranate peel extract on key regulators of ovarian cellular processes *in vitro*

Adriana Kolesarova[1,2]*, Simona Baldovska[1], Ladislav Kohut[1], Jaromir Vasicek[3,4], Eva Ivanisova[5], Julius Arvay[5], Michal Duracka[2] and Shubhadeep Roychoudhury[6]

[1]Institute of Applied Biology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovakia, [2]AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, Nitra, Slovakia, [3]Institute of Farm Animal Genetics and Reproduction, NPPC - Research Institute for Animal Production Nitra, Lužianky, Slovakia, [4]Institute of Biotechnology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovakia, [5]Institute of Food Sciences, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovakia, [6]Department of Life Science and Bioinformatics, Assam University, Silchar, India

In this study, response of ovarian cells (human granulosa cell line HGL5, and human adenocarcinoma cell line OVCAR-3) to short-term pomegranate peel extract (PPE) treatment (for 24 hours in cell culture) was evaluated *in vitro*. Quantitative and qualitative screening of polyphenols revealed punicalagins α and β as major polyphenolic components. Total phenolic content (TPC) was 93.76 mg GAE/g d.w. with a high antioxidant activity of 95.30 mg TEAC/g d.w. In OVCAR-3, PPE treatment inhibited the metabolic activity, and increased cyclin-dependent kinase 1 (CDKN1A, p21) level at the highest dose, but not in HGL5. Flow cytometry analysis could not detect any significant difference between proportions of live, dead, and apoptotic cells in both cell lines. Reactive oxygen species (ROS) revealed an antioxidant effect on HGL5, and a prooxidant effect by stimulating ROS generation in OVCAR-3 cells at the higher doses of PPE. However, in contrast to HGL5, PPE treatment decreased release of growth factors – TGF-β2 and EGF at the highest dose, as well as their receptors TGFBR2 and EGFR in OVCAR-3 cells. PPE also influenced steroidogenesis in granulosa cells HGL5 by stimulating 17β-estradiol secretion at higher doses. In conclusion, the present study highlighted the bioactive compounds in pomegranate peels and the possible mechanisms of action of PPE, shedding light on its promising role in ovarian cancer (chemo)prevention and/or management.

KEYWORDS

HGL5, growth factors, OVCAR-3, *Punica garanatum* L., proliferation, apoptosis, steroidogenesis, reactive oxygen species

1 Introduction

Pomegranate (*Punica granatum* L.), a fruit widely consumed for its taste and nutritional value has garnered scientific attention due to its potential health-promoting properties. It is a rich source of polyphenolic compounds with great bioavailability. While much of the research has focused on the pomegranate's edible arils and juice, the pomegranate peel, a by-product in the processing of pomegranate products, which is usually discarded as a waste, has recently gained recognition for its remarkable health benefits (1–3). Pomegranate peel extract (PPE) has been reported to contain a significant amount of proteins, polysaccharides, minerals, vitamins, dietary fibers, alkaloids, and polyphenols such as flavonoids (catechin, epicatechin, quercetin, rutin, kaempferol, anthocyanins), hydrolyzable tannins (punicalin, punicalagin), and phenolic acids (ellagic acid, gallic acid, caffeic acid, ferulic acid, p-coumaric acid) (1, 4–7). Polyphenolic compounds found in PPE are believed to exert notable antioxidant, anti-inflammatory, antibacterial, and anti-cancer activities (1, 2, 8). Moreover, various preparations of pomegranate (juice, seed oil, peel extract) have been used in clinical studies for their potential therapeutic actions. Pomegranate's polyphenols have shown anticancer effect through the regulation of cellular redox balance, cell cycle arrest in the G2/M phase, induction of apoptosis and DNA damage of cancer cells, as well as by modulation of key signalling pathways (3, 9).

Cancer is one of the major reasons for mortality across the globe. Gynaecological cancers represent a key cause of mortality among women (10), and ovarian cancer is one of the most common gynecological malignancies (11, 12). Reproductive dysfunctions underlie similar causes and mechanisms, including accumulation of reactive oxygen species (ROS) resulting in cellular oxidative stress (13). Studies have focused on the bioactive ingredients of food products that may provide a useful alternative therapeutic approach, particularly based on the capability of phytocompounds to influence reproductive processes and prevent disorders. Therefore, identifying natural phytocompounds with anticancer properties is increasingly emphasized (10, 14, 15). Studies have reported the anticancer potential of pomegranate or its bioactive substances for various cancer types, including breast (16, 17), lung (18, 19), colon (20), skin (21), prostate (22), and cervical (23) cancers. However, studies on ovarian cancer are not sufficient to arrive at any definitive conclusion regarding specific bioactive substances and their mechanisms of action. Thus, understanding of the effects of pomegranate products such as pomegranate peel extract (PPE) and its mechanism of action on ovarian cell models (both non-cancerous and cancerous) will be beneficial both for the society in the fight against ovarian cancer as well as development of beneficial health supplements in the agri-food industry. The present study aimed to identify the polyphenolic substances in pomegranate peel extract (PPE), its total polyphenol content (TPC), antioxidant capacity. Furthermore, the modulatory effects of PPE were determined *in vitro* on key markers of cellular processes related to proliferation, apoptosis, oxidative stress, and steroidogenesis using human ovarian non-cancerous (HGL5) and cancerous (OVCAR-3) cellular models in order to contribute to a better understanding of the mechanism(s) of action of PPE.

2 Materials and methods

2.1 Materials

Pomegranate fruits harvested at the state of complete ripeness were obtained from Spain. All used analytical standards (chlorogenic acid, 4-OH-benzoic acid, trans-caffeic acid, trans-p-coumaric acid, rutin, myricetin, resveratrol, apigenin, genistein, kaempferol), acetonitrile (HPLC gradient grade), methanol (HPLC grade), phosphoric acid (ACS grade), and luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) were obtained from Sigma Aldrich (Sigma-Aldrich Chemie GmbH, Germany). Propidium iodide (Molecular Probes, Switzerland), specific nuclear fluorochrome Yo-Pro-1 (Molecular Probes, Switzerland) and specific membrane marker Annexin V-FITC (Annexin V Apoptosis Detection Kit, Spain) were used for flow cytometry. Other chemicals and solvents used in this study were of analytical grade.

2.2 Extract preparation

PPE was prepared prior to cell culture experiments as previously described (24). Separated pomegranate peels were cut into small pieces and lyophilized. The solid-liquid extraction of 2g grounded pomegranate peel powder was achieved in 20ml non-denatured ethanol (80% v/v) for four hours by horizontal shaker Unimax 2010 (Heidolph Instruments, GmbH, Germany) at room temperature and in the dark. Prepared suspensions were filtered and stored at 4°C.

2.3 High performance liquid chromatography

PPE was filtered through syringe PTFE filter (0.45µm, 25mm) (Agilent Technologies, Germany) and stored at 4°C. Quantitative and qualitative determination of phenolic compounds were performed by HPLC system with diode array detector (HPLC-DAD) instrumentation Agilent Infinity 1260 (Agilent Technologies, Germany). Double deionized water (ddH_2O) was treated (18.2MΩ/cm) in a Simplicity 185 purification system (Millipore SAS, France). Analyses were performed in a Cortecs column (4.6mm x 150mm x 2.7µm) (Waters, USA). Mobile phases consisted of 0.1% H_3PO_4 in ddH_2O (v/v) (A) and acetonitrile (B). The mobile phase flow was 0.6mL/min, and the sample injection was 5µL. The column thermostat was set to 30°C and the samples were kept at 6°C in the sampler manager. The detection wavelength was set at 265nm, 320nm, and 372nm. The compounds were identified by comparing with retention time and UV spectra by running the samples for 30 min after the addition of pure standards (25).

2.4 Total polyphenol content

Determination of TPC was performed by Folin–Ciocalteu's spectrophotometric assay (26). A total of 100µL PPE was mixed

with 0.85mL of Folin-Ciocalteu reagent (Merck, Germany) in a 50mL volumetric flask. After 3 minutes, 5mL of 20% sodium carbonate solution (Sigma Aldrich, USA) was added. The mixture was stirred, and the flask was filled with distilled water to the mark. Obtained solution was incubated at room temperature for 2 hours to allow the development of the characteristic blue color, after which the absorbance was measured at 765nm using a Shimadzu UV-VIS scanning spectrophotometer (Shimadzu, Japan). TPC was expressed in mg of gallic acid equivalents (GAE) per g of dried fruit peel weight (d.w.), based on the calibration curve (R^2 = 0.996).

2.5 Antioxidant activity

Antioxidant activity was determined by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (27) with DPPH• radical (Sigma Aldrich, USA) and methanol (Sigma Aldrich, USA) used to produce a working DPPH solution. 1mL PPE was pipetted into 3.9mL working DPPH solution, stirred, and left in dark for 10 minutes. Antioxidant activity was measured using a Shimadzu UV-VIS scanning spectrophotometer (Shimadzu, Japan) and expressed as mg of Trolox equivalent antioxidant capacity (TEAC) per g of dried fruit peel weight (d.w.), based on the calibration curve (R^2 = 0.994).

2.6 Cell culture

Human ovarian granulosa cells HGL5 were obtained from ABM® (Canada) and human ovarian adenocarcinoma cells OVCAR-3 were obtained from ATCC® (USA). HGL5 cells were cultured in DMEM medium (Sigma-Aldrich, USA) supplemented with 10% FBS (Sigma-Aldrich, USA), 1% antibiotic/antimycotic solution (Invitrogen, USA) at a 37°C and in a 5% CO_2 incubator. OVCAR-3 cells were cultured in RPMI 1640 medium (Gibco-BRL, USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich, USA), 1% antibiotic/antimycotic solution (Invitrogen, CA, USA), 1% non-essential amino acids (Sigma Aldrich, United Kingdom) at 37°C and in a 5% CO_2 incubator. Between 10 and 25 passages of ovarian cells were used in this study (28, 29).

2.7 Pomegranate peel extract treatment to cells

Prior to the experiments, PPE was dissolved in a culture medium and diluted to the desired concentrations. Cells were cultured in plates for 24 hours and treated with PPE (at doses 12.5, 25, 50 and 100 µg/mL). As a positive control (+Control), 80% ethanol in an amount corresponding to the highest used concentration of the respective extract was used and the final ethanol concentration in well was less than 0.1%. All the procedures followed were in accordance with the institutional guidelines.

2.8 Cell viability

Cells (1×10^4 cells/mL/well) were cultured in a 96-well plate and treated with different concentrations of PPE. Cell viability was determined using AlamarBlue reagent (BioSource International, Belgium). After treatment, 1 µL of AlamarBlue reagent was added to each well at the indicated time 4 hours before the endpoint and incubated at 37°C. Resazurin reduction (oxidized indigo blue state into the reduced pink state) was measured by recording the absorbance at 560 nm and 590 nm using a microplate reader (Multiskan FC, ThermoFisher Scientific, Finland) and the results were expressed as percentage of viable cells (30).

2.9 Flow cytometry

Live, apoptotic, and dead cells' percentages were determined by uptake rate and dye retention with a little modification of the method used previously (28). At $5x10^5$ cells/mL per well density cells were seeded in 6-well plates and treated to experimental groups. Yo-Pro-1 and Annexin V-FITC stains were used to detect apoptotic cells. Propidium iodide was used to stain dead cells. After centrifugation at 300xg for 5 minutes, cells were adjusted to $1x10^6$ cells/mL in phosphate buffered saline (without Ca and Mg) and stained with 1µL Yo-Pro-1 solution (100µmol/L) for 15 minutes in the dark at room temperature. According to manufacturer's instructions, Annexin V staining was done. 4µL of propidium iodide (µg/mL) was used to stain cells just prior to flow cytometry analysis FACS Calibur (BD Biosciences, USA). In each sample, at least 50,000 events (cells) were evaluated. Cell Quest Pro (BD Biosciences, USA) software was used for data analyses. The assay identified three separate populations: unstained live cells (Yo-Pro-1^-/PI^- and AnV^-/PI^-), apoptotic cells (Yo-Pro-1^+/PI^- and AnV^+/PI^-), and dead cells (only PI^+). Cultivation with staurosporine was used as a positive control for the purpose of initiating apoptotic processes in cells (Figure 1).

2.10 Enzyme linked immunosorbent assay

At $4x10^5$ cells/mL per well density cells were re-seeded in a 6-well culture plate (Grainer, Germany). Cell supernatants were collected to determine the levels of steroid hormones 17β-estradiol (cat. no. DNOV003) and progesterone (cat. no. DNOV006) by using ELISA kits (NovaTec Immundiagnostica GmbH, Germany). Cell lysates were subjected to determine the levels of human cyclin-dependent kinase inhibitor 1 (CDKN1A, p21; cat. no. EH14267), growth factors – human transforming growth factor β 2 (TGF-β2, cat. no. EH0288) and human epidermal growth factor (EGF, cat. no. EH0009), as well as their receptors (TGF-β receptor type-2 – TGFBR2, cat. no. EH0286; and EGF receptor – EGFR, cat. no. EH0010) according to manufacturer's instructions by using ELISA kits (FineTest, China). ELISA microplates were briefly pre-coated with an antibody, and into the wells the standards, controls, and samples were pipetted. A biotin-conjugated antibody was added to the wells after removal of any

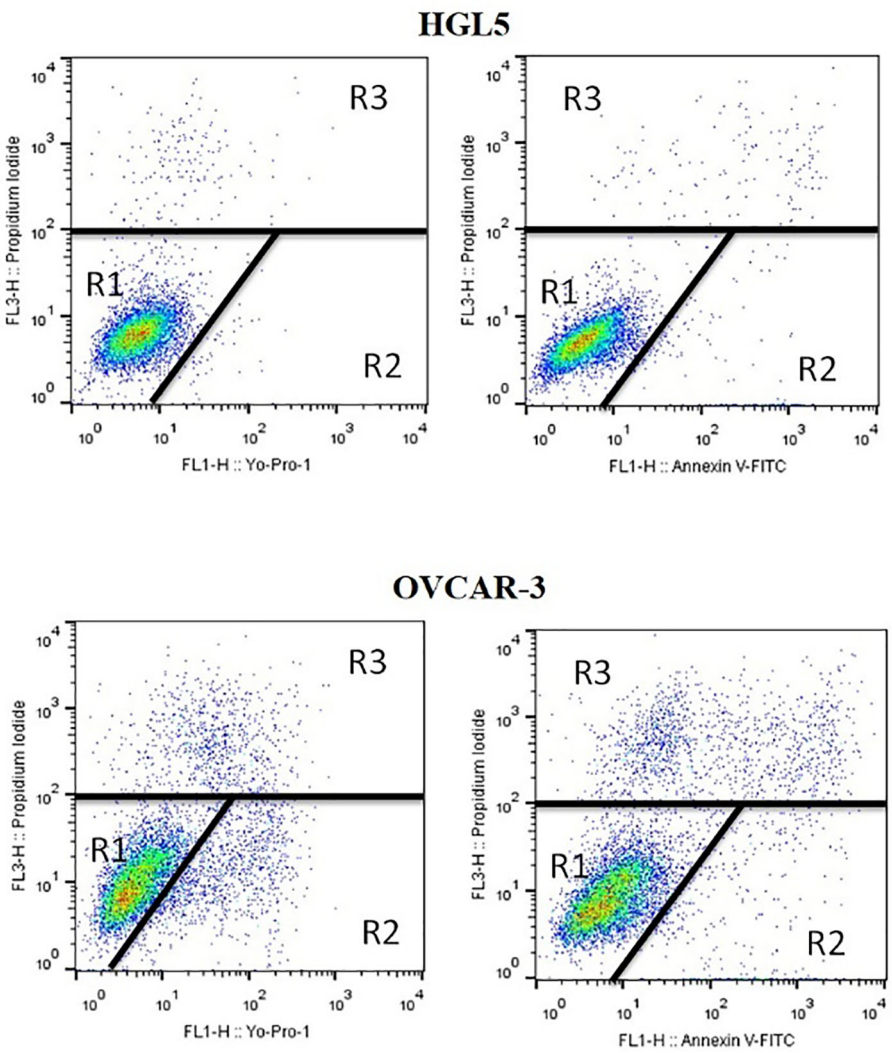


FIGURE 1
Flow cytometry dot plots used for measuring live, dead, and apoptotic cells. R1: unstained live cells ($YP1^-/PI^-$ and AnV^-/PI^-), R2: apoptotic cells ($YP1^+/PI^-$ and AnV^+/PI^-), R3: dead cells (only PI^+).

unbound substance. Streptavidin-conjugated horseradish peroxidase was added to the wells after washing and incubated followed again by washing. Substrate solution was added to the wells thereafter and a stop solution was used to stop color development. Colour intensity was measured spectrophotometrically by using an ELISA microplate reader (Thermo Scientific Multiskan FC, Finland), and results were expressed as mean (29).

2.11 Reactive oxygen species

Intracellular ROS production was detected through quantification by chemiluminometric method. Firstly, at a density of $4x10^4$ cells/mL per well cells were re-seeded into 24-well plate followed by PPE treatment for 24 hours. Thereafter, ROS generation was assessed based on luminol (28). 10µL luminol (5mM) and 400µL experimental sample or control comprised the samples. 400µL of medium, 10µL luminol and 50µL hydrogen peroxide (30%; 8.8 M; Sigma-Aldrich) served as positive control. Glomax Multi+Combined SpectroFluoroLuminometer (Promega Corporation, USA) was used to measure chemiluminescence in 15 cycles of 1 minute each. The results were expressed as relative light units (RLU)/second/10^4 cells (28).

2.12 Statistics

All the experiments were replicated thrice, and data were expressed as mean ± standard error of mean (SEM) followed by analysis of variance (ANOVA) and Dunnett's test. GraphPad Prism 5 program (version 3.02 for Windows; GraphPad Software, USA) was used for further analysis and statistically significant differences were set at $p<0.05$.

3 Results

3.1 Polyphenol content and antioxidant activity of PPE

Quantitative and qualitative screening of polyphenolic compounds revealed ellagitannins punicalagins α (19 007.83 ± 78.77 mg/kg) and β (28 964.55 ± 29.99 mg/kg), and flavonoid rutin (10 789.80 ± 21.15 mg/kg) as most abundant polyphenols in PPE (Table 1). PPE presented a rich source of polyphenols with TPC of 93.76 ± 0.15 mg GAE/g d.w. of pomegranate peels and a total antioxidant capacity of 95.30 ± 0.20 mg TEAC/g d.w. of pomegranate peels.

3.2 PPE treatment inhibits metabolic activity of human ovarian cancer cells *in vitro*

To investigate the effects of PPE on the viability of ovarian cells *in vitro*, non-cancer cells HGL5 and cancer cells OVCAR-3 were treated with PPE for 24 hours. A significant decrease ($p<0.05$) in the number of viable OVCAR-3 cells was observed in all groups treated by PPE as compared to control, with no impact as such on HGL5 cells (Figure 2).

TABLE 1 Screening of the polyphenolic compounds in pomegranate peel extract (PPE).

Phenol compound(s)	Average content in mg/kg d.w.	Concentration (average, mg/2g d.w.)
Punicalagin α	19 007.83 ± 78.77	38.02
Punicalagin β	28 964.55 ± 29.99	57.93
Gallic acid	222.20 ± 0.30	0.44
Ellagic acid	2 265.05 ± 0.73	4.53
Ellagic acid ekvivalent	4 358.78 ± 32.02	8.72
Chlorogenic acid	65.92 ± 0.97	0.13
trans-Caffeic acid	48.26 ± 0.57	0.10
4-hydroxybenzoic acid	589.04 ± 1.58	1.18
trans-p-Coumaric acid	237.63 ± 1.23	0.48
Rutin	10 789.80 ± 21.15	21.58
Myricetin	10.41 ± 0.25	0.02
Resveratrol	768.28 ± 2.32	1.54
Quercetin	12.73 ± 0.50	0.03
Apigenin	452.56 ± 163.6	0.91
Genistein	28.13 ± 0.06	0.06
Kaempferol	1 352.03 ± 3.01	2.70

Data are expressed as mean ± standard error of mean (SEM). HPLC-DAD analysis.

3.3 PPE treatment does not affect the proportion of live, apoptotic, and dead human ovarian cells *in vitro*

Flow cytometry analysis did not confirm any significant change between the proportion of live, apoptotic, and dead ovarian cells after treatment of HGL5 or OVCAR-3 cells with PPE (Table 2).

3.4 PPE treatment increases cyclin-dependent kinase inhibitor 1 in human ovarian cancer cells *in vitro*

To further evaluate the effects of PPE on human ovarian cells, the levels of CDKN1A, p21 as a possible inhibitor of apoptosis after treatment with PPE was measured. No significant changes in the expression of CDKN1A was noted in ovarian granulosa cells HGL5 after PPE treatment. However, the highest concentration of PPE (100 μg/mL) led to a significant increase ($p<0.05$) in CDKN1A level in cancer cells OVCAR-3, as compared to control (Figure 3).

3.5 PPE treatment induces ROS production in human ovarian cancer cells *in vitro*

Generation of ROS, which is closely related to the occurrence of oxidative stress in human ovarian cells was measured to comprehensively understand the mechanism of action of PPE. Interestingly, cell-specific effect of PPE was observed. In case of ovarian granulosa cells HGL5, ROS production was significantly suppressed ($p<0.05$) by PPE at all used concentrations exhibiting an antioxidative effect of PPE. On the other hand, a significant PPE-induced ROS production was noted in cancer cells OVCAR-3 at higher doses (50 and 100 μg/mL) of PPE, as compared to control (Figure 4).

3.6 PPE treatment influences release of growth factors and their receptors by human ovarian cancer cells *in vitro*

Immunological assays were performed to draw the response of ovarian cells to PPE treatment with an emphasis on selected growth factors and their receptors. In contrast to ovarian granulosa cells HGL5 where PPE treatment did not exert any significant effect, PPE treatment decreased the release of growth factors – transforming growth factor β 2 (TGF-β2) and epidermal growth factor (EGF) at the highest dose of 100μg/mL PPE used ($p<0.001$), as well as the expression of their receptors TGFBR2 ($p<0.001$) and EGFR ($p<0.05$) in ovarian cancer cells OVCAR-3, in a dose-specific manner (Figure 5). As assayed by ELISA from cell lysates, the highest dose of 100μg/mL PPE administration in the present study unequivocally showed higher levels of growth factors TGF-β2, EGF, and their receptors (TGFBR2 and EGFR) in OVCAR-3 cells, as compared to control. TGFBR2 expression was significantly higher ($p<0.001$) at other higher doses of PPE treatment to OVCAR-3 cells, too, as compared to control (Figure 5).

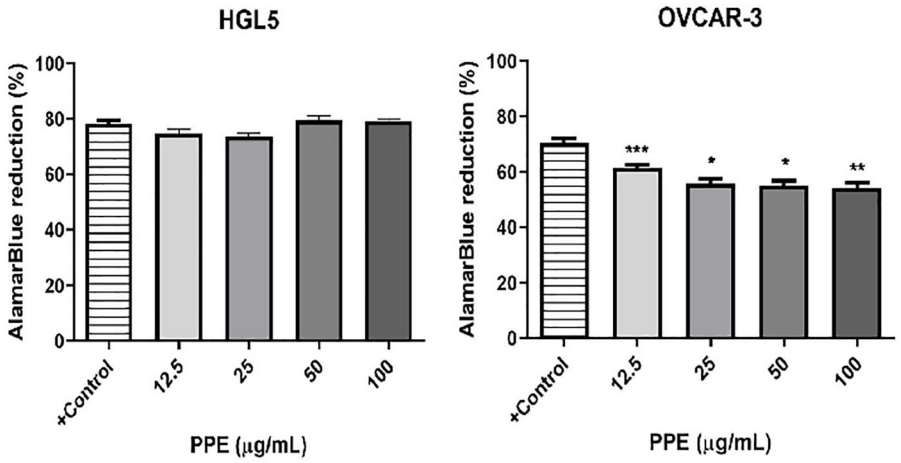


FIGURE 2
Viability of human ovarian granulosa cells HGL5 and cancer cells OVCAR-3 after pomegranate peel extract (PPE) treatment *in vitro*. +Control represents a culture medium with ethanol in an amount corresponding to the highest used concentration of PPE. Statistical differences were tested using one-way analysis of variance (ANOVA) followed by Dunnett 's multiple comparison test. Data are expressed as mean ± standard error of mean (SEM). Statistical differences are indicated from the vehicle (*p<0.05, **p<0.01, ***p<0.001). AlamarBlue test.

3.7 PPE treatment affects steroidogenesis in human ovarian granulosa cells *in vitro*

To evaluate potential effect on steroidogenesis, ELISA assay was performed to determine the release of steroid hormones 17ß-estradiol and progesterone by ovarian granulosa cells HGL5 (but not by ovarian epithelial adenocarcinoma cells OVCAR-3). The results exhibited a decreasing tendency of progesterone secretion (albeit statistically insignificant) at all doses of PPE used in the study. Interestingly, PPE's influence on ovarian steroidogenesis was clear in granulosa cells HGL5 through stimulation ($p<0.05$) of 17β-estradiol secretion at higher doses of 50 and 100µg/mL (Figure 6).

TABLE 2 Live, apoptotic, and dead human ovarian granulosa cells HGL5 and cancer cells OVCAR-3 after pomegranate peel extract (PPE) treatment *in vitro*.

HGL5	YP1/PI	+Control	12.5 µg/mL PPE	25 µg/mL PPE	50 µg/mL PPE	100 µg/mL PPE
Live cells (%)		98.08 ± 0.71	98.03 ± 0.79	97.60 ± 1.17	97.68 ± 1.13	97.80 ± 0.84
Apoptotic cells (%)		0.08 ± 0.07	0.16 ± 0.08	0.11 ± 0.05	0.09 ± 0.04	0.10 ± 0.05
Dead cells (%)		1.84 ± 0.64	1.82 ± 0.77	2.29 ± 1.11	2.23 ± 1.11	2.08 ± 0.78
	AnV/PI					
Live cells (%)		97.13 ± 0.37	96.75 ± 0.95	96.60 ± 0.62	96.23 ± 0.57	95.80 ± 0.62
Apoptotic cells (%)		1.14 ± 0.27	1.20 ± 0.18	1.41 ± 0.27	1.61 ± 0.30	2.05 ± 0.37
Dead cells (%)		1.75 ± 0.57	2.05 ± 1.12	2.02 ± 0.82	2.17 ± 0.71	2.17 ± 0.88
OVCAR-3	YP1/PI	+Control	12.5 µg/mL PPE	25 µg/mL PPE	50 µg/mL PPE	100 µg/mL PPE
Live cells (%)		81.88 ± 1.13	80.50 ± 3.06	79.20 ± 2.42	79.43 ± 3.07	81.00 ± 2.98
Apoptotic cells (%)		9.28 ± 1.79	11.11 ± 1.84	11.39 ± 2.26	11.09 ± 2.19	12.54 ± 3.39
Dead cells (%)		6.82 ± 1.17	9.20 ± 1.76	9.42 ± 2.30	8.05 ± 1.94	7.43 ± 1.93
	AnV/PI					
Live cells (%)		83.03 ± 1.36	81.48 ± 1.37	82.68 ± 1.38	82.98 ± 2.60	82.60 ± 2.20
Apoptotic cells (%)		8.33 ± 1.37	8.76 ± 0.33	8.77 ± 0.39	8.14 ± 1.03	10.66 ± 1.49
Dead cells (%)		8.69 ± 1.34	9.80 ± 1.24	8.60 ± 1.01	8.89 ± 1.70	9.32 ± 1.49

+Control group is represented by cells cultured with ethanol in an amount corresponding to the highest concentration of PPE used. Other experimental groups represent cells treated with PPE in different concentrations (12.5, 25, 50 and 100 µg/mL). Data are expressed as mean ± standard error of mean (SEM). Flow cytometry.

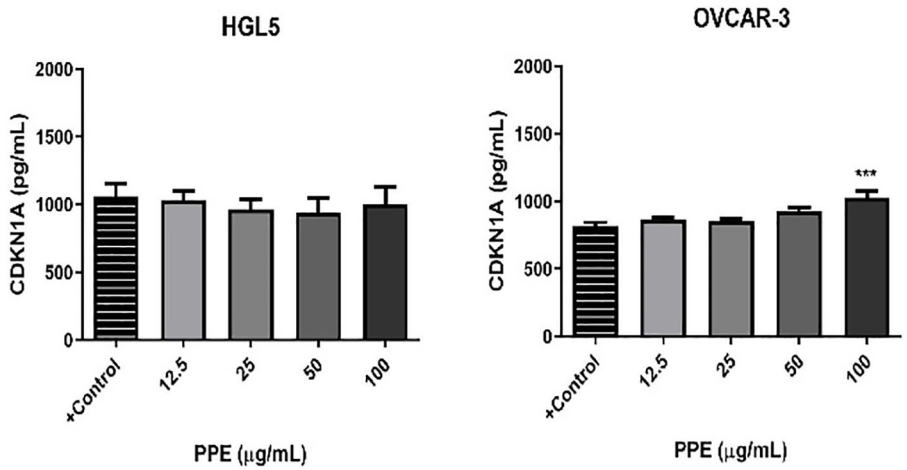


FIGURE 3
Presence of cyclin-dependent kinase 1 (CDKN1A, p21) in human ovarian granulosa cells HGL5 and cancer cells OVCAR-3 after pomegranate peel extract (PPE) treatment *in vitro*. +Control represents a culture medium with ethanol in an amount corresponding to the highest used concentration of PPE. Statistical differences were tested using one-way analysis of variance (ANOVA) followed by Dunnett´s multiple comparison test. Data are expressed as mean ± standard error of mean (SEM). Statistical differences are indicated from the vehicle (***p<0.001). ELISA.

4 Discussion

Pomegranate and its phenolic substances are a subject of increasing scientific interest because of their possible health benefits. Studies have revealed a link between the intake of dietary phytonutrients and the risk of ovarian cancer development (23). Promising cytotoxic, anti-proliferative, and proapoptotic effects of pomegranate have been confirmed experimentally using cancer cells both *in vitro* and *in vivo* (9, 23, 31). Pomegranate peels, usually inedible part of pomegranate fruit is considered as a waste by-product. However, it contains large numbers of polyphenolic compounds (32, 33) and exerts numerous beneficial properties, including anti-cancer activity (34–36). The present study was designed to determine the biological effects of phytonutrients present in pomegranate peel extract (PPE) on human ovarian non-cancerous (HGL5) and cancerous (OVCAR-3) cells *in vitro*.

More than 50% of the bioactive phytocompounds of pomegranate has been reported from its peels. Pomegranate peel largely contains hydrolyzable tannins punicalagins α and β, and punicalins, up to 22-33% (37) and rutin as detected from peel extract (4). Recently, a positive correlation of antioxidant capacity has been established with TPC of pomegranate peels (38). The present study also confirms PPE as an excellent source of polyphenolic compounds with strong antioxidant capacity, among

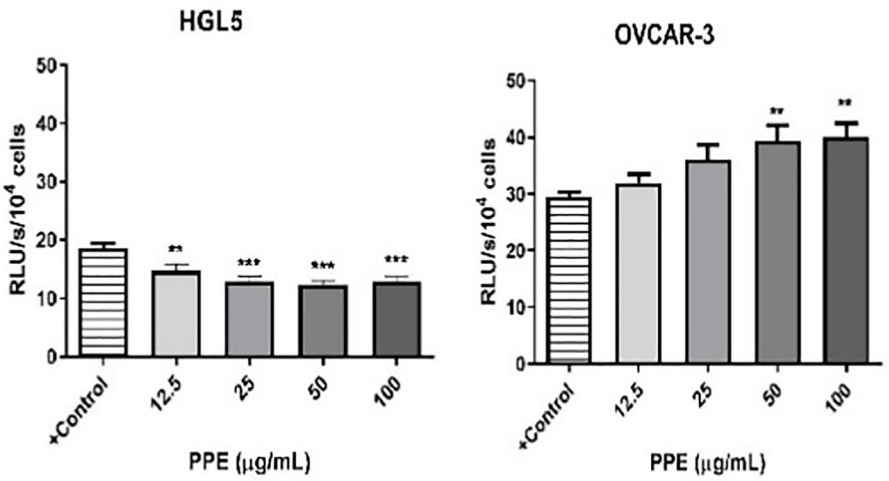


FIGURE 4
Production of reactive oxygen species (ROS) by human ovarian granulosa cells HGL5 and cancer cells OVCAR-3 after pomegranate peel extract (PPE) treatment. +Control represents a culture medium with ethanol in an amount corresponding to the highest used concentration of PPE. Statistical differences were tested using one-way analysis of variance (ANOVA) followed by Dunnett´s multiple comparison test. Data are expressed as mean ± standard error of mean (SEM). Statistical differences are indicated from the vehicle (**p<0.01, ***p<0.001). Chemiluminescence.

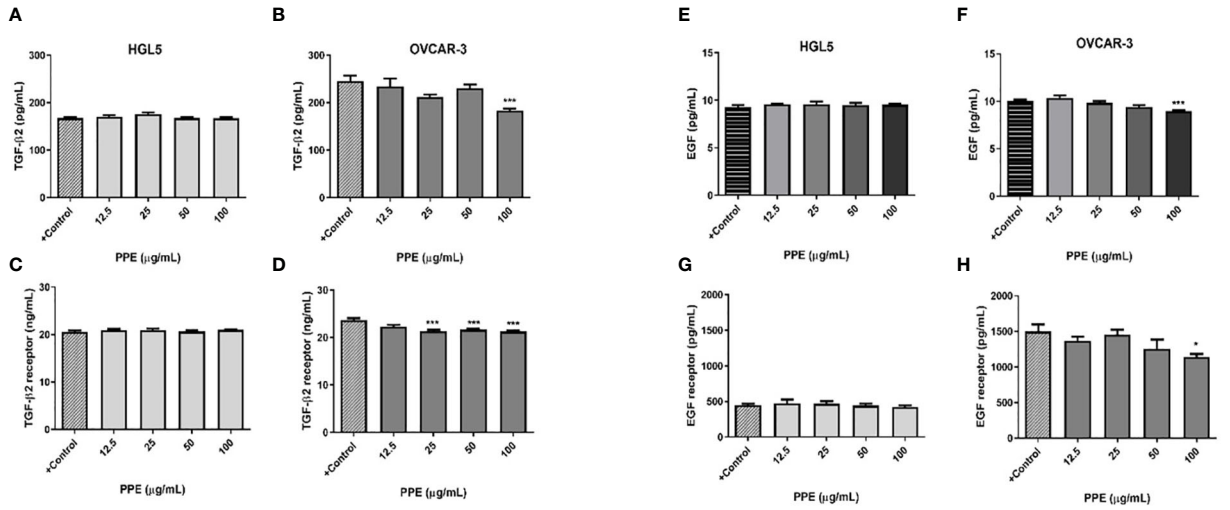


FIGURE 5
Release of growth factors and expression of their receptors by human ovarian granulosa HGL5 and cancer cells OVCAR-3 after pomegranate peel extract (PPE) treatment. TGF-β2 levels in PPE treated HGL5 cells **(A)** and in PPE treated OVCAR-3 cells **(B)**. Expression of TGFBR2 in PPE treated HGL5 cells **(C)** and in PPE treated OVCAR-3 cells **(D)**. EGF levels in PPE treated HGL5 cells **(E)** and in PPE treated OVCAR-3 cells **(F)**. Expression of EGFR in PPE treated HGL5 cells **(G)** and in PPE treated OVCAR-3 cells **(H)**. +Control represents a culture medium with ethanol in an amount corresponding to the highest used concentration of PPE. Statistical differences were tested using one-way analysis of variance (ANOVA) followed by Dunnett´s multiple comparison test. Data are expressed as mean ± standard error of mean (SEM). Statistical differences are indicated from the vehicle (*p<0.05, ***p<0.001). ELISA.

which punicalagins α and β, and flavonoid rutin presented as the main active compounds.

Studies have reported that pomegranate extracts or punicalagin effectively inhibit proliferation of cancer cells (20, 21, 23, 31, 34, 35, 39). In the present study, PPE did not exert any harmful effect on the viability of ovarian granulosa cells HGL5. In addition, it did not seem to induce any change at the nuclear level as it did not impact the percentage of live, apoptotic, or dead ovarian granulosa cells. On the other hand, treatment with PPE at all the doses used in the study led to a reduction in the metabolic activity of ovarian cancer cells OVCAR-3. However, flow cytometry could not confirm any significant changes either at the level of the nucleus or the cell membrane as the number of live, apoptotic, and dead ovarian cancer cells did not differ significantly. Previously, Adaramoye et al. (31) reported a significant anti-proliferative effect of punicalin in human prostate tumor cells PC-3 and LNCaP, which was also associated with the induction of apoptosis. However, less harm was caused to normal prostate cells BPH-1. In SKOV3 human ovary cancer cells, seed extract of pomegranate inhibited the cell growth although the mechanism was not clear (40).

CDKN1A, p21 plays an important role in anti-proliferative or proapoptotic processes and is induced by tumor protein p53 upon

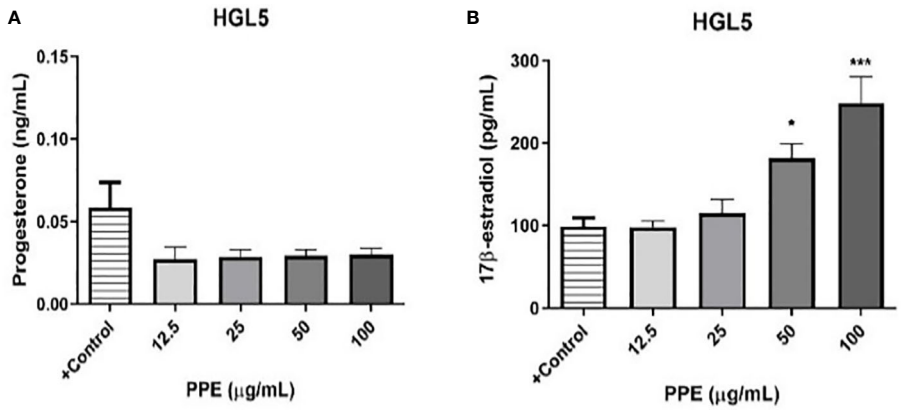


FIGURE 6
Release of progesterone **(A)** and 17β-estradiol **(B)** by human ovarian granulosa cells HGL5 after pomegranate peel extract (PPE) treatment. +Control represents a culture medium with ethanol in an amount corresponding to the highest used concentration of PPE. Statistical differences were tested using one-way analysis of variance (ANOVA) followed by Dunnett´s multiple comparison test. Data are expressed as mean ± standard error of mean (SEM). Statistical differences are indicated from the vehicle (*p<0.05, ***p<0.001). ELISA.

DNA damage or oxidative stress. Additionally, it mediates cell cycle arrest from G1 to S phase, induces apoptosis and is associated with DNA repair (41). Anticancer activity of pomegranate´s punicalagin was associated with cell cycle arrest and increase in p21 expression (42). In a human ovarian cancer cell line A2780, fruit juice as well as the polyphenols of pomegranate such as ellagic acid and luteolin suppressed cell proliferation and migration via downregulation of matrix metalloproteinases (MMP-2 and 9) albeit in a concentration-dependent way (43). The present study reveals similar intracellular events after PPE treatment, when CDKN1A, p21 levels were increased in cancer cells OVCAR-3 without any impact on non-cancer cells HGL5. Recently, urolithin A, a metabolite of ellagitannins, have been reported to inhibit the viability of prostate cancer cells and induce apoptosis by increasing p53 and p21 expression (22). Moreover, ellagic acid present in pomegranate can inhibit the proliferation of breast cancer cells MCF-7 by increasing the expression of cyclin-dependent kinase inhibitors (p21, Cip1, p15 and p19) (44), as well as proliferation of ovarian cells ES-2 and PA-1 by increasing p53 and p21 levels, leading to cell cycle arrest in the G1 phase (45).

Punicalagin present in pomegranate peel can exhibit anticancer activity *in vitro* through cell cycle arrest, regulation of proliferation or survival signals, and catabolic processes such as apoptosis and autophagy (46). In addition, treatment with punicalagin (10 to 100μM) can reduce the viability of cervical cancer cells ME-180 and increase ROS production as well as induce alterations in mitochondrial membrane potential, which can lead to cytotoxic effect on cancer cells (23). Another study reported that punicalagin (12.5 - 200 μM) can affect the viability and proliferation of cervical cancer cells HeLa in a time- and dose-dependent manner by induction of cell cycle arrest in the G1 phase, induction of apoptosis by modulating the expression of apoptosis-associated proteins, downregulating the expression of anti-apoptotic Bcl-2, and upregulating the expression of pro-apoptotic Bax (47). Similarly, punicalin and ellagic acid can promote apoptotic processes in cervical cancer cells Hela and NIH-3T3 by regulating protein expression related to apoptosis (48). Our study also revealed rutin as a flavonoid present in PPE in significant amounts. It can exert antioxidant, proapoptotic, and anti-proliferative activities, increase ROS production and alter Bax/Bcl-2 mRNA expression, and at the same time decrease CDK4 and cyclin D1 expressions, and induce cell cycle arrest in the G0/G1 phase (49).

Oxidative stress refers to disturbance in the balance between the production of ROS and the effectiveness of the antioxidant system, which has been observed in cancer patients, too (50). Oxidative stress is linked with cellular aging and irreversible changes in DNA. It has also been implicated in the progression of degenerative diseases (51). ROS production by PPE treated ovarian cells in the present study confirmed the relationship between the viability of cancer cells and ROS generation. Furthermore, both antioxidant and prooxidant effects of PPE was seen in the present study, depending on the cell type. Based on the results of this study, it may be suggested that PPE can induce oxidative stress in ovarian cancer cells by increasing the ROS levels, which may lead to induction of cytotoxicity. On the contrary, antioxidant effects of PPE have been confirmed by the reduction in ROS production in ovarian granulosa cells HGL5. According to previous studies, punicalagin exhibits strong antioxidant and anti-inflammatory effects and can protect cells by directly scavenging free radicals, ROS and RNS (52, 53). Similarly, the antioxidant and hepatoprotective effects of pomegranate peel powder was earlier confirmed in a biological model of Wistar rats *in vivo*. Pomegranate peel powder was further recommended for use as a component of functional foods (54).

Stimulation by growth factors, such as EGF and TGF plays a key role in the activation of molecular signalling pathways associated with proliferation and cell growth, whereby altered signalling may lead to the development of cancer (44, 55). TGF-β is a secreted cytokine described as a tumor suppressor and TGF-ß2 receptors bind TGF-ß2, thereby engaging the TGF-ß signalling pathway. Mutations in TGF-β signalling pathway occur in various cancer types, including ovarian cancer (56). In the present study, PPE treatment induced changes in the presence of TGFBR2 in a cell-dependent manner. Modulatory activity of PPE in cancer cells OVCAR-3 (but not in HGL5) have been noted by inhibition of TGF-ß2 release, as well as reduction of TGFBR2. This could result in suppression of cell proliferation induced by PPE. Similarly, inhibitory effects of ellagic acid from pomegranate on MCF-7 breast carcinoma cells were found to be mediated by arrest of cell cycle at the G0/G1 phase through the TGF-β/Smads signalling pathway (44). EGF is characterized by overexpression in various cancer cell types, and binding to its receptor EGFR triggers a series of important processes ultimately affecting cell growth, differentiation, and proliferation (57). The current study revealed the suppression of higher EGF levels, as well as EGFR levels after PPE treatment to ovarian cancer cells OVCAR-3, with no impact on non-cancerous HGL5 cells. These findings are indicative of an undeniable effect of PPE on the secretory activity of ovarian cells *in vitro*.

Furthermore, pomegranate possesses strong anti-cancer activity, as exhibited by a variety of mechanisms including anti-estrogenic, anti-proliferative, anti-angiogenetic, anti-inflammatory, and anti-metastatic effects. The prevention or treatment of breast cancer could be associated with inhibition of the mechanisms that govern the estrogen activity, such as the antagonism of the estrogen receptor or the inhibition of estrogen synthesis (58). Ellagitannin-derived compounds present in pomegranate may exert modulatory effect on estrogen synthesis by inhibition of aromatase activity (16). Phytosubstances present in pomegranate peels, especially ellagitannins and punicalagins may play an essential role as possible modulators of steroidogenesis (24, 59, 60). Therefore, the release of steroid hormones by granulosa cells treated with PPE was monitored in the present study. PPE treatment has been found to inhibit the secretion of progesterone (although insignificant statistically) and stimulate that of 17β-estradiol. These results are in line with previous studies indicating that pomegranate is an excellent source of phytoestrogens (61, 62).

In fine, pomegranate peel extract represents an excellent source of polyphenolic substances, including phytoestrogens, and shows potential as a promising chemoprotective agent with efficacy in

cancer cells without harmful effect on non-cancer ovarian cells *in vitro* by regulating several signalling pathways with a remarkable impact on steroidogenesis, cell proliferation, and apoptosis.

5 Conclusions

The present study highlighted the bioactive compounds present in pomegranate peels and the possible mechanisms of action of PPE, shedding light on its promising role in ovarian cancer prevention or management. In this context, PPE presents an excellent source of polyphenolic phytonutrients, mainly punicalagins with strong antioxidant capacity and phytoestrogenic activity on human ovarian granulosa cells *in vitro*. In addition, PPE can exert a cell-specific cytotoxic effect on human ovarian cancer cells by inhibiting growth factors release, metabolic activity, and cell proliferation as well as by stimulating ROS production by ovarian cancer cells without any significant harmful effect on non-cancerous cells. However, further confirmatory studies are essential to understand the therapeutic potential of PPE for paving way to its clinical use.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the studies in accordance with the local legislation and institutional requirements because only commercially available established cell lines were used.

Author contributions

AK: Conceptualization, Formal Analysis, Writing – review & editing. SB: Formal Analysis, Methodology, Writing – original draft. LK: Formal Analysis, Writing – review & editing. JV: Methodology, Writing – review & editing. EI: Methodology, Writing – review & editing. JA: Formal Analysis, Writing – review & editing. MD: Methodology, Writing – review & editing. SR: Formal Analysis, Methodology, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The work was supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic projects APVV-18-0312, and VEGA 1/0266/20.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Mo Y, Ma J, Gao W, Zhang L, Li J, Li J, et al. Pomegranate peel as a source of bioactive compounds: A mini review on their physiological functions. *Front Nutr* (2022) 9:887113. doi: 10.3389/fnut.2022.887113

2. Xiang Q, Li M, Wen J, Ren F, Yang Z, Jiang X, et al. The bioactivity and applications of pomegranate peel extract: A review. *J Food Biochem* (2022) 46(7): e14105. doi: 10.1111/jfbc.14105

3. Teniente SL, Flores-Gallegos AC, Esparza-González SC, Campos-Múzquiz LG, Nery-Flores SD, Rodríguez-Herrera R. Anticancer effect of pomegranate peel polyphenols against cervical cancer. *Antioxidants* (2023) 12(1):127. doi: 10.3390/antiox12010127

4. Abdulla R, Mansur S, Lai H, Ubul A, Sun G, Huang G, et al. Qualitative analysis of polyphenols in macroporous resin pretreated pomegranate husk extract by HPLC-QTOF-MS. *Phytochem Anal* (2017) 28(5):465–73. doi: 10.1002/pca.2695

5. Young JE, Pan Z, Teh HE, Menon V, Modereger B, Pesek JJ, et al. Phenolic composition of pomegranate peel extracts using an liquid chromatography-mass spectrometry approach with silica hydride columns. *J Sep Sci* (2017) 40(7):1449–56. doi: 10.1002/jssc.201601310

6. Gullón P, Astray G, Gullón B, Tomasevic I, Lorenzo JM. Pomegranate peel as suitable source of high-added value bioactives: Tailored functionalized meat products. *Molecules* (2020) 25(12):2859. doi: 10.3390/molecules25122859

7. Pirzadeh M, Caporaso N, Rauf A, Shariati MA, Yessimbekov Z, Khan MU, et al. Pomegranate as a source of bioactive constituents: A review on their characterization, properties and applications. *Crit Rev Food Sci Nutr* (2021) 61(6):982–99. doi: 10.1080/10408398.2020.1749825

8. Viuda-Martos M, Fernández-López J, Pérez-Álvarez JA. Pomegranate and its many functional components as related to human health: a review. *Compr Rev Food Sci Food Saf* (2010) 9(6):635–54. doi: 10.1111/j.1541-4337.2010.00131.x

9. Sharma P, McClees SF, Afaq F. Pomegranate for prevention and treatment of cancer: an update. *Molecules* (2017) 22(1):177. doi: 10.3390/molecules22010177

10. Akkol EK, Dereli FTG, Sobarzo-Sánchez E, Khan H. Roles of medicinal plants and constituents in gynecological cancer therapy: current literature and future directions. *Curr Top Med Chem* (2020) 20(20):1772–90. doi: 10.2174/1568026620666200416084440

11. Siegel RL, Miller KD, Jemal A. Cancer statistics 2018. *CA Cancer J Clin* (2018) 68 (1):7–30. doi: 10.3322/caac.21442

12. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, et al. Ovarian cancer statistics 2018. *CA Cancer J Clin* (2018) 68(4):284–96. doi: 10.3322/caac.21456

13. Wojsiat J, Korczyński J, Borowiecka M, Żbikowska HM. The role of oxidative stress in female infertility and in *vitro* fertilization. *Postepy Hig Med Dosw (Online)* (2017) 71:359–66. doi: 10.5604/01.3001.0010.3820

14. Lang F, Qin Z, Li F, Zhang H, Fang Z, Hao E. Apoptotic cell death induced by resveratrol is partially mediated by the autophagy pathway in human ovarian cancer cells. *PloS One* (2015) 10(6):e0129196. doi: 10.1371/journal.pone.0129196

15. Sirotkin AV, Kolesarova A. *Environmental contaminants and medicinal plants action on female reproduction*. Cambridge, Massachusetts, USA: Academic Press (2022).

16. Adams LS, Zhang Y, Seeram NP, Heber D, Chen S. Pomegranate ellagitannin–derived compounds exhibit antiproliferative and antiaromatase activity in breast cancer cells in *vitro*. *Cancer Prev Res* (2010) 3(1):108–13. doi: 10.1158/1940-6207.CAPR-08-0225

17. Eroglu Ozkan E, Seyhan MF, Kurt Sirin O, Yilmaz-Ozden T, Ersoy E, Hatipoglu Cakmar SD, et al. Antiproliferative effects of Turkish pomegranate (Punica granatum L.) extracts on MCF-7 human breast cancer cell lines with focus on antioxidant potential and bioactive compounds analyzed by LC-MS/MS. *J Food Biochem* (2021) 45 (9):e13904. doi: 10.1111/jfbc.13904

18. Khan N, Afaq F, Kweon MH, Kim K, Mukhtar H. Oral consumption of pomegranate fruit extract inhibits growth and progression of primary lung tumors in mice. *Cancer Res* (2007) 67(7):3475–82. doi: 10.1158/0008-5472.CAN-06-3941

19. Berköz M, Krosniak M. Punicalagin induces apoptosis in A549 cell line through mitochondria-mediated pathway. *Gen Physiol Biophys* (2020) 39(6):557–67. doi: 10.4149/gpb_2020024

20. Sudha T, Mousa DS, El-Far AH, Mousa SA. Pomegranate (Punica granatum) fruit extract suppresses cancer progression and tumor angiogenesis of pancreatic and colon cancer in chick chorioallantoic membrane model. *Nutr Cancer* (2021) 73 (8):1350–6. doi: 10.1080/01635581.2020.1800768

21. Gómez-García FJ, López López A, Guerrero-Sánchez Y, Sánchez Siles M, Martínez Diaz F, Camacho Alonso F. Chemopreventive effect of pomegranate and cocoa extracts on ultraviolet radiation-induced photocarcinogenesis in SKH-1 mice. *PloS One* (2020) 15(4):e0232009. doi: 10.1371/journal.pone.0232009

22. Saleem YIM, Albassam H, Selim M. Urolithin A induces prostate cancer cell death in p53-dependent and in p53-independent manner. *Eur J Nutr* (2020) 59:1607–18. doi: 10.1007/s00394-019-02016-2

23. Zhang L, Chinnathambi A, Alharbi SA, Veeraraghavan VP, Mohan SK, Zhang G. Punicalagin promotes the apoptosis in human cervical cancer (ME-180) cells through mitochondrial pathway and by inhibiting the NF-kB signaling pathway. *Saudi J Biol Sci* (2020) 27(4):1100–6. doi: 10.1016/j.sjbs.2020.02.015

24. Baldovská S, Sláma P, Maruniaková N, Pavlík A, Kohút L, Kolesarova A. Efficacy of phytonutrients from pomegranate peel on human ovarian cells in *vitro*. *J Microbiol Biotechnol Food Sci* (2020) 10(3):511–6. doi: 10.15414/JMBFS.2020.10.3.516

25. Gabriele M, Pucci L, Árvay J, Longo V. Anti-inflammatory and antioxidant effect of fermented whole wheat on TNFα-stimulated HT-29 and NF-κB signaling pathway activation. *J Funct Foods* (2018) 45:392–400. doi: 10.1016/j.jff.2018.04.029

26. Lachman J, Pronek D, Hejtmánková A, Dudjak J, Pivec V, Faitová K. Total polyphenol and main flavonoid antioxidants in different onion (Allium cepa L.) varieties. *Hortic Sci* (2003) 30(4):142–7. doi: 10.17221/3876-HORTSCI

27. Brand-Williams W, Cuvelier ME, Berset CLWT. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci Technol* (1995) 28(1):25–30. doi: 10.1016/S0023-6438(95)80008-5

28. Michalcova K, Roychoudhury S, Halenar M, Tvrda E, Kovacikova E, Vasicek J, et al. *In vitro* response of human ovarian cancer cells to dietary bioflavonoid isoquercitrin. *J Environ Sci Health – B* (2019) 54(9):752–7. doi: 10.1080/03601234.2019.1633214

29. Kolesarova A, Michalcova K, Roychoudhury S, Baldovska S, Tvrda E, Vasicek J, et al. Antioxidative effect of dietary flavonoid isoquercitrin on human ovarian granulosa cells HGL5 in *vitro*. *Physiol Res* (2021) 70(5):745–54. doi: 10.33549/physiolres.934692

30. Baldovska S, Roychoudhury S, Bandik M, Mihal M, Mnahoncakova E, Arvay J, et al. Ovarian steroid hormone secretion by human granulosa cells after supplementation of Sambucus nigra L. extract. *Physiol Res* (2021) 70(5):755. doi: 10.33549/physiolres.934680

31. Adaramoye O, Erguen B, Nitzsche B, Höpfner M, Jung K, Rabien A. Punicalagin, a polyphenol from pomegranate fruit, induces growth inhibition and apoptosis in human PC-3 and LNCaP cells. *Chem Biol Interact* (2017) 274:100–6. doi: 10.1016/j.cbi.2017.07.009

32. Smaoui S, Hlima HB, Mtibaa AC, Fourati M, Sellem I, Elhadef K, et al. Pomegranate peel as phenolic compounds source: Advanced analytical strategies and practical use in meat products. *Meat Sci* (2019) 158:107914. doi: 10.1016/j.meatsci.2019.107914

33. Belgacem I, Li Destri Nicosia MG, Pangallo S, Abdelfattah A, Benuzzi M, Agosteo GE, et al. Pomegranate peel extracts as safe natural treatments to control plant diseases and increase the shelf-life and safety of fresh fruits and vegetables. *Plants* (2021) 10(3):453. doi: 10.3390/plants10030453

34. Modaeinama S, Abasi M, Abbasi MM, Jahanban-Esfahlan R. Anti tumoral properties of Punica granatum (Pomegranate) peel extract on different human cancer cells. *APJCP* (2015) 16(14):5697–701. 10.7314/apjcp.2015.16.14.5697

35. Deng Y, Li Y, Yang F, Zeng A, Yang S, Luo Y, et al. The extract from Punica granatum (pomegranate) peel induces apoptosis and impairs metastasis in prostate cancer cells. *Biomed Pharmacother* (2017) 93:976–84. doi: 10.1016/j.biopha.2017.07.008

36. Ahmadiankia N, Bagheri M, Fazli M. Gene expression changes in pomegranate peel extract-treated triple-negative breast cancer cells. *Rep Biochem Mol Biol* (2018) 7 (1):102.

37. Lansky EP, Newman RA. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *J Ethnopharmacol* (2007) 109 (2):177–206. doi: 10.1016/j.jep.2006.09.006

38. Sabraoui T, Khider T, Nasser B, Eddoha R, Moujahid A, Benbachir M, et al. Determination of punicalagins content, metal chelating, and antioxidant properties of edible pomegranate (Punica granatum L) peels and seeds grown in Morocco. *Int J Food Sci* (2020) 2020:8885889. doi: 10.1155/2020/8885889

39. Cheng X, Yao X, Xu S, Pan J, Yu H, Bao J, et al. Punicalagin induces senescent growth arrest in human papillary thyroid carcinoma BCPAP cells *via* NF-κB signaling pathway. *Biomed Pharmacother* (2018) 103:490–8. doi: 10.1016/j.biopha.2018.04.074

40. Seidi K, Jahanban-Esfahlan R, Abasi M, Abbasi MM. Anti tumoral properties of punica granatum (pomegranate) seed extract in different human cancer cells. *Asian Pac J Cancer Prev* (2016) 17(3):1119–22. doi: 10.7314/apjcp.2016.17.3.1119

41. Kastenhuber ER, Lowe SW. Putting p53 in context. *Cell* (2017) 170(6):1062–78. doi: 10.1016/j.cell.2017.08.028

42. Wang SG, Huang MH, Li JH, Lai FI, Lee HM, Hsu YN. Punicalagin induces apoptotic and autophagic cell death in human U87MG glioma cells. *Acta Pharmacol Sin* (2013) 34(11):1411–9. doi: 10.1038/aps.2013.98

43. Liu H, Zeng Z, Wang S, Li T, Mastriani E, Li QH, et al. Main components of pomegranate, ellagic acid and luteolin, inhibit metastasis of ovarian cancer by down-regulating MMP2 and MMP9. *Cancer Biol Ther* (2017) 18(12):990–9. doi: 10.1080/15384047.2017.1394542

44. Chen HS, Bai MH, Zhang T, Li GD, Liu M. Ellagic acid induces cell cycle arrest and apoptosis through TGF-β/Smad3 signaling pathway in human breast cancer MCF-7 cells. *Int J Oncol* (2015) 46(4):1730–8. doi: 10.3892/ijo.2015.2870

45. Chung YC, Lu LC, Tsai MH, Chen YJ, Chen YY, Yao SP, et al. The inhibitory effect of ellagic acid on cell growth of ovarian carcinoma cells. *Evid. Based Complementary Altern Med* (2013) 2013:306705. doi: 10.1155/2013/306705

46. Berdowska I, Matusiewicz M, Fecka I. Punicalagin in cancer prevention—Via signaling pathways targeting. *Nutrients* (2021) 13(8):2733. doi: 10.3390/nu13082733

47. Tang J, Li B, Hong S, Liu C, Min J, Hu M, et al. Punicalagin suppresses the proliferation and invasion of cervical cancer cells through inhibition of the β-catenin pathway. *Mol Med Rep* (2017) 16(2):1439–44. doi: 10.3892/mmr.2017.6687

48. Gonzalez-Castillo M, Loera MDJ, Ascacio-Valdes J, Rodríguez-Herrera R, Zugasti-Cruz A, Salinas-Santander M, et al. Punicalin and ellagic acid from pomegranate peel extract facilitate apoptotic behaviour in the Hela cell line. *Pak J Pharm Sci* (2021) 34(6):2181–9. doi: 10.36721/PJPS.2021.34.6.REG.2181-2189.1

49. Khan F, Pandey P, Jha NK, Khalid M, Ojha S. Rutin mediated apoptotic cell death in caski cervical cancer cells *via* Notch-1 and Hes-1 downregulation. *Life* (2021) 11(8):761. doi: 10.3390/life11080761

50. Jelic MD, Mandic AD, Maricic SM, Srdjenovic BU. Oxidative stress and its role in cancer. *J Cancer Res Ther* (2021) 17(1):22–8. doi: 10.4103/jcrt.JCRT_862_16

51. Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Nair MG, et al. *In vitro* antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *J Nutr Biochem* (2005) 16(6):360–7. doi: 10.1016/j.jnutbio.2005.01.006

52. Venusova E, Kolesarova A, Horky P, Slama P. Physiological and immune functions of punicalagin. *Nutrients* (2021) 13(7):2150. doi: 10.3390/nu13072150

53. Sihag S, Pal A, Ravikant, Saharan V. Antioxidant properties and free radicals scavenging activities of pomegranate (*Punica granatum* L.) peels: An *in-vitro* study. *Biocatalysis Agric Biotechnol* (2022) 42:102368. doi: 10.1016/j.bcab.2022.102368

54. Ashoush IS, El-Batawy OI, El-Shourbagy GA. Antioxidant activity and hepatoprotective effect of pomegranate peel and whey powders in rats. *Ann Agric Sci* (2013) 58(1):27–32. doi: 10.1016/j.aoas.2013.01.005

55. Hao Y, Baker D, Ten Dijke P. TGF-β-mediated epithelial-mesenchymal transition and cancer metastasis. *Int J Mol Sci* (2019) 20(11):2767. doi: 10.3390/ijms20112767

56. Levy L, Hill CS. Alterations in components of the TGF-β superfamily signaling pathways in human cancer. *Cytokine Growth Factor Rev* (2006) 17(1-2):41–58. doi: 10.1016/j.cytogfr.2005.09.009

57. Björkelund H, Gedda L, Malmqvist M, Andersson K. Resolving the EGF-EGFR interaction characteristics through a multiple-temperature, multiple-inhibitor, real-time interaction analysis approach. *Mol Clin Oncol* (2013) 1(2):343–52. doi: 10.3892/mco.2012.37

58. Moga MA, Dimienescu OG, Bălan A, Dima L, Toma SI, Bîgiu NF, et al. Pharmacological and therapeutic properties of Punica granatum phytochemicals: possible roles in breast cancer. *Molecules* (2021) 26(4):1054. doi: 10.3390/molecules26041054

59. Packova D, Carbonell-BarraChina AA, Kolesarova A. Ellagitannins-compounds from pomegranate as possible effector in steroidogenesis of rabbit ovaries. *Phys Res* (2015) 64(4):583–5. doi: 10.33549/physiolres.932971

60. Baldovská S, Michalcová K, Halenár M, Carbonell-BarraChina AA, Kolesárová A. Polyphenol-rich pomegranate extract as a potential modulator of steroidogenesis in human ovarian cells. *J Microbiol Biotechnol Food Sci* (2019) 8(6):1343–6. doi: 10.15414/jmbfs.2019.8.6.1343-1346

61. Espín JC, Larrosa M, García-Conesa MT, Tomás-Barberán F. Biological significance of urolithins, the gut microbial ellagic acid-derived metabolites: the evidence so far. *Evid. Based Complementary Altern Med* (2013) 2013:270418. doi: 10.1155/2013/270418

62. Shaban NZ, Talaat IM, Elrashidy FH, Hegazy AY, Sultan AS. Therapeutic role of Punica granatum (pomegranate) seed oil extract on bone turnover and resorption induced in ovariectomized rats. *J Nutr Health Aging* (2017) 21:1299–306. doi: 10.1007/s12603-017-0884-5

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 30 November 2023
DOI 10.3389/fendo.2023.1271521

OPEN ACCESS

EDITED BY
Ashu Johri,
Independent Researcher, New York, NY, United States

REVIEWED BY
Shubhadeep Roychoudhury,
Assam University, India
Sonja S. Zafirovic,
VINČA Institute of Nuclear Sciences - National Institute of the Republic of Serbia, Serbia
Mirjana Macvanin,
University of Belgrade, Serbia
Fangdie Ye,
Fudan University, China

*CORRESPONDENCE
Virendra Kumar Yadav
yadava94@gmail.com
Kuang-Yow Lian
kylian@mail.ntut.edu.tw
Ashish Patel
uni.ashish@gmail.com
Dipak Kumar Sahoo
dsahoo@iastate.edu

RECEIVED 02 August 2023
ACCEPTED 17 November 2023
PUBLISHED 30 November 2023

CITATION
Patani A, Balram D, Yadav VK, Lian K-Y, Patel A and Sahoo DK (2023) Harnessing the power of nutritional antioxidants against adrenal hormone imbalance-associated oxidative stress.
Front. Endocrinol. 14:1271521.
doi: 10.3389/fendo.2023.1271521

Harnessing the power of nutritional antioxidants against adrenal hormone imbalance-associated oxidative stress

Anil Patani[1], Deepak Balram[2], Virendra Kumar Yadav[3*], Kuang-Yow Lian[2*], Ashish Patel[3*] and Dipak Kumar Sahoo[4*]

[1]Department of Biotechnology, Smt. S.S. Patel Nootan Science and Commerce College, Sankalchand Patel University, Visnagar, Gujarat, India, [2]Department of Electrical Engineering, National Taipei University of Technology, Taipei, Taiwan, [3]Department of Life Sciences, Hemchandracharya North Gujarat University, Gujarat, India, [4]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA, United States

Oxidative stress, resulting from dysregulation in the secretion of adrenal hormones, represents a major concern in human health. The present review comprehensively examines various categories of endocrine dysregulation within the adrenal glands, encompassing glucocorticoids, mineralocorticoids, and androgens. Additionally, a comprehensive account of adrenal hormone disorders, including adrenal insufficiency, Cushing's syndrome, and adrenal tumors, is presented, with particular emphasis on their intricate association with oxidative stress. The review also delves into an examination of various nutritional antioxidants, namely vitamin C, vitamin E, carotenoids, selenium, zinc, polyphenols, coenzyme Q10, and probiotics, and elucidates their role in mitigating the adverse effects of oxidative stress arising from imbalances in adrenal hormone levels. In conclusion, harnessing the power of nutritional antioxidants has the potential to help with oxidative stress caused by an imbalance in adrenal hormones. This could lead to new research and therapeutic interventions.

KEYWORDS

adrenal hormone imbalance, oxidative stress, nutritional antioxidants, reactive oxygen species, HPT axis

1 Introduction

Adrenal hormone imbalance or dysfunction refers to a condition characterized by aberrant production or regulation of hormones such as cortisol, aldosterone, and dehydroepiandrosterone (DHEA) inside the body. The presence of this imbalance can significantly impact various physiological processes, resulting in a diverse array of health

complications (1). Oxidative stress, which occurs when the body's antioxidant defence systems cannot neutralize reactive oxygen species (ROS), is one of the main causes of these negative effects (abnormal production or regulation of hormones such as cortisol, aldosterone, and DHEA) (2). A study with human cells found that too much glucocorticoid causes too much ROS to be made, which disturbs the balance of metabolic processes and changes the way the vascular endothelium looks and works (3).

Endogenous antioxidant systems control ROS, chemically reactive molecules produced by cellular metabolism (4). However, when there is an imbalance in the adrenal hormones, this delicate balance is disturbed, which results in increased ROS generation and reduced antioxidant defences (5). The aforementioned imbalance may arise due to factors such as chronic anxiety, hormone dysregulation, environmental pollutants, and suboptimal dietary selections (6). Through increased mitochondrial respiration and oxidative phosphorylation, glucocorticoids directly cause oxidative stress in neurons. The incubation of cortical neurons with acute corticosterone resulted in a dose- and time-dependent increase in mitochondrial oxidation, membrane potential, and calcium-holding capacity (7).

Oxidative stress caused by an imbalance in the adrenal hormones has many effects. Oxidative stress can damage lipids, proteins, and DNA, which can cause cellular dysfunction and tissue damage (5). Furthermore, it can turn on inflammatory pathways and mess up the complex signaling networks needed to keep physiology in balance (8). Consequently, adrenal hormone-related diseases like adrenal insufficiency and Cushing's syndrome often show signs of oxidative stress, like fatigue, immune dysfunction, cognitive impairment, and accelerated aging (9).

A crucial part of physiological balance is the complicated relationship between antioxidants in the diet and oxidative stress-induced adrenal hormone imbalance (10). The finely tuned regulation of adrenal hormones can be disrupted by oxidative stress, which is caused by an imbalance between reactive oxygen species and the body's antioxidant defense mechanisms (2). Antioxidants serve an important role in preventing oxidative damage by neutralizing free radicals and protecting the delicate equilibrium of the adrenal glands. Adrenaline hormones such as cortisol and adrenaline, which are essential for stress response and overall hormonal harmony, may be dysregulated when this balance is disrupted. Nutritional antioxidants are bioactive substances found in different foods that can eliminate ROS and boost the body's own antioxidant defences. Some of these molecules are vitamins (like C and E), minerals (like selenium and zinc), phytochemicals (like polyphenols and carotenoids), and other parts of food (11).

The primary objective of this review is to elucidate the mechanisms by which adrenal hormone imbalance induces oxidative stress and investigate the potential contributions of nutritional antioxidants in mitigating such imbalances. Understanding the intricate interplay between adrenal hormone imbalance, oxidative stress, and nutritional antioxidants can give novel insights regarding therapeutic modalities for disorders associated with adrenal hormones, thereby enhancing holistic well-being.

2 Overview of the adrenal gland

The adrenal glands, which are located atop each kidney, are important components of the endocrine system, playing a key role in maintaining homeostasis and responding to stress. Each adrenal gland is divided into two sections: the outer adrenal cortex and the inner adrenal medulla (12). The adrenal cortex is further subdivided into three zones, each of which is responsible for the production of a distinct hormone. Mineralocorticoids, primarily aldosterone, are produced by the outermost zona glomerulosa and regulate electrolyte balance and blood pressure. The zona fasciculata produces glucocorticoids, particularly cortisol, which are involved in glucose metabolism, anti-inflammatory responses, and stress management. Androgens are produced by the innermost zona reticularis, which aids in the development of secondary sexual characteristics (13) (Figure 1).

The adrenal medulla, an extension of the sympathetic nervous system, on the other hand, produces catecholamines such as epinephrine (adrenaline) and norepinephrine (14) (Figure 1), which have widespread impacts on the cardiovascular system, metabolism, and other body systems. They cause the bloodstream to release glucose and fatty acids, priming the body for increased activity. Furthermore, these hormones increase bronchiole dilation, resulting in enhanced oxygen uptake (15). The hormones produced by the adrenal glands are many and diverse, regulating a wide range of physiological functions. Aldosterone influences blood pressure and electrolyte balance through regulating sodium and potassium levels (16). Cortisol has an impact on metabolism, immunological function, and the body's reaction to stress. Androgens play a role in the development of secondary sexual characteristics in men (17).

Furthermore, the adrenal glands produce dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S), which are precursors of sex hormones that influence sexual development and reproductive function. The interplay of these hormones is complex, regulating a wide range of physiological processes and contributing to the body's ability to adapt to both short-term and long-term stressors (18).

3 General overview of adrenal hormone disorders

Adrenal hormone disorders, also called adrenal gland disorders, are characterized by dysfunction or imbalance in the hormones produced by the adrenal glands. The adrenal glands are located on top of the kidneys and are responsible for producing hormones that regulate numerous physiological processes. Common adrenal hormone disorders include adrenal insufficiency, Cushing's syndrome, and adrenal tumors (19).

3.1 Adrenal insufficiency

When the adrenal glands do not produce enough cortisol and, occasionally, aldosterone, it is known as adrenal insufficiency (20). Adrenal insufficiency is a common disorder with multiple causes

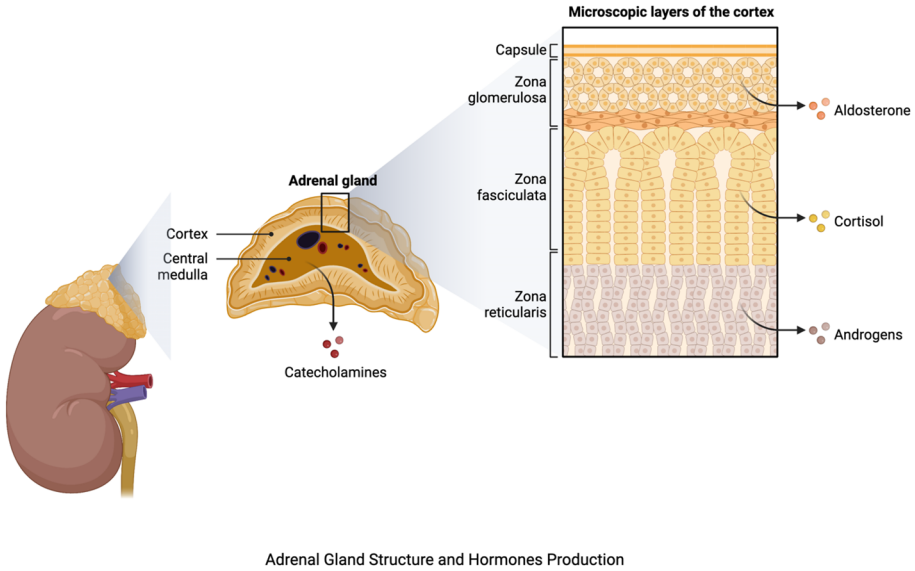


FIGURE 1
Adrenal gland structure and hormone production. The figure was produced with BioRender (Biorender.com; accessed on 29th July 2023).

that can be categorized as primary (adrenal), secondary (pituitary), and tertiary (hypothalamus) forms (21). Primary adrenal insufficiency, often called Addison's disease, is mostly caused by autoimmune adrenal gland damage, but infections and genetic abnormalities can also contribute. Primary adrenal insufficiency is characterized by fatigue, frailty, weight loss, low blood pressure, salt cravings, and skin hyperpigmentation (22). When the pituitary gland is unable to produce enough adrenocorticotropic hormone, which stimulates the adrenal glands to synthesize cortisol, secondary adrenal insufficiency develops (23). Tertiary adrenal insufficiency caused by exogenous steroid medication is common but difficult to diagnose due to its non-specific symptoms (21).

3.2 Cushing's syndrome

Long-term cortisol exposure causes Cushing's syndrome. Exogenous Cushing's syndrome is caused by corticosteroid use, while endogenous is caused by adrenal gland excessive production of cortisol (24). The etiology of endogenous Cushing's syndrome encompasses various factors, including the presence of adrenal tumors (adenomas or carcinomas), pituitary tumors (Cushing's disease), or tumors that generate ACTH elsewhere in the body (25). Cushing's syndrome is linked to severe morbidities and a higher mortality rate. Cardiovascular disease is the leading cause of systemic complications and the leading cause of mortality. The prognosis of the disease is primarily influenced by the diagnostic and therapeutic difficulties that continue to be a significant obstacle (26). Weight gain (especially in the trunk and face), muscle weakness, thinning skin, easily bruising, elevated blood pressure, glucose intolerance, and mood swings are all typical signs of Cushing's syndrome (27).

3.3 Adrenal tumors

Tumors of the adrenal glands can be benign (noncancerous) or malignant (cancerous). Adrenal adenomas are the most prevalent adrenal tumor type and are typically nonfunctional, meaning they do not produce excessive hormones (28). Specific hormones, such as cortisol (which causes Cushing's disease) or aldosterone (which causes primary aldosteronism), can be overproduced as a result of functional adrenal tumors (29). Rare but aggressive malignant tumors known as adrenal carcinomas can produce too much hormone and invade adjacent tissues (30). Clinical assessment, hormone level measurements (such as cortisol, aldosterone, and adrenal androgens ACTH), imaging tests (such as CT scan and MRI) to look for abnormalities in the adrenal glands, and occasionally specialized tests like the dexamethasone suppression test or adrenal vein sampling are used to diagnose adrenal hormone disorders (31). The exact illness and its underlying cause will determine the available treatments. They could include radiation therapy, surgery to remove adrenal tumors, hormone replacement therapy (such as cortisol or aldosterone replacement), or drugs to control symptoms or hormone production (28).

3.4 Adrenal steroidogenesis

The pathophysiology of lethal adrenal disorders is heavily influenced by oxidative stress, and mutations in antioxidant defense genes can have a considerable impact on adrenal steroidogenesis. Because of their high metabolic activity, the adrenal glands, which are required for the production of steroid hormones that regulate different physiological processes, are vulnerable to oxidative damage (32). Excessive oxidative stress

can disrupt adrenal steroidogenesis by disrupting key enzymes involved in hormone synthesis, resulting in cortisol and aldosterone production dysregulation. Mutations in antioxidant defense genes, which are important for reducing oxidative damage, worsen this sensitivity. Such genetic variants weaken cellular defense mechanisms against ROS, raising oxidative stress levels in the adrenal glands. This complex interplay between oxidative stress and genetic factors might lead to the emergence and progression of life-threatening adrenal disorders (33).

4 Oxidative stresses

Oxidative stress occurs when prooxidant molecules like ROS and reactive nitrogen species (RNS) get produced in excess when antioxidant systems are not working efficiently (34, 35). The mitochondrial respiratory chain produces superoxide anion, hydroxyl radical, and hydrogen peroxide during aerobic metabolism (36). RNS includes peroxynitrite-nitrosoperoxycarbonate is produced when peroxynitrite and carbon dioxide react with nitric oxide (NO) (37). Under normal physiological settings, the body makes ROS as a byproduct of metabolism and other cellular processes. Though ROS are important for cell signaling, immune function, and defence against pathogens (38), oxidative stress can be produced by either excessive ROS generation or insufficient antioxidant defence mechanisms (2). Environmental pollutants, exposure to ionizing radiation, certain drugs, chronic inflammation, and lifestyle choices such as excessive alcohol use, a poor diet, and smoking can all contribute to oxidative stress (39). In addition, elevated oxidative stress has been associated with several diseases and conditions, including diabetes, neurological disorders, cardiovascular diseases, and cancer (40).

When ROS levels exceed the antioxidant defences of the body, they can damage lipids, proteins, and DNA. This is also known as oxidative damage, and it can interfere with normal cellular function and contribute to the development of numerous diseases and aging processes (41). This is also linked to Alzheimer's, Parkinson's, cardiovascular diseases, and cancer (42). ROS play a major role in disease development, including cancer, neurodegenerative diseases, cardiovascular diseases, diabetes, and inflammatory diseases. The production of ROS is increased in obesity due to the metabolic burden imposed by excessive macronutrient intake and the availability of substrates (43). Metabolic perturbations in the adipose tissue of individuals with obesity arise as a consequence of mitochondrial dysfunction and endoplasmic reticulum stress within the cellular milieu (44). The accumulation of ROS leads to cellular impairment and subsequently contributes to the pathogenesis of inflammatory and cardiovascular disorders (45). The communication of pro-inflammatory cytokines by mitochondrial ROS serves to reinforce the relationship between OS and inflammation (46).

The human body employs a sophisticated array of antioxidants to combat oxidative stress. These include enzymatic antioxidants such as superoxide dismutase (SOD), catalase, glutathione peroxidase, and non-enzymatic antioxidants, including glutathione (GSH). Antioxidants have the ability to counteract ROS and mitigate cellular damage caused by oxidative stress (47).

The management of oxidative stress is crucial for the maintenance of overall health. A balanced, nutritious diet high in antioxidants, regular exercise, avoiding exposure to environmental toxins, and reducing lifestyle factors known to reduce oxidative stress, whereas smoking and binge drinking, are all effectively increase oxidative stress. Additionally, it's crucial to note that some antioxidant supplements have been investigated for their possible advantages in lowering oxidative stress, while the data for their efficiency is conflicting and should be reviewed with a healthcare provider (39, 48).

5 Adrenal hormonal imbalance-associated oxidative stress

Adrenal hormonal imbalance-related oxidative stress is caused by dysregulation in the finely tuned endocrine system, specifically the adrenal glands (49). The adrenal glands are vital for maintaining physiological homeostasis by secreting hormones such as cortisol and adrenaline. When this equilibrium is upset, either by chronic stress or pathological situations, it can result in the overproduction or underproduction of these hormones, which contributes to oxidative stress (50).

Excess cortisol release, which is frequently associated with chronic stress, activates the glucocorticoid receptor, boosting the creation of reactive oxygen species (ROS) within cells. These ROS molecules, which include superoxide and hydrogen peroxide, cause oxidative damage to cells by destroying lipids, proteins, and DNA (51). Furthermore, disturbed hormonal balance changes antioxidant defense mechanisms, worsening oxidative stress (52).

The mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) pathways have both been associated in adrenal hormonal imbalance-associated oxidative stress. When activated by stress-induced hormonal imbalances, these pathways promote the production of pro-inflammatory cytokines and genes associated with oxidative stress, increasing the overall oxidative burden on cells (53). Furthermore, the hypothalamic-pituitary-adrenal (HPA) axis, a key component in stress response, plays an important role in adrenal hormonal imbalance. Abnormal HPA axis signaling can cause persistent cortisol increase, causing oxidative stress via many mechanisms, including mitochondrial dysfunction and endoplasmic reticulum stress (54). The adrenal glands generate hormones such as glucocorticoids, mineralocorticoids, and androgens, (Figure 1) which play significant roles in regulating various physiological processes (55).

The Table 1 provides a brief overview of the impact of hormonal imbalances on oxidative stress, with a focus on glucocorticoids, mineralocorticoids, and androgens. In the case of glucocorticoids, both excess (Cushing's syndrome) and insufficiency (Addison's disease) contribute to oxidative stress through a variety of mechanisms, such as decreased antioxidant defenses, increased reactive oxygen species (ROS) generation, and impaired mitochondrial function, which ultimately results in chronic inflammation. Mineralocorticoids, whether in excess (Hyperaldosteronism) or in deficiency (Hypoaldosteronism), are linked to oxidative stress, mainly via activation of the renin-angiotensin-aldosterone system (RAAS) and disruption of cellular homeostasis due to sodium and potassium imbalances. Androgens, whether in excess

TABLE 1 Adrenal hormonal imbalance-associated oxidative stress.

Hormone	Imbalance	Effects on Oxidative Stress	Reference
Glucocorticoids	Excess (Cushing's syndrome) or deficiency (Addison's disease)	- Reduced antioxidant defenses: decreased endogenous antioxidant synthesis (e.g., glutathione, SOD, catalase). - Increased ROS generation: Activation of NADPH oxidase stimulates ROS production (superoxide anions, hydrogen peroxide). - Impaired mitochondrial function: Impairment of mitochondrial activity, which results in increased ROS generation within the mitochondria. - Inflammation and oxidative stress: Immune system imbalance and promotion of chronic inflammation, linked to increased oxidative stress.	(33)
Mineralocorticoids	Excess (Hyperaldosteronism) or deficiency (Hypoaldosteronism)	- Excess aldosterone can lead to increased activation of the renin-angiotensin-aldosterone system (RAAS), which is in relation with oxidative stress. - Mineralocorticoid imbalances can disrupt sodium and potassium balance, disrupting cellular homeostasis and causing oxidative stress indirectly.	(56)
Androgens	Excess (Hyperandrogenism) or deficiency (Hypoandrogenism)	- Inflammation and oxidative stress: An imbalance in androgen levels can alter the immunological response and contribute to chronic inflammation, both of which lead to increased oxidative stress. - Mitochondrial dysfunction: Changes in testosterone levels can impair mitochondrial function and increase ROS generation, leading to oxidative stress. - Excess androgen levels can cause oxidative damage to reproductive organs, impacting fertility and reproductive health.	(57)

(Hyperandrogenism) or in deficiency (Hypoandrogenism), cause oxidative stress by affecting immunological responses, fostering chronic inflammation, causing mitochondrial dysfunction, and causing oxidative damage in reproductive organs, thereby affecting fertility and reproductive health (Table 1).

5.1 Glucocorticoids hormone imbalance -associated oxidative stress

In the brain, lungs, and blood cells, the association between glucocorticoids and oxidative stress has been established. Numerous inflammatory and autoimmune disorders are frequently treated with glucocorticoids. The glucocorticoids's excess led to myopathy, osteoporosis, diabetes, and hypertension, among other diseases. All of the previously mentioned pathophysiological conditions are linked to oxidative stress. ROS from glucocorticoids can cause many pathological conditions (58). Dexamethasone, a synthetic glucocorticoid, has been documented to elicit the production of ROS either through direct means or as a consequence of endothelial nitric oxide synthase (eNOS) uncoupling, which is attributed to the constrained availability of tetrahydrofolate (59, 60). When glucocorticoids are produced in excess, they cause glucose levels to rise; this leads to glycation, which increases ROS production; this, in turn, reduces catalase (CAT), glutathione peroxidase (GPx), and SOD levels in the hippocampus, impairing cognitive functions (61). By raising mitochondrial respiration and oxidative phosphorylation, glucocorticoids directly cause neuronal OS (62). A cascade of negative effects is associated with the role of adrenal corticosterone in hippocampus oxidative stress. This disease is characterized by increased lipid peroxidation and protein carbonyl (PC) concentrations, as well as a decrease in antioxidant enzyme activity such as GPx, SOD, and CAT (63). Another study found that short-term exogenous cortisol administration did not enhance juvenile brown trout oxidative stress levels but did increase GSH levels, indicating that the increased GSH may have reduced the formation of ROS (64). Therefore, cortisol may prevent rather than cause oxidative stress and may activate antioxidant defences via genomic pathways in addition to influencing other systems that regulate the formation of pro-oxidants like ROS (65).

Familial glucocorticoid deficiency (FGD) arises due to mutations in the ACTH-receptor components (MC2R, MRAP) or the general steroidogenesis protein (StAR). These mutations lead to an inability of the adrenocortical cells to synthesize glucocorticoids in response to ACTH stimulation. *Nicotinamide Nucleotide Transhydrogenase (NNT)* mutations are responsible for the development of FGD. These mutations were observed to decrease the ability of adrenocortical cells to effectively detoxify ROS. Mutations in NNT result in the manifestation of OS as well as phenotypic and functional abnormalities in mitochondrial activity. These findings provide compelling evidence supporting the important role of NNT in maintaining proper mitochondrial function in cases of adrenocortical insufficiency (66).

5.2 Mineralocorticoids hormone imbalance-associated oxidative stress

According to numerous clinical and investigations in animal models, the most significant physiological mineralocorticoid, aldosterone, causes OS and inflammation in patients with chronic and stable heart failure (67). Aldosterone raises blood pressure, affecting the heart. Mineralocorticoid receptors directly affect cardiac function, electrical conduction, OS, inflammation, and fibrosis, further harming the heart (68). Aldosterone/salt-induced hypertension in rats results in renal damage and an increase in the production of ROS in the renal cortex (69). According to Patni et al. (70) findings, an increase in renal OS causes the induction of apoptosis in the renal tubules. It has been demonstrated that aldosterone stimulates superoxide radical formation in endothelial cells by activating Rec1 (71). The

mineralocorticoid receptor (MR) is activated during both physiological and pathological events since tissue damage, OS, and inflammation are frequent components of disease situations. It has been utilized in clinical trials to treat heart and kidney disease connected to hypertension and other chronic diseases by blocking MR signaling with MR antagonists (MRAs), which suppresses fibrosis in these organs as MRAs likely have cardio-protective effects by directly blocking cardiac and vascular MR (72, 73).

To avoid aldosterone-induced oxidative damage, kidney cells were tested for their potential to up-regulate nuclear factor-erythroid-2-related factor 2 (Nrf2) (74). Aldosterone first activated Nrf2 *in vitro* as an antioxidant response. Although aldosterone-induced oxidative or nitrative stress quickly stimulated antioxidant or detoxifying enzymes such SOD, thioredoxin (TRX), HO-1, or GCSc, this adaptive survival response appeared to be fleeting and overpowered by a chronic increased generation of ROS/RNS. As a result, oxidative DNA damage happened. Additionally, even though Nrf2 activation was seen *in vivo*, aldosterone-treated rat kidneys showed significant DNA damage, showing that the response was insufficient to shield the animals from these side effects (75).

5.3 Androgens hormone imbalance-associated oxidative stress

Testosterone, the main male steroid hormone, causes spermatogenesis and secondary sexual characteristics. Testosterone is anabolic. Usually, it speeds up metabolism. Increased metabolic rate increases O_2 consumption and ROS generation. Thus, testosterone increases OS. However, testosterone's role in OS is controversial. Multiple studies have demonstrated that testosterone induces OS in the muscle, testis, and human placenta (76); others indicate that testosterone has antioxidant properties in the prostate and nervous tissue (77). Testosterone supplements improve the OS parameters in brain tissues and raise antioxidant enzyme levels to reduce oxidative damage. According to *in vitro* research, testosterone treatment in newborn rats specifically protects the cerebellar granule cells from OS-induced cell death. By inhibiting OS, testosterone contributes to the protection of neurons (78). Adrenal androgen imbalances, such as dehydroepiandrosterone (DHEA) and androstenedione, have been linked to oxidative stress in the body. Elevated amounts of these androgens, which are commonly found in conditions such as polycystic ovarian syndrome (PCOS) and some adrenal disorders, can upset the delicate balance of cellular redox processes. This imbalance can result in an excess of reactive oxygen species (ROS), which then overwhelms the body's antioxidant defences, resulting in oxidative stress. This type of oxidative stress is recognized as a key factor in the pathogenesis of a variety of health problems, ranging from metabolic disorders to inflammation-related diseases, emphasizing the importance of understanding and managing adrenal androgen imbalances to mitigate oxidative stress and its associated health consequences (79).

According to, Tam et al. (80) study, androgen deprivation increased ROS anabolism and decreased antioxidant detoxification, which in turn caused OS in the ventral prostate of rats. These researchers discovered that castration caused 4-hydroxynonenal and 8-hydroxy-20-deoxy-guanosine protein adducts in the regressing epithelium, which suggests oxidative damage. Additionally, castration considerably decreased the expression of important antioxidant enzymes (AOEs) (GPx1, thioredoxin, SOD2, and peroxiredoxin 5) and markedly increased the expression of ROS-generating NAD(P)H oxidases. Testosterone supplementation partially repaired oxidative damage in ventral prostate epithelia of castrated rats receiving testosterone replacement therapy had a partial decrease in NAD(P)H oxidase expression but an increase in GPx1, SOD2, peroxiredoxin 5 expression, thioredoxin, CAT, glutathione reductase (GR), c-glutamyl transpeptidase, and glutathione synthetase expression in the regenerating ventral prostate tissue. The augmentation of mitochondrial functionality, alterations in intracellular GSH concentrations, and elevation of c-glutamyl transpeptidase activity collectively facilitated the physiological amplification of androgens, leading to the potentiation of OS in LNCaP human prostate cancer cells that are responsive to androgens (81). Prasad et al. (82) showed that testosterone injection to mice caused the down-regulation of CAT, SOD, GST, and GR in the prostate gland. Androgens lowered the activity of AOEs in the heart of rats (83), while orchidectomy enhanced aortic Cu/ZnSOD (84). Borst et al (85) found that testosterone supplementation significantly increased the revival of cardiac work following ischemia/reperfusion *in vitro*, while orchidectomy significantly lowered rat left ventricular AOE activities. In contrast, Klapcinska et al. (86) showed that castration of male rats lowered the levels of CAT, SOD, GPx, and GR in the left ventricle of the heart, as well as GSH and protein-thiol groups, and increased lipid peroxidation and nitrotyrosine concentrations. Increases in a- and c-tocopherol tissue concentrations in the left ventricle appeared to be a compensatory reaction to the increased OS brought on by gonadectomy. Although androgen replacement restores a healthy serum testosterone level, it decreases left ventricular tissue antioxidant status. The favorable effect of endurance training on SOD and CAT activities was reversed, and myocardial lipid peroxidation was enhanced in adolescent male Wistar rats administered with a high dosage of testosterone (87).

Dehydroepiandrosterone levels gradually decline with age. It has been suggested that DHEA is an effective ageing index with links to geriatric syndromes. There is evidence that low DHEA levels are linked to the beginning and progression of metabolic syndrome and diabetes mellitus, as well as a decrease in bone mineral density (88, 89). Numerous studies have revealed that markers in the oxidative circulation are higher than normal in PCOS patients and that oxidative stress plays a role in the development of PCOS (90). In PCOS, hyperglycemia-induced oxidative stress may be capable of directly promoting hyperandrogenism. The link between plasma testosterone or androstenedione and ROS production suggests this. *In vitro* investigations have revealed that the ovarian steroidogenic enzymes responsible for androgen production are stimulated by oxidative stress and inhibited by antioxidants such as statins (91, 92).

6 Role of nutritional antioxidants in alleviating adrenal hormone imbalance

Nutritional antioxidants are substances that can be found in a variety of foods and are essential in defending the body against free

radical damage, oxidative stress, and the negative impact of reactive oxygen species (ROS). They function by scavenging or neutralizing these harmful chemicals, reducing cellular damage, and improving general health (71). Different types of nutritional antioxidants are depicted in (Figure 2). It was revealed to scavenge hydroxyl radical ($^{\cdot}OH$), superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), nitric oxide ($NO^{\cdot}$), peroxynitrite ($ONOO^-$), singlet oxygen ($^1O_2$) and stimulates antioxidant enzymes (AOEs) SOD, Catalase, GPx, GR by nutritional antioxidants (59, 93) (Figure 3).

6.1 Vitamin C against adrenal hormone imbalance-associated oxidative stress

Water-soluble vitamin C, also referred to as ascorbic acid, is a potent antioxidant. Essential for the preservation of overall well-being, it is imperative due to its involvement in numerous pivotal physiological processes (94). Free radicals are unsteady molecules that can harm cells, resulting in several health issues and hastening aging. Vitamin C functions as an antioxidant that assists in scavenging these free radicals and lowering oxidative stress (95). Vitamin C is required for the production of collagen, a structural protein found in the epidermis, bones, tendons, and blood vessels. Collagen is necessary for the formation of scar tissue, so it plays a crucial role in wound healing. Additionally, vitamin C promotes healthy gums, teeth, and cartilage (96). It is well-known that vitamin C supports immune function. It promotes the production of white blood cells, which are essential for fending off infections and pathogens. It enhances natural killer cells and immune system function (97). Plant-based diets and iron supplements contain non-heme iron, which is better absorbed with vitamin C. Iron deficiency anemia can be prevented by boosting iron absorption with vitamin C (98). Other antioxidants in the body, such as vitamin E, are also renewed by vitamin C. It facilitates the restoration of the antioxidant capacity of vitamin E, which enables it to continue fulfilling its protective function in cell membranes. Citrus fruits like lemons, oranges, and grapefruits, berries like strawberries, kiwi, pineapple, mango, papaya, bell peppers (especially yellow and red), Brussels sprouts, broccoli, and leafy greens like spinach and kale are all excellent sources of vitamin C. Furthermore, achieving this outcome is feasible by administering vitamin C supplements (99).

Excess cortisol production in circumstances such as Cushing's disease or persistent stress can contribute to increased oxidative

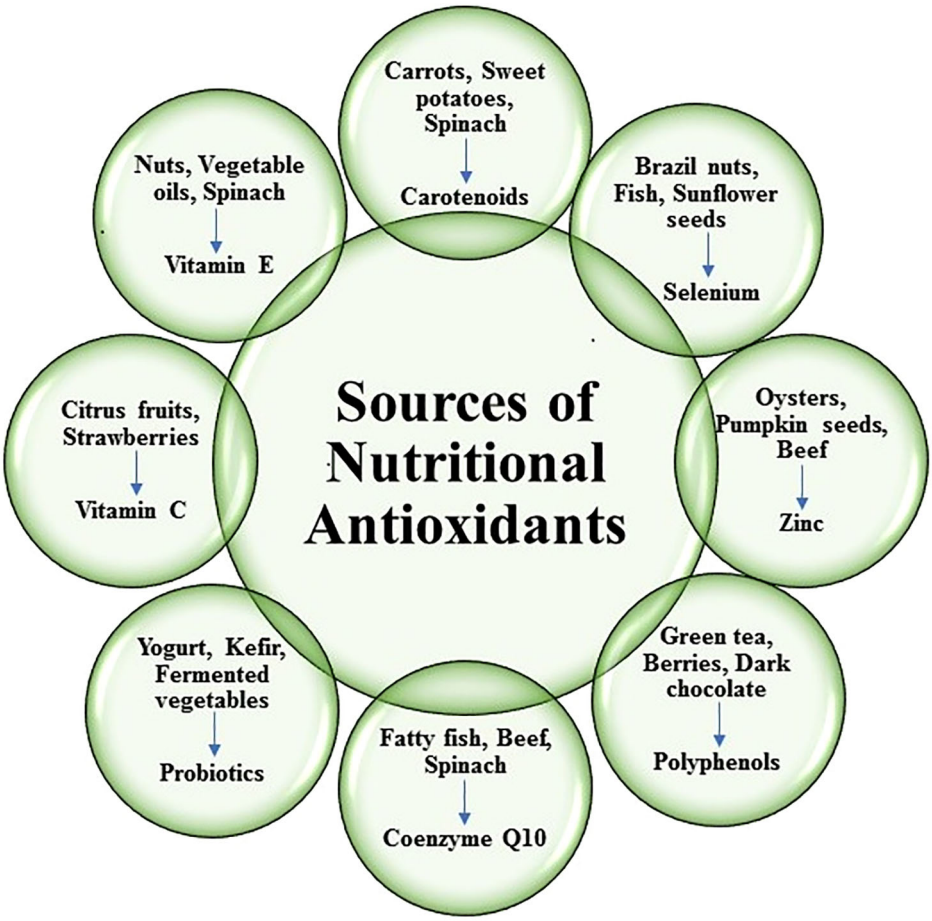


FIGURE 2
Sources of different types of nutritional antioxidants.

10.3389/fendo.2023.1271521

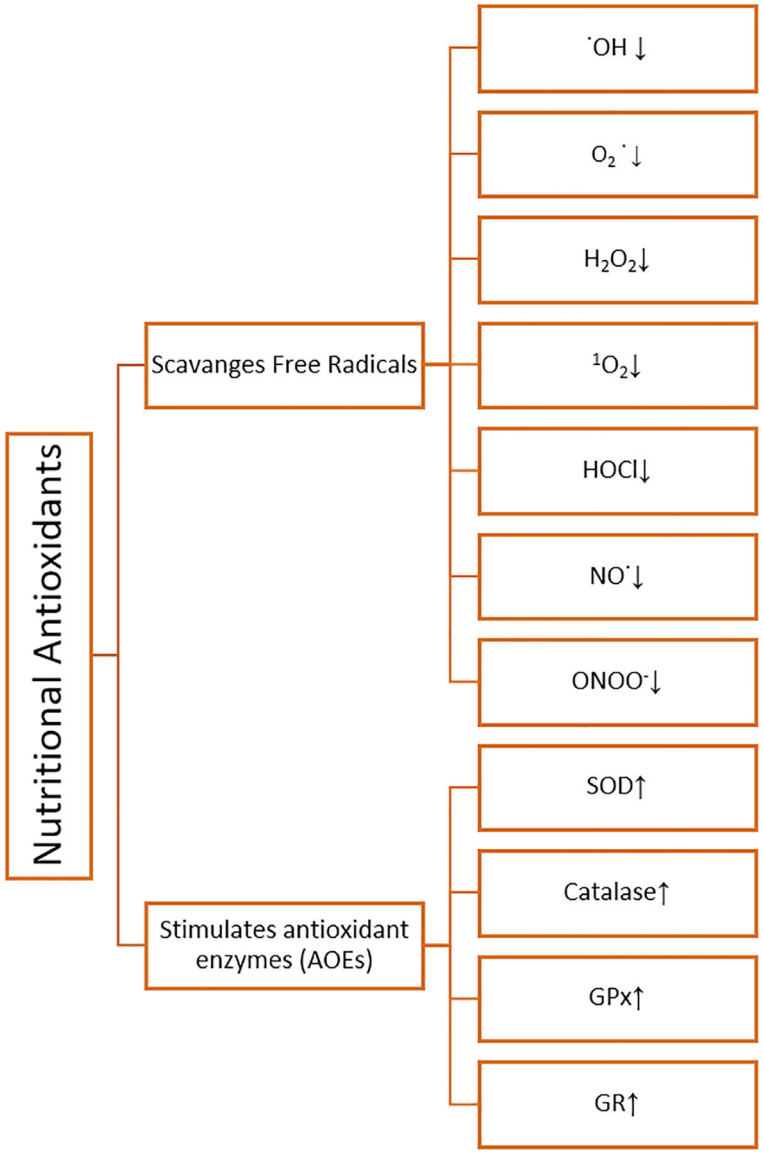


FIGURE 3
Schematic representation of nutritional antioxidant effects.

stress in the body. Vitamin C can help reduce the oxidative stress consequences caused by adrenal hormone imbalance (100).

Vitamin C is a powerful antioxidant capable of scavenging and neutralizing reactive oxygen species (ROS) produced during oxidative stress. It helps stabilize free radicals by giving electrons, preventing them from causing harm to cellular components (95). Vitamin C is essential for regenerating other antioxidants such as vitamin E, glutathione, and coenzyme Q10. These antioxidants also aid in the reduction of oxidative stress and the maintenance of a healthy cellular environment (47). Vitamin C is required for the manufacture of collagen, a protein that provides structure as well as support to a variety of tissues throughout the body, including the adrenal glands. Vitamin C promotes collagen synthesis, which aids in the integrity and function of the adrenal glands, potentially lowering the risk of hormone imbalances. Chronic oxidative stress caused by adrenal hormone abnormalities may weaken the immune system (101). Vitamin C improves immune function by promoting the growth and activity of immune cells like lymphocytes and phagocytes. This can assist the body in fighting infections and other immune-related problems (97). Vitamin C has been demonstrated to influence the body's stress response. It aids in the regulation of cortisol production, the principal stress hormone generated by the adrenal glands. Vitamin C may indirectly assist in minimizing oxidative stress associated with chronic stress by maintaining adrenal health and normalizing cortisol levels (100).

Several studies have revealed a link between stress-related behavior and ascorbic acid. Animal studies show that ascorbic acid reduces stress-induced cortisol production. Ascorbic acid modulates the hypothalamic-pituitary-adrenal (HPA) axis (Figure 4) by directly "braking" cortisol secretion (102). Since ascorbic acid is a cofactor for adrenal cortex enzymes involved in glucocorticoid biosynthesis, this vitamin is necessary for its production (103). By acting as a cofactor

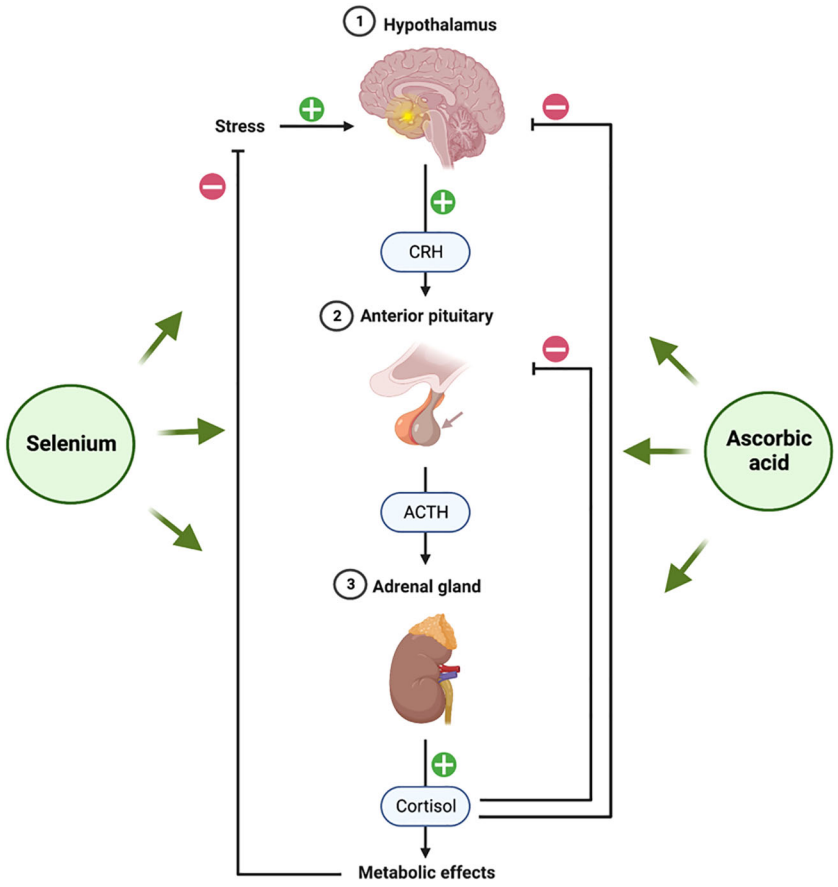


FIGURE 4
Hypothalamic-Pituitary-Adrenal (HPA) axis and role of selenium and ascorbic acid supplements. The figure was produced with BioRender (Biorender.com; accessed on 29th July 2023).

for 11β-hydroxylase, for instance, the ascorbate somewhat increases the conversion of 11-deoxycortisol to cortisol and keeps the cortisol tone at physiological levels (104). Ascorbic acid appears to play a significant part in the stress response, as evidenced by the high amounts of ascorbic acid found in the adrenal glands and the production of ascorbic acid in response to ACTH (100). This is supported by studies showing that ascorbate release occurs before corticosteroid release in the adrenal gland after systemic administration of ACTH to hypophysectomized rats (105), a finding that suggests ascorbate must first be released by the adrenal gland for steroid synthesis (or release) to begin when there is stress.

6.2 Vitamin E against adrenal hormone imbalance-associated oxidative stress

A class of fat-soluble substances with antioxidant capabilities is referred to as vitamin E. It can be found in seeds, leafy green vegetables, nuts, and vegetable oils. There exist two principal variants of vitamin E, namely alpha-tocopherol and gamma-tocopherol. It is worth noting, however, that the term "vitamin E" encompasses a group of eight distinct compounds. These compounds are present in a variety of food sources and are also accessible in the form of dietary supplements (106). Vitamin E antioxidants serve to protect cells against the detrimental effects of free radicals. Free radicals are responsible for inducing cellular damage and contributing to the process of aging. Vitamin E mitigates oxidative stress inside the human body through its ability to counteract the detrimental effects of free radicals (39). Vitamin E is predominantly acknowledged as a lipid-soluble antioxidant, whereby it operates within lipid-rich compartments of cellular structures, such as cell membranes, to mitigate lipid oxidation. Furthermore, it is widely believed that the immune system and gene expression may experience advantageous effects (107).

Adrenal hormone imbalance can increase oxidative stress in the body, and some evidence suggests that vitamin E may protect against it (108). As an antioxidant, Vitamin E neutralizes free radicals and ROS generated during cellular metabolism. Adrenal hormone imbalance can enhance ROS production, causing oxidative damage to cells and tissues. Vitamin E aids in the scavenging of these damaging chemicals, thereby lowering oxidative stress (109). It has been demonstrated that vitamin E affects the body's hormone levels, especially those of the adrenal glands. Vitamin E may assist in regulating adrenal gland activity and perhaps lessen the production of stress-related chemicals like cortisol by fostering hormonal equilibrium. This may indirectly lessen the oxidative stress brought

on by the imbalance of adrenal hormones (110). Chronic adrenal hormone imbalance can cause the body to become inflamed, increasing the risk of oxidative stress. Due to its anti-inflammatory qualities, vitamin E may help lessen inflammation brought on by an imbalance in adrenal hormones. Vitamin E can indirectly lower oxidative stress levels by reducing inflammation (111). Cell membranes are protected from oxidative damage by vitamin E. Unbalanced levels of adrenal hormones can worsen oxidative stress within cells, which can harm biological components. Vitamin E helps maintain appropriate cellular function and lessens oxidative stress-related damage by maintaining the integrity of cell membranes (112).

The consideration of the impact of glucocorticoids on the production of free radicals in the context of stressful situations holds significant importance. Long-term treatment of glucocorticoids is associated with oxidative brain damage in primates, which has been demonstrated (113). According to Al-Sowayan (114), vitamin E therapy reduces exposure to neurotransmitters that cause hypotension by boosting total glutathione, hydrosulfide groups, and selenium levels in the liver and serum. Vitamin E's chain-breaking antioxidant activities minimize oxidative damage by scavenging free radicals (115).

6.3 Carotenoids against adrenal hormone imbalance-associated oxidative stress

A class of pigments known as carotenoids can be found in many different fruits, plants, and other living things. They have been known for being antioxidants and are important for human nutrition (116). In the human body, carotenoids function as antioxidants to help shield cells from injury from harmful compounds known as free radicals. Oxidative stress is a result of free radicals and is linked to several chronic diseases and the aging process. Carotenoids combat these free radicals, minimizing oxidative damage and boosting general health (117). It is worth noting that a mere fraction of the extensive repertoire of carotenoids, exceeding 600 in number, are habitually ingested by people in general. Beta-carotene, lycopene, lutein, zeaxanthin, and astaxanthin are a few well-known carotenoids. Every carotenoid has different antioxidant capabilities and potential health advantages. Many fruits and vegetables contain large amounts of carotenoids. Beta-carotene is abundant in orange and yellow fruits and vegetables like carrots, sweet potatoes, mangoes, and apricots. Watermelons and tomatoes both contain significant levels of lycopene. Zeaxanthin and lutein are found in leafy green vegetables like kale and spinach. Seafood frequently contains astaxanthin, especially salmon and shrimp (118).

Due to their antioxidant action, carotenoids have been linked to many health advantages. Particularly concentrated in the retina, lutein, and zeaxanthin help prevent age-related macular degeneration (AMD) and cataracts. Beta-carotene and astaxanthin, in particular, can help prevent UV damage to the skin and enhance its appearance (119). Carotenoids reduce inflammation and boost immune cell function, boosting immunity. Certain carotenoids, such as lycopene, have been linked to a reduced risk of heart disease by protecting blood vessels from oxidative injury. Carotenoids are absorbed differently depending on food preparation (cooking, chopping, etc.), the presence of dietary lipids, and individual metabolic differences. Carotenoids are more readily absorbed when consumed with a modest amount of fat. In order to exert their full effects, certain carotenoids, such as beta-carotene, must be converted into vitamin A in the body (120).

Carotenoids have been studied for their possible advantages in lowering oxidative stress and promoting general health because of their well-known antioxidant qualities. Although there is little direct study on the benefits of carotenoids directly on oxidative stress related to oxidative stress linked with adrenal hormone imbalance, their antioxidant and anti-inflammatory characteristics may have beneficial impacts (121).

The body uses carotenoids like beta-carotene, lycopene, and lutein as powerful antioxidants. They aid in scavenging dangerous reactive oxygen species (ROS) and free radicals produced by cellular metabolism. Oxidative stress arises due to increased ROS production, instigated by an aberration in the equilibrium of adrenal hormones. Carotenoids possess the ability to scavenge ROS, thereby mitigating the deleterious effects of oxidative damage (122). Unbalanced levels of adrenal hormones can cause chronic inflammation, which is intimately related to oxidative stress. Anti-inflammatory characteristics found in carotenoids make them useful for controlling the inflammatory response. Carotenoids may indirectly lower oxidative stress levels linked to adrenal hormone imbalance by lowering inflammation (123). Unbalanced adrenal hormones can impact immunological performance and perhaps exacerbate oxidative damage. Through the stimulation of immune cell activity and the modulation of immunological responses, carotenoids have been demonstrated to support immune system function. An immune system that is in good health is better able to tolerate oxidative stress and minimize its effects (124). Although the direct effects of carotenoids on adrenal hormones have not been thoroughly investigated, they may indirectly influence the body's hormonal balance. Certain hormones, particularly sex hormones, are partly produced and metabolized by carotenoids. Carotenoids may benefit adrenal hormone imbalance by promoting hormonal equilibrium, potentially lowering oxidative stress (125). When combined with other antioxidants like vitamins E and C, carotenoids can offer more antioxidant protection (126).

Depression is significantly influenced by carotenoid-cleaving enzymes, which are involved in the metabolism of carotenoids. It should be noted that the oxidative degradation of carotenoids, facilitated by carotenoid oxygenases, results in the formation of apocarotenoids. Retinal, retinol, retinoic acid, and abscisic acid are examples of apocarotenoids. By hyperactivating the hypothalamic-pituitary-adrenal (HPA) axis, retinoic acid, the active form of vitamin A, has been related to depressed behavior. Retinoic acid can cause suicide in sensitive people (127, 128). According to a study, eating foods high in carotene and vitamin C is linked to less severe depressive symptoms (129). Lower carotenoid levels may also be a result of bad eating habits linked to obesity and overweight, which have been linked to an enhanced risk of depression due to inflammation or HPA axis dysregulation (10).

6.4 Selenium against adrenal hormone imbalance-associated oxidative stress

Selenium is an indispensable trace mineral that serves as a potent antioxidant in the body. It exerts a protective effect on

cellular structures by synergistic interactions with other antioxidants, such as vitamin E, thereby mitigating the deleterious impact of free radicals and oxidative stress-induced damage (130). Numerous antioxidant enzymes, such as glutathione peroxidase, which works to scavenge free radicals and lessen oxidative cell damage, require selenium as a cofactor. Free radicals can damage cells and cause chronic diseases like cancer, heart disease, and neurological disorders (131). By scavenging these free radicals, selenium reduces oxidative stress. In order to maintain a strong immune system, selenium is essential. It aids in controlling immunological responses, improves immune cell performance, and encourages the formation of antibodies (132). The synthesis and metabolism of thyroid hormones depend on selenium. It converts inactive thyroid hormone (T4) into active thyroid hormone (T3) to sustain proper thyroid function. Selenium has been investigated for its potential role in lowering the risk of specific cancers, including skin, lung, prostate, and colorectal cancers (133). As an antioxidant, it can aid in preventing DNA damage to cells and stop the development of cancer cells. By lowering oxidative stress, enhancing blood vessel function, and reducing inflammation, selenium may benefit heart health. These outcomes may assist in reducing the risk of cardiovascular conditions, such as heart disease and stroke. The health of the male reproductive system depends on selenium. It contributes to sperm production and aids in preserving the sperm cells' structural integrity. It has been demonstrated that selenium supplementation enhances sperm motility and lessens sperm DNA damage (134). The recommended daily selenium intake varies depending on various factors, such as age, gender, and specific medical conditions. The recommended daily intake (RDA) for adults is about 55 micrograms per day. It is crucial to remember that consuming too much selenium can be hazardous, so stick to the recommended dosages. Brazil nuts, organ meats like liver and kidney, whole grains, eggs, and poultry are all excellent sources of selenium (135).

Selenium's antioxidant capabilities and function in promoting the activity of antioxidant enzymes may have implications for lowering oxidative stress, even though there is little direct study on its impact on adrenal hormone imbalance-related oxidative stress (136).

Several antioxidant enzymes, such as thioredoxin reductases and glutathione peroxidases, require selenium in order to function. These enzymes are essential for scavenging ROS and guarding cells against oxidative damage. Increased ROS formation from adrenal hormone imbalance causes oxidative stress. To strengthen the body's defence against oxidative stress, selenium aids in activating these antioxidant enzymes (137). The formation of glutathione, a potent antioxidant and detoxifying molecule, requires selenium. Glutathione is a key component of cellular antioxidant defence mechanisms and aids in the reduction of oxidative stress. Selenium shortage might hinder the production of glutathione, thereby aggravating the oxidative stress brought on by an imbalance in adrenal hormones. The generation and function of glutathione are supported by adequate selenium levels, promoting antioxidant defence (138). Unbalanced adrenal hormones can impact immunological performance and perhaps exacerbate oxidative damage. Immunomodulatory characteristics of selenium are well recognized, and it supports healthy immune system operation. A healthy immune system can better manage oxidative stress and lessen its harmful effects (139). Thyroid hormone metabolism, which is closely related to the control of adrenal hormones, involves selenium. The thyroid and hormonal equilibrium in the body may be supported by maintaining healthy selenium levels. Selenium may indirectly help to reduce the oxidative stress brought on by the imbalance of adrenal hormones by encouraging hormonal equilibrium (140).

Endocrine components of the "fight or flight" stress response include the hypothalamic-pituitary-adrenal (HPA) axis (Figure 4) (141). Corticotropin-releasing hormone (CRH) from the hypothalamus causes the anterior pituitary to release ACTH in response to stress. The production of corticosteroids, including the glucocorticoid class of stress hormones, is then triggered by ACTH acting on the adrenal gland. Almost every tissue in the body contains the GC receptor (GCR) (142). Because of their ability to reduce inflammation, GCs are frequently given for a wide range of ailments and diseases (143). Selenium appears to have a significant protective effect against the harm and dysfunction brought on by excessive activation of the HPA axis. Our research group has conducted a recent assessment of the progress made in investigating the relationship that has been extensively studied in the brain using rodent models in recent years (144).

A comprehensive investigation on porcine subjects has yielded noteworthy findings about the impact of selenium insufficiency on the antioxidant capacity and subsequent induction of oxidative stress within adrenal tissue. This phenomenon has been observed to occur through the mediation of the toll-like receptor 4 (TLR4)/NF-kB pathway. This observation contributes to the expanding body of evidence regarding the potential involvement of selenium in modulating the physiological functions of the adrenal gland (145). The observed correlation between selenium deficiency and reduced levels of miR-30d-R_1, a microRNA (miRNA) known for its inhibitory effect on TLR4 expression, implies a potential link between the dysregulation of the TLR4/NF-kB pathway and the onset of inflammatory processes (146). It is noteworthy to observe that the overexpression of TLR4 in human adrenocortical cells resulted in a reduction in the production of cortisol and aldosterone (147). Consequently, selenium has the potential to facilitate the functioning of the HPA axis through its ability to induce a mechanism of downregulation of TLR4 miRNA, thereby promoting the synthesis of adrenal steroids. The observed phenomenon of reduced corticosterone secretion due to selenium deficiency can be attributed to the blunting of the adrenal response to ACTH (139). The involvement of selenoproteins in the development of the HPA axis represents a captivating correlation between selenium and the physiological reaction to stress. During the developmental process of neuroendocrine cells, an intriguing observation was made about activating the Selenot gene in the adrenal medulla (148).

6.5 Zinc against adrenal hormone imbalance-associated oxidative stress

The immune system, cell division, and growth are just a few of the basic activities in the body that zinc is crucial for. Despite its relative

lack of recognition as an antioxidant, zinc exhibits antioxidant properties and plays a crucial role in bolstering the body's overarching antioxidant defense mechanisms (149). By scavenging damaging free radicals, zinc functions as an antioxidant to help protect cells from oxidative stress. Free radicals are unstable molecules that can harm cells and speed up the aging process. They also have a role in several disorders. Zinc is an antioxidant that helps to stabilize these free radicals and stop them from doing any harm. In addition to having antioxidant qualities, zinc also helps several enzymes involved in antioxidant defence systems function. It is an essential part of the enzyme SOD, which assists in converting superoxide radicals into less dangerous molecules (150). Additionally, zinc helps in the production of metallothionein, a protein that helps control metal levels and protects against oxidative damage. Enriching zinc consumption is crucial for the body to have a healthy antioxidant system. Oysters, red meat, chicken, beans, nuts, and whole grains are healthy food sources of zinc. Additional zinc supplements are available, which may be advantageous for people with specific health issues or zinc deficiency (151).

Zinc's antioxidant capabilities and involvement in hormonal balance may have implications for lowering oxidative stress, even though there is little direct study on its effects on adrenal hormone imbalance-related oxidative damage (150).

Superoxide dismutase and catalase are two antioxidant enzymes that utilize zinc as a cofactor. These enzymes aid in reducing oxidative stress and neutralizing reactive oxygen species (ROS). Unbalanced levels of adrenal hormones can increase the generation of ROS, which increases the risk of oxidative damage. The body's defence against oxidative stress is aided by zinc because of its role in the functioning of antioxidant enzymes (152). The synthesis, secretion, and metabolism of numerous hormones, particularly adrenal hormones, depend heavily on zinc. The proper production and control of these hormones can be hampered by an imbalance in the adrenal hormones. Zinc may indirectly lessen the oxidative stress brought on by an imbalance in adrenal hormones by promoting hormonal equilibrium (153). Unbalanced levels of adrenal hormones might affect how well the immune system works, thereby increasing oxidative stress. Zinc is important for immune system health and influences the growth and functioning of immune cells. A healthy immune system can better manage oxidative stress and lessen its harmful effects (154). Zinc aids in DNA repair processes and promotes healthy cellular operation. DNA can be harmed by oxidative stress, which can also harm other biological components. Zinc's role in DNA repair promotes cellular health by preserving the integrity of genetic information and minimizing damage brought on by oxidative stress (155). Inflammation, which is strongly related to oxidative stress, can be brought on by a chronic adrenal hormone imbalance. The anti-inflammatory qualities of zinc make it possible to control the inflammatory response. Zinc may indirectly help lower oxidative stress levels linked to an imbalance in adrenal hormones by reducing inflammation (156).

Additionally, research has shown that cortisol impacts micronutrient metabolism, particularly magnesium, zinc, and selenium. Cortisol increases the expression of genes for metallothionein and ZIP-14, which accumulate zinc in the liver and adipose tissue, promoting hypozincemia in obese people (157).

Morais et al. (158) performed a correlation analysis to determine if cortisol affected zinc, magnesium, and selenium homeostasis in study participants. The plasma and erythrocyte zinc levels did not correlate with urine cortisol levels. Cortisol/cortisone ratio and erythrocyte zinc levels also correlated negatively. This study found hypozincemia in obese women due to elevated cortisol levels, which promote the production of metallothionein and ZIP-14 genes.

6.6 Polyphenols against adrenal hormone imbalance-associated oxidative stress

A class of naturally occurring substances called polyphenols can be found in a wide range of plant-based foods, such as fruits, vegetables, whole grains, herbs, and spices. Since they are recognized for having antioxidant capabilities, they can aid in preventing free radical damage to the body's cells. Polyphenols are dietary antioxidants important for preserving general health and preventing chronic disorders (159).

By scavenging free radicals, polyphenols function as powerful antioxidants. Free radicals, characterized by their inherent instability, possess the capacity to inflict cellular damage and contribute significantly to the pathogenesis of various medical conditions, including but not limited to cancer, cardiovascular illnesses, and neurodegenerative disorders (160). By scavenging free radicals, polyphenols function as powerful antioxidants. Free radicals are unsteady molecules that can harm cells and play a role in the emergence of a number of illnesses, such as cancer, heart disease, and neurological disorders (161). Polyphenol-rich diets may have several positive health effects. Reducing inflammation, preventing cardiovascular disease, promoting brain health, boosting immunological function, and maybe lowering the risk of some cancers are a few of these (160). Inflammatory pathways in the body have been proven to be modulated by polyphenols, which help to lessen chronic inflammation, which is linked to a number of diseases like heart disease, obesity, and some types of cancer. Polyphenols may have biological effects besides their antioxidant action, such as encouraging good gut bacteria, enhancing blood sugar regulation, and promoting healthy aging processes (161).

Fruits, vegetables, tea, coffee, and cocoa are just a few examples of plant-based meals rich in polyphenols, a broad set of substances. Due to their anti-inflammatory and antioxidant capabilities, which may help lower oxidative stress and enhance general health, they have attracted much interest. While there is little direct evidence on how polyphenols affect the oxidative stress brought on by adrenal hormone imbalance, their antioxidant and anti-inflammatory properties may have implications for reducing oxidative stress (162).

Strong antioxidants, polyphenols can trap and deactivate free radicals and reactive oxygen species (ROS) produced by oxidative stress. Unbalanced levels of adrenal hormones can increase the generation of ROS, which increases the risk of oxidative damage. By quenching these detrimental chemicals and shielding cells from oxidative damage, polyphenols can help decrease oxidative stress (160). Inflammation, which is strongly related to oxidative stress, can be brought on by a chronic adrenal hormone imbalance. The

inflammatory response can be modulated by polyphenols, which have anti-inflammatory effects. Polyphenols may indirectly help lower oxidative stress levels linked to an imbalance in adrenal hormones by reducing inflammation (163). Polyphenols have been demonstrated to alter the body's signalling systems and hormone levels. Polyphenols may have indirect impacts on hormone control even though their direct effects on adrenal hormones are not fully understood. Polyphenols may potentially assist in lowering oxidative stress linked to adrenal hormone imbalance by supporting hormonal equilibrium (164). Unbalanced levels of the adrenal hormones can cause mitochondria to malfunction and produce more ROS. It has been demonstrated that polyphenols enhance mitochondrial function and defend them against oxidative damage. Polyphenols support cellular health by protecting mitochondrial health and lowering oxidative stress (163). Superoxide dismutase (SOD) and catalase are two examples of endogenous antioxidant enzymes that can be stimulated by polyphenols. These enzymes are essential for reducing ROS and preserving redox equilibrium. Polyphenols may offer extra defence against oxidative stress related to an imbalance in adrenal hormones by increasing the activity of these antioxidant enzymes (165).

Polyphenols activate the redox-sensitive transcription factor nuclear factor erythroid 2-related factor-2 (Nrf2) (166). Contrarily, research suggests that polyphenols can modify the glucocorticoid receptor's (GR) activity. In fact, GR and FK506 binding protein 5 (FKBP5) expression can be changed by the polyphenolic flavonoid icariin, which enhances GR stability and lessens GR sensitivity to GC *in vivo* (167). More research is required in this area since manipulation of the GR regulatory system is currently an intriguing target for the treatment of stress-related illnesses (168).

6.7 Coenzyme Q10 against adrenal hormone imbalance-associated oxidative stress

A naturally occurring substance in the body is coenzyme Q10 (CoQ10). CoQ10 plays a pivotal role in generating adenosine triphosphate (ATP), which serves as the primary energy source for cells. Additionally, CoQ10 performs the role of an antioxidant, assisting in preventing cell deterioration brought on by harmful molecules known as free radicals. CoQ10 acts as an antioxidant to combat free radicals and stop oxidative stress, which can cause cellular damage and be a factor in several health issues (169). In order to stabilize free radicals and lessen their potential for harm, it donates electrons. Small levels of coenzyme Q10 are included in some meals, including meat, fish, and whole grains. However, CoQ10 production by the body tends to decrease with aging, and some diseases or drugs might further lower its levels. CoQ10 supplements are therefore offered to support optimal levels of this substance. CoQ10 has been researched for its possible health advantages in several illnesses, even though it is primarily known for its role in energy production and as an antioxidant (170).

Despite the limited body of research investigating the specific impact of CoQ10 on oxidative stress resulting from imbalances in adrenal hormones, it's inherent antioxidant properties and involvement in cellular energy metabolism suggest potential efficacy in mitigating oxidative stress (171).

CoQ10 is a powerful antioxidant that protects cells from the damage that free radicals and reactive oxygen species (ROS) cause when they combine with oxygen. Dysregulation of adrenal hormones can lead to increased production of ROS, hence contributing to oxidative stress. CoQ10 helps eliminate these harmful molecules, which lowers oxidative stress and keeps the health of cells (172). CoQ10 is an important part of the electron transport chain, which is a process that helps cells make energy (ATP). A lack of adrenal hormones can change how cells use energy, which can increase oxidative stress. CoQ10 may help restore cellular balance and lower oxidative stress by helping cells make energy more efficiently (173). CoQ10 can make antioxidants like vitamin E, vitamin C, and glutathione, which are important parts of the body's antioxidant defence system, from scratch. By regenerating and reusing these antioxidants, CoQ10 makes them more effective at fighting oxidative stress caused by an imbalance in adrenal hormones (174). A lack of adrenal hormones that lasts for a long time can cause inflammation, which is linked to oxidative stress. CoQ10 has anti-inflammatory qualities and can help control the way the body reacts to inflammation. CoQ10 may indirectly help reduce oxidative stress by reducing inflammation (51). CoQ10 keeps cell parts, like cell walls and mitochondria, from getting damaged by oxidation. Unbalanced adrenal hormones can cause oxidative stress in cells, which can damage the structures of cells. CoQ10 helps keep cell walls and mitochondria working well, which reduces damage caused by oxidative stress (175).

When it comes to disorders with the pituitary and adrenal glands, there is proof of mitochondrial dysfunction in people with Cushing's syndrome. This is shown in respiratory chain complex enzyme activity (176) and oxidative stress (measured by total antioxidant capacity and plasma 15-F2t-isoprostane) (177). However, there haven't been many studies on how CoQ10 affects pituitary and adrenal function in endocrine therapy. Some pituitary/adrenal problems may be associated with low levels of CoQ10 in the blood, according to preliminary investigations (178). Plasma CoQ10 levels were evaluated in six patients with ACTH-dependent adrenal hyperplasia, 19 with secondary solitary hypoadrenalism, and 19 with concurrent hypothyroidism (multiple pituitary deficits). Compared to numerous pituitary deficits and ACTH-dependent adrenal hyperplasia, CoQ10 levels were considerably lower in secondary isolated hypoadrenalism. Patients with acromegaly apparently have lower plasma CoQ10 levels (179).

6.8 Probiotics against adrenal hormone imbalance-associated oxidative stress

Typically, probiotics are not regarded as a direct source of antioxidants. However, some probiotic strains have been demonstrated to have indirect antioxidant benefits or to be able to improve the body's antioxidant state via a variety of mechanisms (180).

A healthy, diversified gut flora, which is important for the digestion of food components, can be maintained with the use of

probiotics. Some helpful bacteria in the stomach can accelerate the breakdown and creation of bioactive molecules with antioxidant capabilities from dietary antioxidants, such as polyphenols found in fruits and vegetables. The availability and potency of antioxidants in the body may increase as a result of this metabolic activity (181). It has been demonstrated that probiotics can indirectly lower oxidative stress by enhancing gut health and lowering inflammation. Probiotics can assist in maintaining gut barrier function, preventing the transfer of risky bacteria and endotoxins into the bloodstream, and reducing systemic inflammation by supporting a healthy gut microbiota. This can then support general antioxidant defence mechanisms and minimize oxidative stress (182). Probiotics can increase the body's natural synthesis and activity of endogenous antioxidants. For instance, it has been discovered that specific probiotic strains enhance the production of glutathione, a potent antioxidant that aids in neutralizing free radicals and the defence of cells against oxidative damage (180). Probiotics can interact with intestinal mucosal cells and other tissues to enhance their antioxidant defences. They can affect cellular signaling pathways that produce antioxidant enzymes like SOD and catalase, which remove free radicals and minimize oxidative stress (183).

Probiotics, known as beneficial microorganisms that promote a healthy gut microbiota, have exhibited promising potential in modulating the equilibrium of adrenal hormones and mitigating challenges associated with oxidative stress. The interaction between the gut microbiota and the adrenal glands is reciprocal, which means that each can have an impact on the other's operation. Dysbiosis, a condition characterized by perturbations in the composition and function of the gastrointestinal microbiota, has been implicated in various pathological states, encompassing abnormalities of adrenal hormone levels and elevated oxidative stress (184).

Probiotics support a balanced population of good bacteria in the gut, enhancing overall gut health (Figure 5). Better nutrient absorption, especially of antioxidants and other vital micronutrients necessary for preventing oxidative stress, is made possible by a healthy gut lining (185). Probiotic supplementation has been shown to exert a modulatory effect on the body's inflammatory and immunological responses. Chronic inflammation can disturb the equilibrium of the adrenal hormones and is linked to increased oxidative stress. Probiotics may indirectly aid adrenal gland function and lessen oxidative stress by lowering inflammation (186). In the gut, some probiotic strains can create antioxidants. For instance, it has been demonstrated that certain *Lactobacillus* and *Bifidobacterium* species produce antioxidants like glutathione, which can help mitigate oxidative stress and protect against cell damage (187). Probiotics can convert polyphenols present in fruits and vegetables, for example, into bioactive compounds with antioxidant properties. This transformation engenders heightened accessibility and potency of antioxidants, thereby fostering equilibrium in adrenal hormone levels and mitigating the impact of oxidative stress (180). Probiotics can influence the body's response to stress and help regulate cortisol levels. By promoting a balanced stress response, probiotics may assist in regulating adrenal hormone production and mitigating the harmful effects of stress-induced oxidative stress (188).

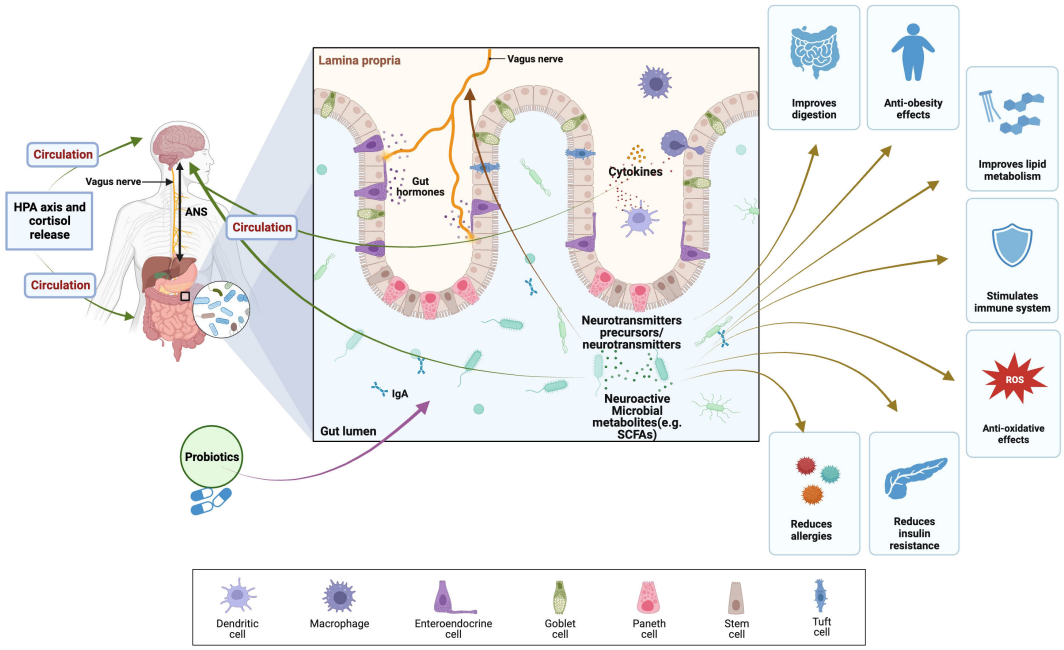


FIGURE 5
Health benefits of probiotics and their effects on brain, gut, and microbiome (BGM) axis modulating of HPA axis and cortisol release. The BGM axis network of routes that facilitate the exchange of information and signals encompasses neuronal elements (vagus nerve, neurotransmitters, and enteric nervous system), the HPA axis, and stress hormones like cortisol. Furthermore, immune mechanisms, specifically cytokines, contribute to this complex interplay. (SCFAs), Short-chain fatty acids; (ANS), autonomic nervous system; (ROS), reactive oxygen species; (HPA axis), Hypothalamic–pituitary–adrenal axis. The figure was produced with BioRender (Biorender.com; accessed on 30th Oct 2023).

Bidirectional connections within the brain-gut-microbiome (BGM) axis have been demonstrated in several preclinical and clinical research studies (189, 190). The communication between gut microbes and the central nervous system is facilitated through a complex network of interconnected channels, which encompass the nervous, endocrine, and immune signaling mechanisms. The brain possesses the capability to exert influence on the structural and functional organization of the gut microbiota. This influence is primarily mediated by the autonomic nervous system (ANS), which regulates various aspects, including gut permeability, regional gut motility, intestinal transit and secretion. The HPA axis operates under the fundamental mechanism of negative feedback and assumes a pivotal function in eliciting the body's stress response and governing various physiological processes, encompassing digestion, immune system functionality, and energy equilibrium (191). Stress hormones induce the disruption of tight junctions, consequently leading to increased permeability of the intestinal barrier (192). Dietary probiotic supplementation has been shown to offer potential alleviation of the HPA axis response to acute stress (193). For example, the administration of a probiotic treatment containing *L. farciminis* in a murine model has been reported to effectively mitigate the stress-induced hyperpermeability, endotoxemia, and, thus, ameliorating the stress response of the HPA axis.

Moreover, recent research findings have indicated that using probiotics and prebiotics, which serve as agents that regulate the composition of the gastrointestinal microbiota, may offer potential advantages in mitigating the manifestations of stress-related infertility (194). The beneficial effects of probiotics on infertility associated with stress have been attributed to various mechanisms, such as regulating the HPA axis, modulation of the immune response, and restoring microbial homeostasis (184). The HPA axis plays a pivotal role in maintaining reproductive health by governing the intricate regulation of the stress response. The HPA axis can be disrupted by stress, resulting in variations in cortisol levels, the primary stress hormone (49). Multiple studies have provided evidence indicating that probiotics possess the ability to modulate the HPA axis and reduce cortisol levels, thereby alleviating the adverse impact of stress on reproductive health (194). Under stressful circumstances, *Lactobacillus casei* strain Shirota (LcS) may prevent cortisol hypersecretion and physical symptoms, possibly through reducing stress reactivity in the paraventricular nucleus (PVN) and vagal afferent signaling to the brain (195). According to Nasri et al. (196), selenium and probiotic co-administration to women with polycystic ovary syndrome (PCOS) reduced modified Ferriman Gallwey (mF-G) scores and total testosterone.

The role of nutritional antioxidants in alleviating adrenal hormone imbalance is depicted in (Figure 6) (Table 2).

7 Search strategy

This review paper's search strategy included a thorough study of scientific databases such as PubMed, Scopus, Google Scholar, and Web of Science, utilizing a combination of keywords and controlled

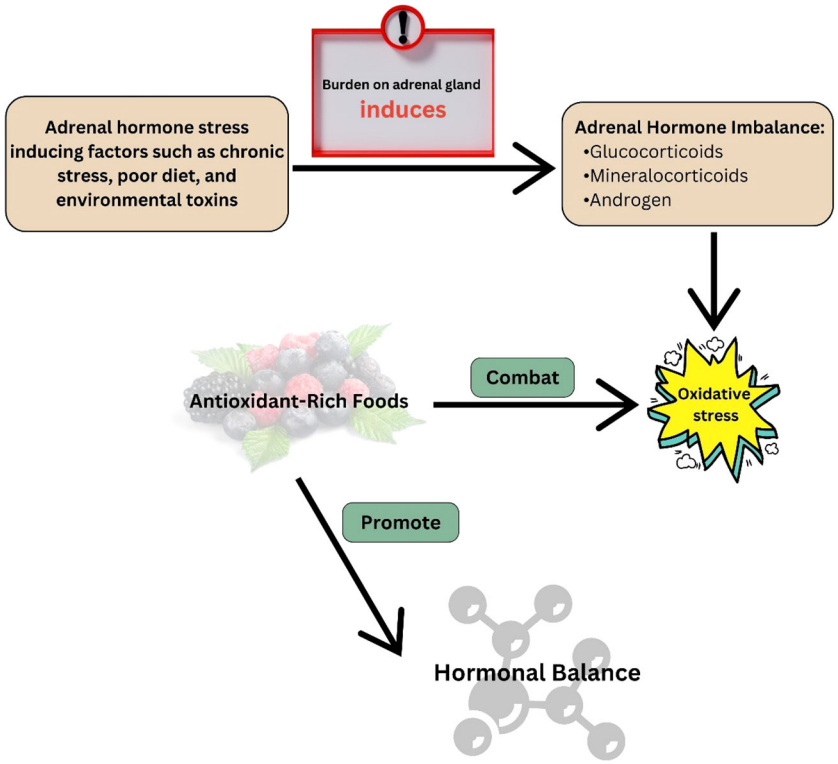


FIGURE 6
Role of nutritional antioxidants in alleviating adrenal hormone imbalance.

10.3389/fendo.2023.1271521

TABLE 2 The role of nutritional antioxidants in Adrenal hormone function.

Nutritional Antioxidant	Role in Adrenal Hormone Function	Reference
Vitamin C	- Supports the production of androgens, glucocorticoids, and mineralocorticoids - Serves as a cofactor in the process by which cholesterol is transformed into pregnenolone	(103)
Vitamin E	- Protects adrenal cells from oxidative stress - Possibly plays a function in regulating cortisol levels	(197)
Carotenoids	- Carotenoids contained in several foods, beta-carotene and lycopene, act as antioxidants - Aiding in the reduction of oxidative stress in the adrenal glands	(10)
Selenium	Important in the synthesis of selenoproteins such as glutathione peroxidase, which protects adrenal cells from oxidative damage.	(144)
Zinc	- Zinc is required for the synthesis, release, and general function of adrenal hormones - As an antioxidant, it protects cells from oxidative stress.	(198)
Polyphenols	Reduce oxidative damage and inflammation in the adrenal glands to help with adrenal hormone balance.	(161)
Coenzyme Q10	- Plays a critical function in the cellular energy production process - Supports the overall function of the adrenal glands and may lessen oxidative stress.	(179)
Probiotics	- Indirectly altering adrenal hormone balance and encouraging optimal function by mitigating oxidative stress and inflammation.	(199)

vocabulary terms. The most commonly used terms were "oxidative stress," "oxidative stress," "nutritional antioxidants," "reactive oxygen species," and "adrenal hormone imbalance." The search was refined using Boolean operators (AND, OR) to ensure relevance to the review's focus on the potential of nutritional antioxidants against oxidative stress linked with adrenal hormone imbalance. Furthermore, specific terms relating to adrenal gland function, such as "glucocorticoids," "mineralocorticoids," "androgens," and "adrenal hormone disorders" such as "adrenal insufficiency," "Cushing's syndrome," and "adrenal tumors," were added to collect relevant literature. The search included experimental and clinical investigations, as well as review papers, to provide a full picture of the current level of knowledge on the topic. The inclusion criteria included publications published in the previous decade, and the search approach was iterative, with continual refinement based on the identified literature until a thorough selection of relevant studies was obtained.

8 Conclusions

In conclusion, the effectiveness of nutritional antioxidants in combating oxidative stress caused by adrenal hormone imbalance is undeniable. Antioxidants, such as vitamin C, vitamin E, carotenoids, selenium, zinc, polyphenols, coenzyme Q10, and probiotics, play vital roles in mitigating the negative effects of oxidative stress on adrenal hormone balance. By mitigating dangerous free radicals and reducing oxidative damage, these antioxidants can aid in the restoration and maintenance of adrenal hormone function. In addition, the article discusses adrenal hormone abnormalities such as adrenal insufficiency, Cushing's syndrome, and adrenal tumors. The findings imply that utilizing the efficacy of dietary antioxidants may offer therapeutic approaches for alleviating oxidative stress associated with adrenal hormone abnormalities, opening up new areas for investigation.

Author contributions

AnP: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. DB: Data curation, Formal Analysis, Resources, Software, Writing – review & editing. VY: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. K-YL: Resources, Software, Supervision, Validation, Writing – review & editing. AsP: Formal Analysis, Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing. DS: Conceptualization, Formal Analysis, Funding acquisition, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Lotfi CFP, Kremer JL, Dos Santos Passaia B, Cavalcante IP. The human adrenal cortex: growth control and disorders. *Clinics* (2018) 73(suppl 1):e473s. doi: 10.6061/clinics/2018/e473s

2. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: harms and benefits for human health. *Oxid Med Cell Longev* (2017) 2017:8416763. doi: 10.1155/2017/8416763

3. Bjelaković G, Beninati S, Pavlović D, Kocić G, Jevtović T, Kamenov B, et al. Glucocorticoids and oxidative stress. *J Basic Clin PhysiolPharmacol* (2007) 18(2):115–27. doi: 10.1515/jbcpp.2007.18.2.115

4. Vona R, Pallotta L, Cappelletti M, Severi C, Matarrese P. The impact of oxidative stress in human pathology: focus on gastrointestinal disorders. *Antioxidants* (2021) 10 (2):201. doi: 10.3390/antiox10020201

5. Roychoudhury S, Chakraborty S, Choudhury AP, Das A, Jha NK, Slama P, et al. Environmental factors-induced oxidative stress: hormonal and molecular pathway disruptions in hypogonadism and erectile dysfunction. *Antioxidants* (2021) 10(6):837. doi: 10.3390/antiox10060837

6. Madison A, Kiecolt-Glaser JK. Stress, depression, diet, and the gut microbiota: human-bacteria interactions at the core of psychoneuroimmunology and nutrition. *Curr Opin Behav Sci* (2019) 28:105–10. doi: 10.1016/j.cobeha.2019.01.011

7. Du J, Wang Y, Hunter R, Wei Y, Blumenthal R, Falke C, et al. Dynamic regulation of mitochondrial function by glucocorticoids. *Proc Natl Acad Sci U.S.A.* (2009) 106 (9):3543–8. doi: 10.1073/pnas.0812671106

8. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* (2017) 9(6):7204–18. doi: 10.18632/oncotarget.23208

9. Salvador J, Gutierrez G, Llavero M, Gargallo J, Escalada J, López J. Endocrine disorders and psychiatric manifestations. In: Portincasa P, Frühbeck G, Nathoe HM, editors. *Endocrinology and Systemic Diseases. Endocrinology*. Cham: Springer (2019). p. 311–45. doi: 10.1007/978-3-319-66362-3_12-1

10. Rasmus P, Kozłowska E. Antioxidant and anti-inflammatory effects of carotenoids in mood disorders: an overview. *Antioxidants* (2023) 12(3):676. doi: 10.3390/antiox12030676

11. Pérez-Torres I, Castrejón-Téllez V, Soto ME, Rubio-Ruiz ME, Manzano-Pech L, Guarner-Lans V. Oxidative stress, plant natural antioxidants, and obesity. *Int J Mol Sci* (2021) 22(4):1786. doi: 10.3390/ijms22041786

12. Gligorijevic N, Kaljevic M, Radovanovic N, Jovanovic F, Joksimovic B, Singh S, et al. Adrenal abscesses: A systematic review of the literature. *J Clin Med* (2023) 12 (14):4601. doi: 10.3390/jcm12144601

13. Dumontet T, Martinez A. Adrenal androgens, adrenarche, and zona reticularis: A human affair? *Mol Cell Endocrinol* (2021) 528:111239. doi: 10.1016/j.mce.2021.111239

14. Gordan R, Gwathmey JK, Xie LH. Autonomic and endocrine control of cardiovascular function. *World J Cardiol* (2015) 7(4):204–14. doi: 10.4330/wjc.v7.i4.204

15. Bennett JM, Reeves G, Billman GE, Sturmberg JP. Inflammation-nature's way to efficiently respond to all types of challenges: implications for understanding and managing "the epidemic" of chronic diseases. *Front Med (Lausanne)* (2018) 5:316. doi: 10.3389/fmed.2018.00316

16. Tsilosani A, Gao C, Zhang W. Aldosterone-regulated sodium transport and blood pressure. *Front Physiol* (2022) 13:770375. doi: 10.3389/fphys.2022.770375

17. Stamou MI, Colling C, Dichtel LE. Adrenal aging and its effects on the stress response and immunosenescence. *Maturitas* (2023) 168:13–9. doi: 10.1016/j.maturitas.2022.10.006

18. Turcu A, Smith JM, Auchus R, Rainey WE. Adrenal androgens and androgen precursors-definition, synthesis, regulation and physiologic actions. *Compr Physiol* (2014) 4(4):1369–81. doi: 10.1002/cphy.c140006

19. Pignatti E, Flück CE. Adrenal cortex development and related disorders leading to adrenal insufficiency. *Mol Cell Endocrinol* (2021) 527:111206. doi: 10.1016/j.mce.2021.111206

20. Bouillon R. Acute adrenal insufficiency. *Endocrinol Metab Clin* (2006) 35 (4):767–75. doi: 10.1016/j.ecl.2006.09.004

21. Husebye ES, Pearce SH, Krone NP, Kämpe O. Adrenal insufficiency. *Lancet* (2021) 397(10274):613–29. doi: 10.1016/S0140-6736(21)00136-7

22. Nieman LK, Chanco Turner ML. Addison's disease. *Clin Dermatol* (2006) 24 (4):276–80. doi: 10.1016/j.clindermatol.2006.04.006

23. Salvatori R. Adrenal insufficiency. *JAMA* (2005) 294(19):2481–8. doi: 10.1001/jama.294.19.2481

24. Newell-Price J, Bertagna X, Grossman AB, Nieman LK. Cushing's syndrome. *Lancet* (2006) 367(9522):1605–17. doi: 10.1016/S0140-6736(06)68699-6

25. Lacroix A, Feelders RA, Stratakis CA, Nieman LK. Cushing's syndrome. *Lancet* (2015) 386(9996):913–27. doi: 10.1016/S0140-6736(14)61375-1

26. Pivonello R, De Martino MC, De Leo M, Lombardi G, Colao A. Cushing's syndrome. *Endocrinol Metab Clin North Am* (2008) 37(1):135–49. doi: 10.1016/j.ecl.2007.10.010

27. Kirk LF Jr., Hash RB, Katner HP, Jones T. Cushing's disease: clinical manifestations and diagnostic evaluation. *Am Fam Physician* (2000) 62(5):1119–27.

28. Fassnacht M, Arlt W, Bancos I, Dralle H, Newell-Price J, Sahdev A, et al. Management of adrenal incidentalomas: European Society of Endocrinology Clinical Practice Guideline in collaboration with the European Network for the Study of Adrenal Tumors. *Eur J Endocrinol* (2016) 175(2):G1–G34. doi: 10.1530/EJE-16-0467

29. Vaduva P, Bonnet F, Bertherat J. Molecular basis of primary aldosteronism and adrenal cushing syndrome. *J Endocr Soc* (2020) 4(9):bvaa075. doi: 10.1210/jendso/bvaa075

30. Else T, Kim AC, Sabolch A, Raymond VM, Kandathil A, Caoili EM, et al. Adrenocortical carcinoma. *Endocr Rev* (2014) 35(2):282–326. doi: 10.1210/er.2013-1029

31. Bancos I, Hahner S, Tomlinson J, Arlt W. Diagnosis and management of adrenal insufficiency. *Lancet Diabetes Endocrinol* (2015) 3(3):216–26. doi: 10.1016/S2213-8587(14)70142-1

32. Corkery-Hayward M, Metherell LA. Adrenal dysfunction in mitochondrial diseases. *Int J Mol Sci* (2023) 24(2):1126. doi: 10.3390/ijms24021126

33. Prasad R, Kowalczyk JC, Meimaridou E, Storr HL, Metherell LA. Oxidative stress and adrenocortical insufficiency. *J Endocrinol* (2014) 221(3):R63–73. doi: 10.1530/JOE-13-0346

34. Kochman J, Jakubczyk K. The influence of oxidative stress on thyroid diseases. *Antioxidants* (2021) 10:1442. doi: 10.3390/antiox10091442

35. Prajapati D, Patani A, Jain T, Patel A, Singh S. ROS responsive silica nanoparticles for controlled and targeted drug delivery. In: Chawla S, Singh S, Husen A, editors. *Smart Nanomaterials Targeting Pathological Hypoxia. Smart Nanomaterials Technology*. Singapore: Springer (2023). p. 327–46. doi: 10.1007/978-981-99-1718-1_17

36. Macvanin MT, Gluvic Z, Zafirovic S, Gao X, Essack M, Isenovic ER. The protective role of nutritional antioxidants against oxidative stress in thyroid disorders. *Front Endocrinol* (2023) 13:1092837. doi: 10.3389/fendo.2022.1092837

37. Mangge H, Becker K, Fuchs D, Gostner JM. Antioxidants, inflammation and cardiovascular disease. *World J Cardiol* (2014) 6:462–77. doi: 10.4330/wjc.v6.i6.462

38. Bardaweel SK, Gul M, Alzweiri M, Ishaqat A, ALSalamat HA, Bashatwah RM. Reactive oxygen species: the dual role in physiological and pathological conditions of the human body. *Eurasian J Med* (2018) 50(3):193–201. doi: 10.5152/eurasianjmed.2018.17397

39. Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, et al. Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Front Physiol* (2020) 11:694. doi: 10.3389/fphys.2020.00694

40. Singh A, Kukreti R, Saso L, Kukreti S. Oxidative stress: A key modulator in neurodegenerative diseases. *Molecules* (2019) 24(8):1583. doi: 10.3390/molecules24081583

41. Liu Z, Ren Z, Zhang J, Chuang CC, Kandaswamy E, Zhou T, et al. Role of ROS and nutritional antioxidants in human diseases. *Front Physiol* (2018) 9:477. doi: 10.3389/fphys.2018.00477

42. Lehrer S, Rheinstein PH. Alzheimer's disease and parkinson's disease may result from reactivation of embryologic pathways silenced at birth. *Discov Med* (2021) 31 (163):89–94.

43. Turrens JF, Boveris A. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. *Biochem J* (1980) 191:421–7. doi: 10.1042/bj1910421

44. Zimmermann MB, Aeberli I. Dietary determinants of subclinical inflammation, dyslipidemia and components of the metabolic syndrome in overweight children: a review. *Int J Obes (Lond)* (2008) 32 Suppl 6:S11–18. doi: 10.1038/ijo.2008.202

45. Mancini A, Di Segni C. Thyroid hormones, oxidative stress, and inflammation. *Mediators Inflammation* (2016) 2016:6757154. doi: 10.1155/2016/6757154

46. Siti HN, Kamisah Y, Kamsiah J. The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review). *VasculPharmacol* (2015) 71:40–56. doi: 10.1016/j.vph.2015.03.005

47. Tan BL, Norhaizan ME, Liew WP, Sulaiman Rahman H. Antioxidant and oxidative stress: A mutual interplay in age-related diseases. *Front Pharmacol* (2018) 9:1162. doi: 10.3389/fphar.2018.01162

48. Patani A, Patel A, Prajapati D, Khare N, Singh S. Various agriculture crop plant-based bioactive compounds and their use in nanomaterial synthesis and applications. In: Husen A, editor. *Nanomaterials from Agricultural and Horticultural Products. Smart Nanomaterials Technology*. Singapore: Springer (2023). p. 323–241. doi: 10.1007/978-981-99-3435-5_12

49. Hinds JA, Sanchez ER. The role of the hypothalamus–pituitary–adrenal (HPA) axis in test-induced anxiety: assessments, physiological responses, and molecular details. *Stresses* (2022) 2(1):146–55. doi: 10.3390/stresses2010011

50. Hannibal KE, Bishop MD. Chronic stress, cortisol dysfunction, and pain: a psychoneuroendocrine rationale for stress management in pain rehabilitation. *Phys Ther* (2014) 94(12):1816–25. doi: 10.2522/ptj.20130597

51. Juszczyk G, Mikulska J, Kasperek K, Pietrzak D, Mrozek W, Herbet M. Chronic stress and oxidative stress as common factors of the pathogenesis of depression and alzheimer's disease: the role of antioxidants in prevention and treatment. *Antioxidants (Basel)* (2021) 10(9):1439. doi: 10.3390/antiox10091439

52. Di Domenico M, Pinto F, Quagliuolo L, Contaldo M, Settembre G, Romano A, et al. The role of oxidative stress and hormones in controlling obesity. *Front Endocrinol (Lausanne)* (2019) 10:540. doi: 10.3389/fendo.2019.00540

53. Sahoo DK, Heilmann RM, Paital B, Patel A, Yadav VK, Wong D, et al. Oxidative stress, hormones, and effects of natural antioxidants on intestinal inflammation in inflammatory bowel disease. *Front Endocrinol (Lausanne)* (2023) 14:1217165. doi: 10.3389/fendo.2023.1217165

54. Herman JP, McKlveen JM, Ghosal S, Kopp B, Wulsin A, Makinson R, et al. Regulation of the hypothalamic-pituitary-adrenocortical stress response. *Compr Physiol* (2016) 6(2):603–21. doi: 10.1002/cphy.c150015

55. Sheng JA, Bales NJ, Myers SA, Bautista AI, Roueinfar M, Hale TM, et al. The hypothalamic-pituitary-adrenal axis: development, programming actions of hormones, and maternal-fetal interactions. *Front Behav Neuro Sci* (2021) 14:601939. doi: 10.3389/fnbeh.2020.601939

56. Ames MK, Atkins CE, Pitt B. The renin-angiotensin-aldosterone system and its suppression. *J Vet Intern Med* (2019) 33(2):363–82. doi: 10.1111/jvim

57. Traish A, Bolanos J, Nair S, Saad F, Morgentaler A. Do androgens modulate the pathophysiological pathways of inflammation? Appraising the contemporary evidence. *J Clin Med* (2018) 7(12):549. doi: 10.3390/jcm7120549

58. Sahoo DK, Chainy GBN. Hormone-linked redox status and its modulation by antioxidants. *VitamHorm* (2023) 121:197–246. doi: 10.1016/bs.vh.2022.10.007

59. Chainy GBN, Sahoo DK. Hormones and oxidative stress: an overview. *Free Radic Res* (2020) 54(1):1–26. doi: 10.1080/10715762.2019.1702656

60. Sugino N, Hirosawa-Takamori M, Zhong L, Telleria CM, Shiota K, Gibori G. Hormonal regulation of copper-zinc superoxide dismutase and manganese superoxide dismutase messenger ribonucleic acid in the rat corpus luteum: induction by prolactin and placental lactogens. *Biol Reprod* (1998) 59(3):599–605. doi: 10.1095/biolreprod59.3.599

61. Memarzia A, Khazdair MR, Behrouz S, Gholamnezhad Z, Jafarnezhad M, Saadat S, et al. Experimental and clinical reports on anti-inflammatory, antioxidant, and immunomodulatory effects of Curcuma longa and curcumin, an updated and comprehensive review. *Biofactors* (2021) 47(3):311–50. doi: 10.1002/biof.1716

62. Spiers JG, Chen HJ, Sernia C, Lavidis NA. Activation of the hypothalamic-pituitary-adrenal stress axis induces cellular oxidative stress. *Front Neurosci* (2015) 8:456. doi: 10.3389/fnins.2014.00456

63. Lee KH, Cha M, Lee BH. Neuroprotective effect of antioxidants in the brain. *Int J Mol Sci* (2020) 21(19):7152. doi: 10.3390/ijms21197152

64. Birnie-Gauvin K, Peiman KS, Larsen MH, Aarestrup K, Willmore WG, Cooke SJ. Short-term and long-term effects of transient exogenous cortisol manipulation on oxidative stress in juvenile brown trout. *J Exp Biol* (2017) 220(Pt 9):1693–700. doi: 10.1242/jeb.155465

65. Bisht S, Dada R. Oxidative stress: Major executioner in disease pathology, role in sperm DNA damage and preventive strategies. *Front Biosci (Schol Ed)* (2017) 9(3):420–47. doi: 10.2741/s495

66. Weinberg-Shukron A, Abu-Libdeh A, Zhadeh F, Carmel L, Kogot-Levin A, Kamal L, et al. Combined mineralocorticoid and glucocorticoid deficiency is caused by a novel founder nicotinamide nucleotide transhydrogenase mutation that alters mitochondrial morphology and increases oxidative stress. *J Med Genet* (2015) 52 (9):636–41. doi: 10.1136/jmedgenet-2015-103078

67. Mocan M, MocanHognogi LD, Anton FP, Chiorescu RM, Goidescu CM, Stoia MA, et al. Biomarkers of inflammation in left ventricular diastolic dysfunction. *Dis Markers* (2019) 2019:7583690. doi: 10.1155/2019/7583690

68. Lang F. On the pleotropic actions of mineralocorticoids. *Nephron Physiol* (2014) 128(1-2):1–7. doi: 10.1159/000368263

69. Ayuzawa N, Fujita T. The mineralocorticoid receptor in salt-sensitive hypertension and renal injury. *J Am Soc Nephrol* (2021) 32(2):279–89. doi: 10.1681/ASN.2020071041

70. Patni H, Mathew JT, Luan L, Franki N, Chander PN, Singhal PC. Aldosterone promotes proximal tubular cell apoptosis: role of oxidative stress. *Am J Physiol Renal Physiol* (2007) 293(4):F1065–71. doi: 10.1152/ajprenal.00147.2007

71. Martemucci G, Costagliola C, Mariano M, D'andrea L, Napolitano P, D'Alessandro AG. Free radical properties, source and targets, antioxidant consumption and health. *Oxygen* (2022) 2(2):48–78. doi: 10.3390/oxygen2020006

72. Tesch GH, Young MJ. Mineralocorticoid receptor signaling as a therapeutic target for renal and cardiac fibrosis. *Front Pharmacol* (2017) 8:313. doi: 10.3389/fphar.2017.00313

73. Prajapati DH, Patel MD, Mehta HH, Patel DA, Dave DP, Patel JR, et al. A review on patient diet management for chronic kidney disease based on clinical trials. *IJRESM* (2022) 5(11):174–81.

74. Su X, Wang S, Zhang H, Yang G, Bai Y, Liu P, et al. Sulforaphane prevents angiotensin II-induced cardiomyopathy by activation of Nrf2 through epigenetic modification. *J Cell Mol Med* (2021) 25(9):4408–19. doi: 10.1111/jcmm.16504

75. Brinks R, Wruck CJ, Schmitz J, Schupp N. Nrf2 activation does not protect from aldosterone-induced kidney damage in mice. *Antioxidants* (2023) 12(3):777. doi: 10.3390/antiox12030777

76. Ramya S, Poornima P, Jananisri A, Geofferina IP, Bavyataa V, Divya M, et al. Role of hormones and the potential impact of multiple stresses on infertility. *Stresses* (2023) 3(2):454–74. doi: 10.3390/stresses3020033

77. Imran M, Ghorat F, Ul-Haq I, Ur-Rehman H, Aslam F, Heydari M, et al. Lycopene as a natural antioxidant used to prevent human health disorders. *Antioxidants* (2020) 9(8):706. doi: 10.3390/antiox9080706

78. Yan W, Kang Y, Ji X, Li S, Li Y, Zhang G, et al. Testosterone upregulates the expression of mitochondrial ND1 and ND4 and alleviates the oxidative damage to the nigrostriatal dopaminergic system in orchiectomized rats. *Oxid Med Cell Longev* (2017) 2017:1202459. doi: 10.1155/2017/1202459

79. Witchel SF, Oberfield SE, Peña AS. Polycystic ovary syndrome: pathophysiology, presentation, and treatment with emphasis on adolescent girls. *J Endocr Soc* (2019) 3 (8):1545–73. doi: 10.1210/js.2019-00078

80. Tam NN, Gao Y, Leung YK, Ho SM. Androgenic regulation of oxidative stress in the rat prostate: involvement of NAD(P)H oxidases and antioxidant defense machinery during prostatic involution and regrowth. *Am J Pathol* (2003) 163(6):2513–22. doi: 10.1016/S0002-9440(10)63606-1

81. Ripple MO, Henry WF, Rago RP, Wilding G. Prooxidant-antioxidant shift induced by androgen treatment of human prostate carcinoma cells. *J Natl Cancer Inst* (1997) 89(1):40–8. doi: 10.1093/jnci/89.1.40

82. Prasad S, Kalra N, Singh M, Shukla Y. Protective effects of lupeol and mango extract against androgen induced oxidative stress in Swiss albino mice. *Asian J Androl* (2008) 10(2):313–8. doi: 10.1111/j.1745-7262.2008.00313.x

83. Argenziano M, Tiscornia G, Moretta R, Casal L, Potilinski C, Amorena C, et al. Arrhythmogenic effect of androgens on the rat heart. *J Physiol Sci* (2017) 67(1):217–25. doi: 10.1007/s12576-016-0459-y

84. Blanco-Rivero J, Sagredo A, Balfagón G, Ferrer M. Orchidectomy increases expression and activity of Cu/Zn-superoxide dismutase, while decreasing endothelial nitric oxide bioavailability. *J Endocrinol* (2006) 190(3):771–8. doi: 10.1677/joe.1.06887

85. Borst SE, Quindry JC, Yarrow JF, Conover CF, Powers SK. Testosterone administration induces protection against global myocardial ischemia. *HormMetab Res* (2010) 42(2):122–9. doi: 10.1055/s-0029-1241843

86. Kłapcińska B, Jagsz S, Sadowska-Krepa E, Górski J, Kempa K, Langfort J. Effects of castration and testosterone replacement on the antioxidant defense system in rat left ventricle. *J Physiol Sci* (2008) 58(3):173–7. doi: 10.2170/physiolsci.RP002208

87. Szabó R, Börzsei D, Kupai K, Hoffmann A, Gesztelyi R, MagyarinéBerkó A, et al. Spotlight on a new heme oxygenase pathway: testosterone-induced shifts in cardiac oxidant/antioxidant status. *Antioxidants* (2019) 8(8):288. doi: 10.3390/antiox8080288

88. Samaras N, Samaras D, Frangos E, Forster A, Philippe J. A review of age-related dehydroepiandrosterone decline and its association with well-known geriatric syndromes: is treatment beneficial? *Rejuvenation Res* (2013) 16(4):285–94. doi: 10.1089/rej.2013.1425

89. Powrie YSL, Smith C. Central intracrine DHEA synthesis in ageing-related neuroinflammation and neurodegeneration: therapeutic potential? *J Neuroinflamm* (2018) 15:289. doi: 10.1186/s12974-018-1324-0

90. Mohammadi M. Oxidative stress and polycystic ovary syndrome: A brief review. *Int J Prev Med* (2019) 10:86. doi: 10.4103/ijpvm.IJPVM_576_17

91. Shabbir S, Khurram E, Moorthi VS, Eissa YTH, Kamal MA, Butler AE. The interplay between androgens and the immune response in polycystic ovary syndrome. *J Transl Med* (2023) 21(1):259. doi: 10.1186/s12967-023-04116-4

92. González F, Sia CL, Shepard MK, Rote NS, Minium J. Hyperglycemia-induced oxidative stress is independent of excess abdominal adiposity in normal-weight women with polycystic ovary syndrome. *Hum Reprod* (2012) 27(12):3560–8. doi: 10.1093/humrep/des320

93. Hardeland R, Pandi-Perumal SR. Melatonin, a potent agent in antioxidative defense: actions as a natural food constituent, gastrointestinal factor, drug and prodrug. *NutrMetab (Lond)* (2005) 2:22. doi: 10.1186/1743-7075-2-22

94. Hemilä H. Vitamin C and infections. *Nutrients* (2017) 9(4):339. doi: 10.3390/nu9040339

95. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev* (2010) 4(8):118–26. doi: 10.4103/0973-7847.70902

96. Chambial S, Dwivedi S, Shukla KK, John PJ, Sharma P. Vitamin C in disease prevention and cure: an overview. *Indian J Clin Biochem* (2013) 28(4):314–28. doi: 10.1007/s12291-013-0375-3

97. Carr AC, Maggini S. Vitamin C and immune function. *Nutrients* (2017) 9(11):1211. doi: 10.3390/nu9111211

98. Piskin E, Cianciosi D, Gulec S, Tomas M, Capanoglu E. Iron absorption: factors, limitations, and improvement methods. *ACS Omega* (2022) 7(24):20441–56. doi: 10.1021/acsomega.2c01833

99. Jideani AI, Silungwe H, Takalani T, Omolola AO, Udeh HO, Anyasi TA. Antioxidant-rich natural fruit and vegetable products and human health. *Int J Food Prop* (2021) 24(1):41–67. doi: 10.1080/10942912.2020.1866597

100. Marik PE. Vitamin C: an essential "stress hormone" during sepsis. *J Thorac Dis* (2020) 12(Suppl 1):S84–8. doi: 10.21037/jtd.2019.12.64

101. Pullar JM, Carr AC, Vissers MCM. The roles of vitamin C in skin health. *Nutrients* (2017) 9(8):866. doi: 10.3390/nu9080866

102. Brody S. High-dose ascorbic acid increases intercourse frequency and improves mood: a randomized controlled clinical trial. *Biol Psychiatry* (2002) 52:371–4. doi: 10.1016/S0006-3223(02)01329-X

103. Patak P, Willenberg HS, Bornstein SR. Vitamin C is an important cofactor for both adrenal cortex and adrenal medulla. *Endocr Res* (2004) 30:871–5. doi: 10.1081/ERC-200044126

104. Jenkins JS. The effect of ascorbic acid on adrenal steroid synthesis in Vitro. *Endocrinology* (1962) 70:267–71. doi: 10.1210/endo-70-2-267

105. Moritz B, Schmitz AE, Rodrigues ALS, Dafre AL, Cunha MP. The role of vitamin C in stress-related disorders. *J NutrBiochem* (2020) 85:108459. doi: 10.1016/j.jnutbio.2020.108459

106. Shahidi F, Pinaffi-Langley ACC, Fuentes J, Speisky H, de Camargo AC. Vitamin E as an essential micronutrient for human health: Common, novel, and unexplored dietary sources. *Free Radic Biol Med* (2021) 176:312–21. doi: 10.1016/j.freeradbiomed.2021.09.025

107. Lee GY, Han SN. The role of vitamin E in immunity. *Nutrients* (2018) 10 (11):1614. doi: 10.3390/nu10111614

108. Ciarcià G, Bianchi S, Tomasello B, Acquaviva R, Malfa GA, Naletova I, et al. Vitamin E and non-communicable diseases: A review. *Biomedicines* (2022) 10 (10):2473. doi: 10.3390/biomedicines10102473

109. Pincemail J, Meziane S. On the potential role of the antioxidant couple vitamin E/selenium taken by the oral route in skin and hair health. *Antioxidants (Basel)* (2022) 11(11):2270. doi: 10.3390/antiox11112270

110. Sahoo DK, Roy A, Chainy GB. Protective effects of vitamin E and curcumin on L-thyroxine-induced rat testicular oxidative stress. *Chem Biol Interact* (2008) 176(2-3):121–8. doi: 10.1016/j.cbi.2008.07.009

111. Singh U, Devaraj S, Jialal I. Vitamin E, oxidative stress, and inflammation. *Annu Rev Nutr* (2005) 25:151–74. doi: 10.1146/annurev.nutr.24.012003.132446

112. Sun Y, Ma A, Li Y, Han X, Wang Q, Liang H. Vitamin E supplementation protects erythrocyte membranes from oxidative stress in healthy Chinese middle-aged and elderly people. *Nutr Res* (2012) 32(5):328–34. doi: 10.1016/j.nutres.2012.03.012

113. Dhama K, Latheef SK, Dadar M, Samad HA, Munjal A, Khandia R, et al. Biomarkers in stress related diseases/disorders: diagnostic, prognostic, and therapeutic values. *Front Mol Biosci* (2019) 6:91. doi: 10.3389/fmolb.2019.00091

114. Al-Sowayan NS. Possible modulation of nervous tension-induced oxidative stress by vitamin E. *Saudi J Biol Sci* (2020) 27(10):2563–6. doi: 10.1016/j.sjbs.2020.05.018

115. Ponnampalam EN, Kiani A, Santhiravel S, Holman BWB, Lauridsen C, Dunshea FR. The importance of dietary antioxidants on oxidative stress, meat and milk production, and their preservative aspects in farm animals: antioxidant action, animal health, and product quality—Invited review. *Animals* (2022) 12(23):3279. doi: 10.3390/ani12233279

116. Maoka T. Carotenoids as natural functional pigments. *J Nat Med* (2020) 74 (1):1–16. doi: 10.1007/s11418-019-01364-x

117. Swapnil P, Meena M, Singh SK, Dhuldhaj UP, Marwal A. Vital roles of carotenoids in plants and humans to deteriorate stress with its structure, biosynthesis, metabolic engineering and functional aspects. *Curr Plant Biol* (2021) 26:100203. doi: 10.1016/j.cpb.2021.100203

118. Black HS, Boehm F, Edge R, Truscott TG. The benefits and risks of certain dietary carotenoids that exhibit both anti- and pro-oxidative mechanisms-A comprehensive review. *Antioxidants (Basel)* (2020) 9(3):264. doi: 10.3390/antiox9030264

119. Mrowicka M, Mrowicki J, Kucharska E, Majsterek I. Lutein and zeaxanthin and their roles in age-related macular degeneration-neurodegenerative disease. *Nutrients* (2022) 14(4):827. doi: 10.3390/nu14040827

120. Crupi P, Faienza MF, Naeem MY, Corbo F, Clodoveo ML, Muraglia M. Overview of the potential beneficial effects of carotenoids on consumer health and well-being. *Antioxidants (Basel)* (2023) 12(5):1069. doi: 10.3390/antiox12051069

121. Saini RK, Prasad P, Lokesh V, Shang X, Shin J, Keum YS, et al. Carotenoids: dietary sources, extraction, encapsulation, bioavailability, and health benefits-A review of recent advancements. *Antioxidants (Basel)* (2022) 11(4):795. doi: 10.3390/antiox11040795

122. Elvira-Torales LI, García-Alonso J, Periago-Castón MJ. Nutritional importance of carotenoids and their effect on liver health: A review. *Antioxidants (Basel)* (2019) 8 (7):229. doi: 10.3390/antiox8070229

123. Kabir MT, Rahman MH, Shah M, Jamiruddin MR, Basak D, Al-Harrasi A, et al. Therapeutic promise of carotenoids as antioxidants and anti-inflammatory agents in neurodegenerative disorders. *BioMed Pharmacother* (2022) 146:112610. doi: 10.1016/j.biopha.2021.112610

124. Iddir M, Brito A, Dingeo G, Fernandez Del Campo SS, Samouda H, La Frano MR, et al. Strengthening the Immune System and Reducing Inflammation and Oxidative Stress through Diet and Nutrition: Considerations during the COVID-19 Crisis. *Nutrients* (2020) 12(6):1562. doi: 10.3390/nu12061562

125. Coulter AA, Greenway FL, Zhang D, Ghosh S, Coulter CR, James SL, et al. Naringenin and β-carotene convert human white adipocytes to a beige phenotype and elevate hormone- stimulated lipolysis. *Front Endocrinol (Lausanne)* (2023) 14:1148954. doi: 10.3389/fendo.2023.1148954

126. Darvin ME, Lademann J, von Hagen J, Lohan SB, Kolmar H, Meinke MC, et al. Carotenoids in human skin*In vivo*: antioxidant and photo-protectant role against external and internal stressors. *Antioxidants (Basel)* (2022) 11(8):1451. doi: 10.3390/antiox11081451

127. Bremner JD, Shearer KD, McCaffery PJ. Retinoic acid and affective disorders: The evidence for an association. *J Clin Psychiatry* (2012) 73:37–50. doi: 10.4088/JCP.10r05993

128. Ludot M, Mouchabac S, Ferreri F. Inter-relationships between isotretinoin treatment and psychiatric disorders: Depression, bipolar disorder, anxiety, psychosis and suicide risks. *World J Psychiatry* (2015) 5:222–7. doi: 10.5498/wjp.v5.i2.222

129. Oishi J, Doi H, Kawakami N. Nutrition and depressive symptoms in community-dwelling elderly persons in Japan. *Acta Med Okayama* (2009) 63:9–17. doi: 10.18926/AMO/31854

130. Xiao J, Khan MZ, Ma Y, Alugongo GM, Ma J, Chen T, et al. The antioxidant properties of selenium and vitamin E; their role in periparturient dairy cattle health regulation. *Antioxidants* (2021) 10(10):1555. doi: 10.3390/antiox10101555

131. Tinggi U. Selenium: its role as antioxidant in human health. *Environ Health Prev Med* (2008) 13(2):102–8. doi: 10.1007/s12199-007-0019-4

132. Avery JC, Hoffmann PR. Selenium, selenoproteins, and immunity. *Nutrients* (2018) 10(9):1203. doi: 10.3390/nu10091203

133. Gorini F, Sabatino L, Pingitore A, Vassalle C. Selenium: an element of life essential for thyroid function. *Molecules* (2021) 26(23):7084. doi: 10.3390/molecules26237084

134. Shalihat A, Hasanah AN, Mutakin, Lesmana R, Budiman A, Gozali D. The role of selenium in cell survival and its correlation with protective effects against cardiovascular disease: A literature review. *BioMed Pharmacother* (2021) 134:111125. doi: 10.1016/j.biopha.2020.111125

135. Kipp AP, Strohm D, Brigelius-Flohé R, Schomburg L, Bechthold A, Leschik-Bonnet E, et al. Revised reference values for selenium intake. *J Trace Elem Med Biol* (2015) 32:195–9. doi: 10.1016/j.jtemb.2015.07.005

136. Sahoo DK, Roy A, Bhanja S, Chainy GB. Hypothyroidism impairs antioxidant defence system and testicular physiology during development and maturation. *Gen Comp Endocrinol* (2008) 156(1):63–70. doi: 10.1016/j.ygcen.2007.11.007

137. Zoidis E, Seremelis I, Kontopoulos N, Danezis GP. Selenium-dependent antioxidant enzymes: actions and properties of selenoproteins. *Antioxidants (Basel)* (2018) 7(5):66. doi: 10.3390/antiox7050066

138. Andrade IGA, Suano-Souza FI, Fonseca FLA, Lago CSA, Sarni ROS. Selenium levels and glutathione peroxidase activity in patients with ataxia-telangiectasia: association with oxidative stress and lipid status biomarkers. *Orphanet J Rare Dis* (2021) 16(1):83. doi: 10.1186/s13023-021-01732-5

139. Toh P, Nicholson JL, Vetter AM, Berry MJ, Torres DJ. Selenium in bodily homeostasis: hypothalamus, hormones, and highways of communication. *Int J Mol Sci* (2022) 23(23):15445. doi: 10.3390/ijms232315445

140. Sahoo DK. Testicular protection from thyroid hormone mediated oxidative stress. *WebmedCentral Reprod* (2013) 4(5):WMC004252. doi: 10.9754/journal.wmc.2013.004252

141. McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev* (2007) 87:873–904. doi: 10.1152/physrev.00041.2006

142. Juszczak GR, Stankiewicz AM. Glucocorticoids, genes and brain function. *Prog Neuro-Psychopharmacol Biol Psychiatry* (2018) 82:136–68. doi: 10.1016/j.pnpbp.2017.11.020

143. Vandewalle J, Luypaert A, De Bosscher K, Libert C. Therapeutic mechanisms of glucocorticoids. *Trends Endocrinol Metab* (2018) 29:42–54. doi: 10.1016/j.tem.2017.10.010

144. Torres DJ, Alfulaij N, Berry MJ. Stress and the brain: an emerging role for selenium. *Front Neurosci* (2021) 15:666601. doi: 10.3389/fnins.2021.666601

145. Kaixin Z, Xuedie G, Jing L, Yiming Z, Khoso PA, Zhaoyi L, et al. Selenium-deficient diet induces inflammatory response in the pig adrenal glands by activating TLR4/NF-κB pathway *via* miR-30d-R. *Metallomics* (2021) 13:mfab037. doi: 10.1093/mtomcs/mfab037

146. Wang C, Zhang Y, Luo J, Ding H, Liu S, Amer S, et al. Identification of miRNomes reveals ssc-miR-30d-R_1 as a potential therapeutic target for PRRS viral infection. *Sci Rep* (2016) 6:24854. doi: 10.1038/srep24854

147. Lai F, Zhou G, Mai S, Qin X, Liu W, Zhang Y, et al. Sini decoction improves adrenal function and the short-term outcome of septic rats through downregulation of adrenal toll-like receptor 4 expression. *Evid Based ComplementAlternat Med* (2018) 2018:5186158. doi: 10.1155/2018/5186158

148. Abid H, Cartier D, Hamieh A, François-Bellan AM, Bucharles C, Pothion H, et al. AMPK activation of PGC-1α/NRF-1-dependent SELENOT gene transcription promotes PACAP-induced neuroendocrine cell differentiation through tolerance to oxidative stress. *Mol Neurobiol* (2018) 56:4086–101. doi: 10.1007/s12035-018-1352-x

149. Gombart AF, Pierre A, Maggini S. A review of micronutrients and the immune system–working in harmony to reduce the risk of infection. *Nutrients* (2020) 12(1):236. doi: 10.3390/nu12010236

150. Sahoo DK. Protocols for evaluating antioxidant defence and oxidative stress parameters in rat testis. *WebmedCentral Biochem* (2013) 4(5):WMC004265. doi: 10.9754/journal.wmc.2013.004265

151. Maywald M, Rink L. Zinc in human health and infectious diseases. *Biomolecules* (2022) 12(12):1748. doi: 10.3390/biom12121748

152. Lee SR. Critical role of zinc as either an antioxidant or a prooxidant in cellular systems. *Oxid Med Cell Longev* (2018) 2018:9156285. doi: 10.1155/2018/9156285

153. Baltaci AK, Mogulkoc R, Baltaci SB. Review: The role of zinc in the endocrine system. *Pak J Pharm Sci* (2019) 32(1):231–9.

154. Wu Q, Gao ZJ, Yu X, Wang P. Dietary regulation in health and disease. *Signal Transduct Target Ther* (2022) 7(1):252. doi: 10.1038/s41392-022-01104-w

155. Yildiz A, Kaya Y, Tanriverdi O. Effect of the interaction between selenium and zinc on DNA repair in association with cancer prevention. *J Cancer Prev* (2019) 24 (3):146–54. doi: 10.15430/JCP.2019.24.3.146

156. Olechnowicz J, Tinkov A, Skalny A, Suliburska J. Zinc status is associated with inflammation, oxidative stress, lipid, and glucose metabolism. *J Physiol Sci* (2018) 68 (1):19–31. doi: 10.1007/s12576-017-0571-7

157. Morais JBS, Dias TMDS, Cardoso BEP, de Paiva Sousa M, Sousa TGV, Araújo DSC, et al. Adipose tissue dysfunction: impact on metabolic changes? *HormMetabRes* (2022) 54(12):785–94. doi: 10.1055/a-1922-7052

158. Morais JBS, Severo JS, Beserra JB, de Oiveira ARS, Cruz KJC, de Sousa Melo SR, et al. Association between cortisol, insulin resistance and zinc in obesity: a mini-review. *Biol Trace Elem Res* (2019) 191(2):323–30. doi: 10.1007/s12011-018-1629-y

159. Michalak M. Plant-derived antioxidants: significance in skin health and the ageing process. *Int J Mol Sci* (2022) 23(2):585. doi: 10.3390/ijms23020585

160. Rudrapal M, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA, Alomary MN, et al. Dietary polyphenols and their role in oxidative stress-induced human diseases: insights into protective effects, antioxidant potentials and mechanism(s) of action. *Front Pharmacol* (2022) 13:806470. doi: 10.3389/fphar.2022.806470

161. Bié J, Sepodes B, Fernandes PCB, Ribeiro MHL. Polyphenols in health and disease: gut microbiota, bioaccessibility, and bioavailability. *Compounds* (2023) 3 (1):40–72. doi: 10.3390/compounds3010005

162. Zhang Z, Li X, Sang S, McClements DJ, Chen L, Long J, et al. Polyphenols as plant-based nutraceuticals: health effects, encapsulation, nano-delivery, and application. *Foods* (2022) 11(15):2189. doi: 10.3390/foods11152189

163. Gessner DK, Ringseis R, Eder K. Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. *J AnimPhysiolAnimNutr (Berl)* (2017) 101(4):605–28. doi: 10.1111/jpn.12579

164. Shabbir U, Tyagi A, Elahi F, Aloo SO, Oh DH. The potential role of polyphenols in oxidative stress and inflammation induced by gut microbiota in alzheimer's disease. *Antioxidants (Basel)* (2021) 10(9):1370. doi: 10.3390/antiox10091370

165. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria J Med* (2018) 54 (4):287–93. doi: 10.1016/j.ajme.2017.09.001

166. Gopinath K, Sudhandiran G. Naringin modulates oxidative stress and inflammation in 3-nitropropionic acid-induced neurodegeneration through the activation of nuclear factor-erythroid 2-related factor-2 signalling pathway. *Neuroscience* (2012) 227:134–43. doi: 10.1016/j.neuroscience

167. Wei K, Xu Y, Zhao Z, Wu X, Du Y, Sun J, et al. Icariin alters the expression of glucocorticoid receptor, FKBP5 and SGK1 in rat brains following exposure to chronic mild stress. *Int J Mol Med* (2016) 38(1):337–44. doi: 10.3892/ijmm.2016.2591

168. Donoso F, Ramírez VT, Golubeva AV, Moloney GM, Stanton C, Dinan TG, et al. Naturally derived polyphenols protect against corticosterone-induced changes in primary cortical neurons. *Int J Neuropsychopharmacol* (2019) 22(12):765–77. doi: 10.1093/ijnp/pyz052

169. Sifuentes-Franco S, Sánchez-Macías DC, Carrillo-Ibarra S, Rivera-Valdés JJ, Zuñiga LY, Sánchez-López VA. Antioxidant and anti-inflammatory effects of coenzyme Q10 supplementation on infectious diseases. *Healthcare (Basel)* (2022) 10 (3):487. doi: 10.3390/healthcare10030487

170. Barcelos IP, Haas RH. CoQ10 and aging. *Biol (Basel)* (2019) 8(2):28. doi: 10.3390/biology8020028

171. Saini R. Coenzyme Q10: The essential nutrient. *J Pharm Bioallied Sci* (2011) 3 (3):466–7. doi: 10.4103/0975-7406.84471

172. Silva SVE, Gallia MC, Luz JRDD, Rezende AA, Bongiovanni GA, Araujo-Silva G, et al. Antioxidant effect of coenzyme Q10 in the prevention of oxidative stress in arsenic-treated CHO-K1 cells and possible participation of zinc as a pro-oxidant agent. *Nutrients* (2022) 14(16):3265. doi: 10.3390/nu14163265

173. Gutierrez-Mariscal FM, Arenas-de Larriva AP, Limia-Perez L, Romero-Cabrera JL, Yubero-Serrano EM, López-Miranda J. Coenzyme Q10 supplementation for the reduction of oxidative stress: clinical implications in the treatment of chronic diseases. *Int J Mol Sci* (2020) 21(21):7870. doi: 10.3390/ijms21217870

174. Hajiluian G, Heshmati J, Jafari Karegar S, Sepidarkish M, Shokri A, Shidfar F. Diabetes, age, and duration of supplementation subgroup analysis for the effect of coenzyme Q10 on oxidative stress: A systematic review and meta-analysis. *Complement Med Res* (2021) 28(6):557–70. doi: 10.1159/000515249

175. López-Lluch G. Coenzyme Q homeostasis in aging: Response to non-genetic interventions. *Free Radic Biol Med* (2021) 164:285–302. doi: 10.1016/j.freeradbiomed

176. Ježková J, Ďurovcová V, Wenchich L, Hansíková H, Zeman J, Hána V, et al. The relationship of mitochondrial dysfunction and the development of insulin resistance in Cushing's syndrome. *Diabetes MetabSyndrObes* (2019) 12:1459–71. doi: 10.2147/DMSO.S209095

177. Karamouzis I, Berardelli R, D'Angelo V, Fussotto B, Zichi C, Giordano R, et al. Enhanced oxidative stress and platelet activation in patients with Cushing's syndrome. *Clin Endocrinol (Oxf)* (2015) 82(4):517–24. doi: 10.1111/cen.12524

178. Mancini A, Leone E, Silvestrini A, Festa R, Di Donna V, De Marinis L, et al. Evaluation of antioxidant systems in pituitary-adrenal axis diseases. *Pituitary* (2010) 13 (2):138–45. doi: 10.1007/s11102-009-0213-z

179. Mantle D, Hargreaves IP. Coenzyme Q10 and endocrine disorders: an overview. *Antioxidants* (2023) 12(2):514. doi: 10.3390/antiox12020514

180. Wang Y, Wu Y, Wang Y, Xu H, Mei X, Yu D, et al. Antioxidant properties of probiotic bacteria. *Nutrients* (2017) 9(5):521. doi: 10.3390/nu9050521

181. Rodríguez-Daza MC, Pulido-Mateos EC, Lupien-Meilleur J, Guyonnet D, Desjardins Y, Roy D. Polyphenol-mediated gut microbiota modulation: toward prebiotics and further. *Front Nutr* (2021) 8:689456. doi: 10.3389/fnut.2021.689456

182. Wang X, Zhang P, Zhang X. Probiotics regulate gut microbiota: an effective method to improve immunity. *Molecules* (2021) 26(19):6076. doi: 10.3390/molecules26196076

183. Feng T, Wang J. Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a systematic review. *Gut Microbes* (2020) 12(1):1801944. doi: 10.1080/19490976.2020

184. Sabit H, Kassab A, Alaa D, Mohamed S, Abdel-Ghany S, Mansy M, et al. The effect of probiotic supplementation on the gut–brain axis in psychiatric patients. *Curr Issues Mol Biol* (2023) 45(5):4080–99. doi: 10.3390/cimb45050260

185. Hemarajata P, Versalovic J. Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. *Therap Adv Gastroenterol* (2013) 6(1):39–51. doi: 10.1177/1756283X12459294

186. Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. Mechanisms of action of probiotics. *Adv Nutr* (2019) 10(Suppl1):S49–S66.. doi: 10.1093/advances/nmy063

187. Biswas S, Banerjee ER. Probiotic treatment of inflammatory bowel disease: Its extent and intensity. *World J Immunol* (2022) 12(2):15–24. doi: 10.5411/wji.v12.i2.15

188. Anker-Ladefoged C, Langkamp T, Mueller-Alcazar A. The potential impact of selected bacterial strains on the stress response. *Healthcare (Basel)* (2021) 9(5):494. doi: 10.3390/healthcare9050494

189. Martin CR, Osadchiy V, Kalani A, Mayer EA. The Brain-Gut-Microbiome Axis. *Cell Mol Gastroenterol Hepatol* (2018) 6(2):133. doi: 10.1016/J.JCMGH.2018.04.003

190. Sahoo DK, Borcherding DC, Chandra L, Jergens AE, Atherly T, Bourgois-Mochel A, et al. Differential Transcriptomic Profiles Following Stimulation with Lipopolysaccharide in Intestinal Organoids from Dogs with Inflammatory Bowel Disease and Intestinal Mast Cell Tumor. *Cancers* (2022) 14(14):3525. doi: 10.3390/CANCERS14143525

191. Neuman H., Debelius JW, Knight R., Koren O. Microbial endocrinology: the interplay between the microbiota and the endocrine system. *FEMS Microbiol Rev* (2015) 39(4):509–21. doi: 10.1093/FEMSRE/FUU010

192. Tsigos C, Chrousos GP. Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *J Psychosom Res* (2002) 53(4):865–71. doi: 10.1016/S0022-3999(02)00429-4

193. Ait-Belgnaoui A, Durand H, Cartier C, Chaumaz G, Eutamene H, Ferrier L, et al. Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. *Psychoneuroendocrinology* (2012) 37 (11):1885–95. doi: 10.1016/J.PSYNEUEN.2012.03.024

194. Rani K, Kaur G, Ali SA. Probiotic-prebiotic therapeutic potential: a new horizon of microbial biotherapy to reduce female reproductive complications. *Pharma Nutr* (2023) 11:100342. doi: 10.1016/j.phanu.2023.100342

195. Takada M, Nishida K, Kataoka-Kato A, Gondo Y, Ishikawa H, Suda K, et al. Probiotic *Lactobacillus casei* strain Shirota relieves stress-associated symptoms by modulating the gut-brain interaction in human and animal models. *Neurogastroenterol Motil* (2016) 28(7):1027–36. doi: 10.1111/nmo.12804

196. Nasri K, Jamilian M, Rahmani E, Bahmani F, Tajabadi-Ebrahimi M, Asemi Z. The effects of synbiotic supplementation on hormonal status, biomarkers of inflammation and oxidative stress in subjects with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *BMC Endocr Disord* (2018) 18 (1):21. doi: 10.1186/s12902-018-0248-0

197. Aşır F, Nergiz Y, Pala A. Vitamin E protected the mouse adrenal gland against immobilization stress. *Pol J Vet Sci* (2022) 25(3):447–54. doi: 10.24425/pjvs.2022.142029

198. Nasiadek M, Stragierowicz J, Klimczak M, Kilanowicz A. The role of zinc in selected female reproductive system disorders. *Nutrients* (2020) 12(8):2464. doi: 10.3390/nu12082464

199. Zheng Y, Zhang L, Bonfili L, de Vivo L, Eleuteri AM, Bellesi M. Probiotics supplementation attenuates inflammation and oxidative stress induced by chronic sleep restriction. *Nutrients* (2023) 15(6):1518. doi: 10.3390/nu15061518

frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 04 January 2024
DOI 10.3389/fendo.2023.1245512

OPEN ACCESS

EDITED BY
Luna Samanta,
Ravenshaw University, India

REVIEWED BY
Chris Scott,
Charles Sturt University, Australia
Sudhanshu Kumar Bharti,
Patna University, India

*CORRESPONDENCE
Adriana Kolesarova
adriana.kolesarova@uniag.sk

RECEIVED 23 June 2023
ACCEPTED 11 December 2023
PUBLISHED 04 January 2024

CITATION
Kohut L, Baldovska S, Mihal M, Belej L, Sirotkin AV, Roychoudhury S and Kolesarova A (2024) The multiple actions of grape and its polyphenols on female reproductive processes with an emphasis on cell signalling.
Front. Endocrinol. 14:1245512.
doi: 10.3389/fendo.2023.1245512

The multiple actions of grape and its polyphenols on female reproductive processes with an emphasis on cell signalling

Ladislav Kohut[1], Simona Baldovska[2], Michal Mihal[1], Lubomir Belej[3], Alexander V. Sirotkin[4], Shubhadeep Roychoudhury[5] and Adriana Kolesarova[1,2]*

[1]Institute of Applied Biology, Slovak University of Agriculture in Nitra, Nitra, Slovakia, [2]AgroBioTech Research Center, Slovak University of Agriculture in Nitra, Nitra, Slovakia, [3]Institute of Food Sciences, Slovak University of Agriculture in Nitra, Nitra, Slovakia, [4]Department of Zoology and Anthropology, Constantine the Philosopher University, Nitra, Slovakia, [5]Department of Life Science and Bioinformatics, Assam University, Silchar, India

Grapes are an economically important fruit crop, and their polyphenols (mainly phenolic acids, flavanols, flavonols, anthocyanins, proanthocyanidins, and stilbenes) can exert a wide range of health benefits as an interesting and valuable dietary supplement for natural complementary therapy. However, their potential physiological and therapeutic actions on reproductive processes have not been sufficiently elucidated. This evidence-based study presents current knowledge of grape extracts and polyphenols, as well as their properties and therapeutical actions in relation to female reproduction in a nutshell. Grape extract, and its polyphenols such as resveratrol, proanthocyanidin B2 or delphinidin may influence female reproductive physiology and pathology, as well as regulate multiple signaling pathways related to reproductive hormones, steroid hormones receptors, intracellular regulators of oxidative stress and subsequent inflammation, apoptosis, and proliferation. Their role in the management of ovarian cancer, age-related reproductive insufficiency, ovarian ischemia, PCOS, or menopausal syndrome has been indicated. In particular, the potential involvement of grapeseed extracts and/or proanthocyanidin B2 and delphinidin on ovarian steroidogenesis, oocyte maturation, and developmental capacity has been implicated, albeit at different regulatory levels. Grape polyphenols exert a wide range of health benefits posing grape extract as an interesting and valuable dietary supplement for natural complementary therapy. This evidence-based study focuses on the actions of grapeseed extract and grape polyphenols on female reproductive processes at various regulatory levels and multiple signalling pathways by regulating reproductive hormones (GnRH, gonadotropins, prolactin, steroid hormones, IGFBP), steroid receptors, markers of proliferation and apoptosis. However, lack of knowledge of standardized dosages so far limits their clinical application despite the wide range of their biological and therapeutic potentials.

KEYWORDS

grapeseed extract, phenolic compounds, proanthocyanidin, resveratrol, delphinidin, female reproduction, steroid hormones, proliferation

1 Introduction

Grape is an economically important and one of the most grown fruits worldwide (1). Most of the production (about 80% of the yield) is used for wine making (2). An important by-product of grape processing – grape pomace is the most important residual after juice extraction or wine making and consists of peel, seed, stem, and pulp (3). Grape pomace is considered a rich source for the extraction of a wide range of valuable phytonutrients, which exhibit a variety of bioactivities, such as antioxidant, anti-inflammatory, cardioprotective, anti-aging, antimicrobial and anti-cancer properties (4–9). Bioactive substances including proanthocyanidins, anthocyanins, phenolic acids, stilbenes, and flavonols are abundant in grape by-products (10) that can help in prevention or management of several conditions such as inflammatory conditions characterized by bowel disruption and the involvement of the immune system and colorectal cancer. Grape by-products can promote remarkable effects in reducing pro-inflammatory, pro-oxidative, and proliferative actions in inflammatory bowel diseases and colorectal cancer both *in vivo* and *in vitro* (10). Moreover, bioactive substances, such as resveratrol (11), anthocyanidins like delphinidin (12) and procyanidin such as procyanidin B2 (13) are valuable in multiple industries, including pharmaceuticals, agri-food, or cosmetics (13–15). Another abundant by-product of winemaking is grapeseed oil, which is processed from grapeseeds and presents an excellent source of γ-tocotrienol, and α-tocopherol. It also contains fatty acids mainly linoleic, oleic, palmitic, and stearic acids, as well as polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs) (16). Furthermore. secondary plant metabolites such as polyphenols are produced by the grape berries during the growth in reaction to environmental stressors. They form significant components of red wines that enhance the sensory qualities and antioxidant capacity (17). Red wine polyphenols comprise newly generated ones during the winemaking process (such as highly polymerized polyphenols) in addition to those found in grapes as mentioned earlier many of which are recognized to possess beneficial impacts on health (17). Although several studies have summarized the most known physiological and therapeutic effects of grapes and their by-products (12, 15, 18–20), the action of grape extract and grape polyphenols on reproductive processes has not been sufficiently elucidated yet. The present evidence-based study summarizes the current knowledge concerning the provenance, properties, as well as physiological and therapeutic actions of grape extract and grape polyphenols on various cellular processes with a focus on female reproduction.

2 Provenance, bioactive substances and physiological actions

Grapes (*Vitis vinifera* L.) present an important source of phenolic compounds including phenolic acids, tannins, coumarins, flavonoids, flavones, and stilbenes (7). Grape pomace also contains neutral polysaccharides (30%), pectic substances (20%), and insoluble proanthocyanidins (15%) (21).

Among grape pomace compounds with high nutraceutical value, polyphenols (phenolic acids, flavanols, flavonols, anthocyanins, proanthocyanidins, and stilbenes) are the most interesting due to their bioactive properties (22–24). One of the most efficient bioactive compounds found in grape skin, seeds, and wine is stilbenoid resveratrol (25, 26), widely known primarily for its phytoestrogenic and antioxidant activities (27). Additionally, polyphenolic pigments anthocyanidins, including delphinidin, mainly extracted from grape skins, are responsible for many of the red-orange to blue-violet colors (28). Grapeseeds contain proanthocyanidins, which are composed of epicatechin and monomeric catechin, gallic acid, and polymeric and oligomeric proanthocyanidins (29). Interestingly, proanthocyanidins present more powerful free radical scavengers than vitamins C, E, or β-carotene (30). Monomeric and dimeric flavanols, as well as mono- and diglycosides have been identified in grapeseed extracts. Diglycosylated flavanol dimers have been detected in grape skin extracts, too. The concentration of the mono- and diglycosides depends largely on the grape variety and grape source (31). Major bioactive compounds present in different parts of grape and grape products are given in Table 1.

Grape polyphenols are effective inhibitors of enzymes linked with various ailments. Findings indicate an inverse relationship between the consumption of grapes or grape products and the development of age-related complications including cardiovascular disorders with an estimated 6–7% reduction in deaths from cardiovascular disorders (7). Studies demonstrated biological activities including antioxidant, cardioprotective, anti-cancer, anti-inflammatory, anti-aging, and antimicrobial properties exerted by grape polyphenols such as anthocyanins, flavanols, flavonols, and resveratrol. Chromatographic analysis confirmed the presence of 19 phytochemicals. The prominent compound was catechin followed by gallic acid, caftaric acid, and epicatechin (4–6, 48). Moreover, skin protection, antidiabetic, immunomodulatory and anti-neurodegenerative activities as well as hepatoprotective and neuroprotective effects using phenolic compounds gathered form grape ethanol extract have been reported (11, 15, 18, 48).

Oxidative stress has been associated with the pathogenesis of several chronic diseases and inflammatory processes. Polyphenols are strong antioxidants that act as a defense barrier against free radicals, as well as non-radical oxidants (49). Phenolic acids, stilbenoids, tannins, quinones, coumarins and flavonoids from grapes have the potential to enhance the oxidant capacity of cells stimulating enzymatic expression and reducing the reactive oxygen species (ROS) by either inhibiting their production or by directly scavenging them or via xenobiotic detoxification. For example, administration of Bordo grape juice to human test subjects, led to elevation of antioxidant activities and lowering of blood glucose (50). Grape polyphenols, particularly flavanols can maintain cellular protein homeostasis (proteostasis). Since impaired proteostasis is closely involved in all amyloid diseases, grapeseed extracts may be a valuable therapeutic agent for the prevention and/or management of neurodegenerative diseases (51). The antimicrobial activity against Gram-positive bacteria and antioxidant properties could

10.3389/fendo.2023.1245512

TABLE 1 Major bioactive compounds in different parts of grape and grape products.

Grape part/product	Bioactive compounds	References
Seeds	catechin epicatechin epicatechin-3-O-gallate proanthocyanidins procyanidin B2 dimeric procyanidin gallic acid	[15, 29, 32–36]
Skin	epigallocatechin kaempferol myricetin trans-resveratrol quercetin proanthocyanidins ellagic acid	[15, 32, 33, 37–40]
Leaves	kaempferol myricetin quercetin gallic acid ellagic acid	[32, 41]
Stems	quercetin 3-O-glucuronide rutin astilbin trans-resveratrol	[15, 38, 42, 43]
Raisin	hydroxymethylfurfural hydroxycinnamic acid	[41, 44, 45]
Red wine	catechin cyanidin-3-glucoside peonidin-3-glucoside cetunidin-3-glucoside delphinidin-3-glucoside resveratrol quercetin hydroxycinnamic acid	[43, 45–47]

be associated with phenolic compounds found in grape stems (52). Resveratrol isolated from grape stems was applied on hepatocellular carcinoma Hep-G2 (hepatoma G2) cells, breast adenocarcinoma MCF-7 cells, colon carcinoma HCT116 cells, and lymphoblastic leukemia cells (1301). After treatment, it was shown that resveratrol possesses anti-proliferative and apoptotic effects (53). Anthocyanidins have been found to possess anti-aging and anti-inflammatory properties (28). Lim and Song (12) described the possible use of delphinidin according to its effect on different types of cancers and various chronic diseases. For the study, they used ovarian adenocarcinoma cells (SKOV3) which were then treated with delphinidin alone or with various inhibitors of cell signaling proteins.

Grape pomace contains a high level of antioxidants with the ability to counteract chronic inflammatory symptoms which was demonstrated on colorectal adenocarcinoma-derived intestinal epithelial cell line Caco-2 after grape pomace ethanolic extract treatment (54). Additionally, grapeseeds contain several flavonoids and non-flavonoids which can exert antioxidant and anti-inflammatory activities. Beneficial effects of grapeseed extract in relation to oxidative stress and metabolic disorders such as insulin resistance have been associated with the modulation of plasma adipokines in mammals (55, 56). Grapeseed supplementation has the potential to scavenge oxygen free radicals in the egg yolk in mammals and chicken, as well as it can reduce oxidative damage in the liver in rats (57, 58). It has been reported that grapeseed proanthocyanidin extract possess anti-inflammatory and antioxidant activities (13, 59, 60), and can reduce cytotoxicity as well as genotoxicity (49), including decreasing oxidative damage induced by aflatoxins (61). Proanthocyanidin B2 found in grapeseed present one of the most valuable components of grapeseed extract and can be used due to its protective action against oxidative stress and development of cardiovascular diseases demonstrated on human umbilical vein endothelial cells (HUVEC) (62, 63). In this regard, grape extracts and their polyphenols exhibit protective effects against different toxins and a variety of mechanisms of their action, disturbing physiological homeostasis through increase in superoxide dismutase (SOD) levels and glutathione peroxidase activities, as well as decrease in malondialdehyde (MDA) levels or activation of the nuclear erythroid 2-related factor 2/ARE pathway demonstrated on PC12 rat cells (26). Possible physiological and therapeutic actions of grape polyphenols depending on their bioactive substances are presented in Table 2.

3 Effect on female reproductive processes

Reproductive dysfunctions can be indicated by a negative correlation between muscle growth and reproductive effectiveness (76, 77). Exposure to oxidative stress can lead to the inflammation initiation which is the trigger of multiple reproductive disorders, including ovarian cancer or multiple reproductive defects, such as oocyte mutation, polycystic ovary syndrome (PCOS), endometriosis, as well as can affect ovarian folliculogenesis, oocyte maturation and the release of sex hormones (78–80). Bioactive phytonutrients are known to impart several properties such as anti-inflammatory and antioxidant activities that may have a beneficial impact on reproductive functions (81, 82). Polyphenols can pass through various protective barriers in reproductive organs, which can possibly affect their physiological functions (11, 83, 84). Moreover, grape seed extract had positive impact on improving fertility in golden laying hens (85). Although few studies have been carried out on reproductive cells we have summarized the available information related to the effects of grape polyphenols on female reproductive organs and their (dys)functions in a nutshell. The action on female reproductive processes is presented in Figure 1.

3.1 Effect on ovaries

Oxidative stress plays an important role in ovarian aging and can lead to decline of fertility in animals and humans (71, 86). According to Shen et al. (87), apoptotic processes induced by oxidative stress in granulosa cells are considered a major cause of

TABLE 2 Physiological and therapeutic actions of grape polyphenols.

Action(s)	Bioactive compounds	References
Anti-inflammatory	catechin epicatechin kaempferol resveratrol procyanidin	[34, 39, 40, 47]
Anti-proliferative Anti-cancer	catechin epicatechin-3-O-gallate resveratrol procyanidin B2 malvidin-3-glucoside	[4, 5, 34–36, 41, 45, 64]
Antioxidant	catechin epicatechin-3-O-gallate rutin myricetin kaempferol quercetin resveratrol gallic acid	[39–41, 43, 45, 64, 65]
Neuroprotective	procyanidin B2 malvidin-3-glucoside peonidin-3-glucoside	[35, 45]
Anti-diabetic	epigallocatechin rutin myricetin quercetin	[37, 40, 43]
Anti-bacterial	catechin epicatechin quercetin resveratrol	[65, 66]
Action on female reproductive processes	myricetin resveratrol procyanidin B2 delphinidin	[8, 12, 13, 59, 60, 67–75]

follicular atresia. It has been reported that polyphenols can improve the amount and quality of oocytes in mice and humans which was demonstrated after treating oocytes (71, 88). Beneficial effects of grapeseed extract on oocyte maturation and early development based on the mean numbers of cleavage, morula, and blastocyst rates have been observed in sheep (89). Grapeseed procyanidin B2 can positively affect oocyte viability in mice and promote their maturation and developmental capacity (74). The use of grapeseed extract could also be effective in the prevention or treatment of PCOS. Short-term grapeseed extract treatment provided a beneficial impact on PCOS positive women's metabolic status (90). Furthermore, grapeseed extract can exert a positive impact on health in reproductive insufficiency and menopause and, also prevent negative morphological changes in ovaries due to reproductive ageing (71, 91). This effect could be due to the presence of proanthocyanidin B2, which has been observed in rat ovaries as a possible protection against age-dependent degenerative changes (11, 71). Some studies have described the protective role of proanthocyanidin B2 from grapeseed against damage to rat ovarian tissue induced by ischemia or ischemia/reperfusion (70, 71, 92). Grapeseed extract can affect resistance to chemotherapy and reduce human ovarian cancer cell growth (13). Delphinidin such as a member of the anthocyanidin family and a natural pigment in grapes may be a pivotal therapeutic target for the prevention of epithelial ovarian cancer (12). Grapeseed ethanol extract, as well as proanthocyanidin B2 can modulate human granulosa cell functions, including steroidogenesis, and can exert phytoestrogenic activity with a positive effect on steroid hormone production in human granulosa cells (93). Available data suggest the potential of using maternal diet supplemented with grapeseed extract in the improvement of egg quality in hens. Furthermore, grapeseed extract can ameliorate egg quality by decreasing the rate of double yolk eggs and by improving the size of normal eggs and the elasticity of the shell (60). In contrast, supplementation of dietary polyphenol resveratrol could not impact egg production and egg quality related to the shell, yolk, and albumen in quails (67). In hens, grapeseed proanthocyanidins have been reported to play an important role in the prevention of ovarian aging process by reducing oxidative stress (71).

3.2 Effect on uterus

Colitti et al. (94) described the possible impact of grapeseed extract on endometrial functions. In heifers, grapeseed extract (oral administration) affected the expression of several genes in the uterine endometrium. In addition, anti-inflammatory properties of resveratrol present in grapes can contribute to the prevention of endometriosis. This well-known phytonutrient has been considered a novel drug in endometriosis prevention and/or treatment (72, 95). Resveratrol has also been reported to modulate the response of endometrium to progesterone and estrogen during decidualization and reinforce hormone action during human endometrial stromal cell (ESC) differentiation, which could lead to improvement of women's health (73). Another study provided evidence of promising chemopreventive properties of proanthocyanidins in grapeseeds against cervical cancer. Proanthocyanidin B2 can suppress cervical cancer proliferation and growth and induce apoptosis through the mitochondrial signaling pathway (68). Thus, available literature so far suggests the impact of grape polyphenols on uterine endometrium, decidualization, and their potential to prevent and/or treat endometriosis and cervical cancer. Physiological and therapeutic actions of grape polyphenols on female reproductive processes are presented in Table 3.

4 Regulation of female reproductive processes

Grape, grape extract and their bioactive polyphenols can affect female reproductive processes via extracellular regulators and multiple intracellular signaling pathways. Their mechanism(s) of actions on female reproductive processes have been studied insufficiently, however, there are some studies describing the possible mechanism(s) of effect on female reproduction (11).

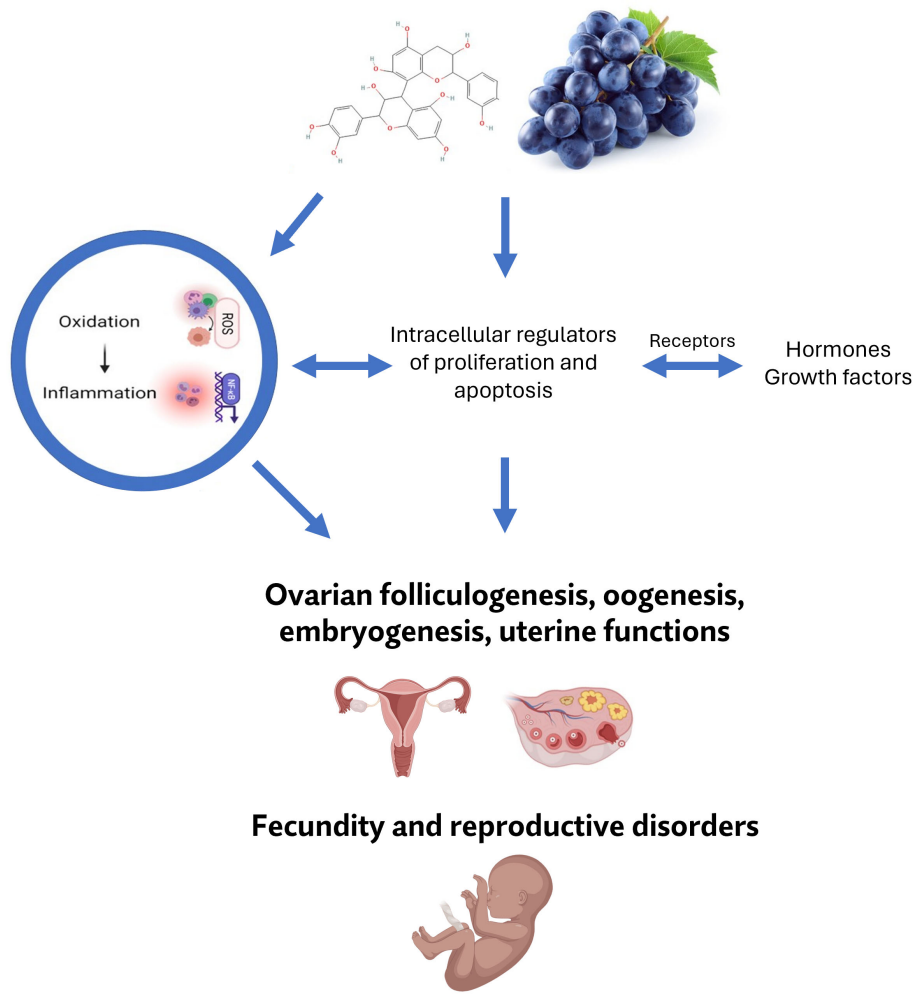


FIGURE 1
Possible involvement of bioactive constituents of grape (mainly its polyphenols) in female reproductive processes, and their pathological alterations.

4.1 Hormonal regulation and steroidogenesis

Phenolic compounds present in grapes can affect the essential regulators of reproductive processes, including hypothalamic neurohormones (GnRH, oxytocin, LH and FSH), steroid hormones (estradiol, progesterone, testosterone) and prostaglandins (96). Furthermore, due to the chemical similarity of polyphenols to the structure of estrogens, they may exert hormone-like effects (estrogen-agonistic or antagonistic) by binding or activating estrogen receptors (ERα and ERß) (11, 97). A flavonol myricetin present in red wine can block insulin-like growth factor I (IGF-I)-induced progesterone production by granulosa cells and stimulate IGF-I induced estradiol production (75). Similarly, resveratrol can increase prolactin and IGF-I binding protein 1 (IGFBP1) release, which can result in enhanced decidualization of human embryonic stem cells (ESCs) *in vitro* (73). Grapeseed extract can influence insulin sensitivity by increasing insulin receptors expression and stimulation (98).

Regarding the effect on steroidogenesis, grape extracts, as well as grapeseed proanthocyanidin B2 improved progesterone and estradiol secretion and this was associated with a higher level of the cholesterol carriers, steroidogenic acute regulatory protein (StAR), cyclic adenosine monophosphate response element-binding protein (CREB), and mitogen-activated protein kinases extracellular signal-regulated kinases 1/2 (MAPK ERK1/2) phosphorylation in both primary luteinized human granulosa cells (hGC) and human tumor granulosa cells (KGN). Taken together, GSE and GSPB2 *in vitro* treatments decrease oxidative stress and increase steroidogenesis without affecting cell proliferation and viability in human granulosa cells (93).Another study described the ability of grapeseed extract to modulate an aromatase inhibitor *in vitro* as well as *in vivo* in aromatase-transfected MCF-7 (MCF-7aro) BC xenograft mice (99). Oral administration of grapeseed to heifers can alter progesterone release during estrous cycle after daily oral administration of grapeskin extract for 3 weeks (94). A flavonol myricetin present in red wine can directly affect ovarian function, including steroidogenesis in bovine granulosa cells and theca cells *in vitro*. These cells were gathered from non-pregnant beef cows. Moreover, myricetin has been able to reduce some of the inhibitory effects of mycotoxins on granulosa cell functions (75).

TABLE 3 Physiological and therapeutic actions of grape polyphenols on female reproductive processes.

Bioactive compound (s)/ extract	Experimental model(s)	Effect(s)	Reference (s)
Procyanidin B2	a type 1 diabetes mouse oocytes	reducing oxidative stress; promoting mitochondrial function; improving oocyte quality, maturation, and embryo development	[74]
	porcine granulosa cells	reducing oxidative stress; inhibiting of H_2O_2-induced apoptosis	[59]
	ICR mice granulosa cells	improving cell viability; inducing apoptosis; reducing oxidative stress	[70]
Procyanidin	human ovarian cancer cells A2780 and A2780/T	enhancing cytotoxicity; suppressing inflammation	[13]
Proanthocyanidins	human cervical cancer cells HeLa and SiHa	inhibiting cell growth; inducing apoptosis	[68]
Grape seed proanthocyanidin extract (GSPE)	hyline brown hen ovaries	reducing oxidative stress; preventing ovarian aging	[71]
Lipophilic grape seed proanthocyanidin (LGSP)	human cervical cancer cells HeLa; HeLa-derived xenograft zebrafish model	increasing ROS production; inhibiting cell growth; inducing apoptosis	[8]
Myricetin	bovine granulosa cells (GC) and theca cells (TC)	direct effect on steroidogenesis; reducing of inhibitory effects of mycotoxins	[75]
Delphinidin	ovarian cancer cells SKOV3	inhibiting of cell proliferation	[12]
Resveratrol	human endometriotic implants in nude mice	inhibiting of endometriosis lesions	[95]
	Sprague-Dawely rat ovaries	protecting and restoring ovarian functions after radiotherapy-induced premature ovarian failure (POF); suppressing of inflammation	[69]
	immortalized human endometrial stromal cell (t-HESC)	enhancing decidualization; decreasing cell proliferation	[73]

(Continued)

TABLE 3 Continued

4.2 Proliferation and apoptosis

Grape polyphenols may affect ovarian cell functions and physiological processes. Interestingly, it has been demonstrated that grapeseed proanthocyanidin B2 may play an important role in the regulation of apoptosis and proliferation in the ovaries (71). In addition, grapeseed proanthocyanidin B2 treatment can inhibit hydrogen peroxide (H_2O_2)-induced apoptosis in granulosa cells possibly via let-7a upregulation, resulting in protective effect and promotion of viability of porcine granulosa cells (59). Furthermore, grapeseed extracts can inhibit Akt phosphorylation, which can regulate multiple cellular processes such as cell proliferation, survival, and metabolism (100). Another grape constituent, delphinidin inhibits ovarian cancer cell proliferation via inactivation of PI3K/AKT and ERK1/2 mitogen-activated protein kinase signaling pathway, which could be a pivotal therapeutic target for the prevention of epithelial ovarian cancer (12).

Lipophilic grapeseed proanthocyanidin can exert an anti-proliferative effect on cervical cancer HeLa cells by increasing ROS production, resulting in the induction of cellular apoptosis, and cell cycle arrest in the G2/M phase. Proanthocyanidin can reduce mitochondrial membrane potential, upregulate Bax/Bcl-2 ratio, increase the release of cytochrome c, and activate caspase-3 and poly(ADP-Ribose)polymerase (PARP), and thus it can induce apoptotic processes in cervical cancer cells through the intrinsic mitochondrial/caspase-mediated pathway (8). Higher concentrations (50 to 100 µg/mL) of grapeseed extract and proanthocyanidin B2 can inhibit cell proliferation in a human ovarian granulosa-like tumor cell line KGN and hGCs, associated with decrease in cyclin D2 level and an increase in p21 and p27 levels to induce cell cycle arrest in G1 phase (93). While proanthocyanidin B2 did not influence nuclear and cytoplasmic apoptosis in porcine granulosa cells (59), it inhibited the ovarian cancer cell viability and enhanced the resistance to chemotherapy (13). Moreover, both grapeseed extract and proanthocyanidin B2 can increase the cleaved caspase-3 level and impair Bcl-2-associated death promoter protein (BAD) phosphorylation, resulting in cell death. Thus, both can inhibit the expression of intracellular markers (MAP kinase, cyclin D2, Akt phosphorylation), and promote the

expression of proliferation inhibitors or apoptotic markers (p21, p27) in ovarian granulosa and ovarian cancer cells (13, 93, 101).

4.3 Oxidative stress

It is known that oxidative stress is a key promoter of reproductive alterations that can negatively affect ovarian functions through apoptosis induction. Moreover, it can dysregulate the expression of related genes (59, 87). ROS production can lead to oxidative stress affecting ovarian functions. Thanks to its protective properties against oxidative stress, grapeseed proanthocyanidin B2 can prevent ovarian aging by oxidative stress suppression in hens (71). In addition, proanthocyanidins can improve oocyte quality, viability, and maturation, as well as developmental capacity by inhibiting ROS production in murine oocytes (74). Dietary grapeseed extract supplementation can reduce ROS levels in egg yolk suggesting a reduction in both oxidative stress and lipid peroxidation in reproductive broiler hens (60). Additionally, a decrease in lipid peroxidation level and an increase in antioxidant capacity in egg yolk have been observed in laying hens, fed with grape pomace flour (102–104). Furthermore, grapeseed extract and grape polyphenols, such as resveratrol and proanthocyanidin B2 may suppress oxidative stress in non-cancerous and cancerous granulosa cells by promoting antioxidant enzymes (59, 93, 105). Moreover, grapeseed extract may exert a negative prooxidant or beneficial antioxidant effect through modulation of NOX actions (106) and possess the ability to regulate ROS production in human granulosa cells. At low concentrations (0.1 to 10 μg/mL), it can reduce oxidative stress by decreasing ROS content and NOX4 expression (93).

Hypothalamic–pituitary–adrenal axis is activated by stress, which can increase glucocorticoid secretion and disrupt the ovarian cycle (94). Maternal dietary supplementation of grapeseed extract can reduce plasma and tissue oxidative stress associated with the modulation of adipokines content in plasma and peripheral tissues in broiler hens (93).

Regarding the anti-inflammatory activity of grapes, a study indicated that grapeseed extract may reduce the expression of pro-inflammatory interleukins in rats suffering from PCOS (107). Furthermore, resveratrol acts in counteracting the inflammatory signaling pathway associated with radiotherapy-induced premature ovarian failure. Resveratrol has been reported to ameliorate cell damage in ovary induced by ionizing radiation and have a protective effect on endometriosis via downregulation of prostaglandins, interleukins, and stimulating inflammation transcription factor NF-κB (69, 72). Furthermore, resveratrol activates SIRT1 expression, resulting in the inhibition of poly (ADP-Ribose)polymerase-1 (PARP-1) and NF-κB expression-mediated inflammatory cytokines, as well as can restore ovarian function by increasing anti-Müllerian hormone (AMH) levels (69). Similarly, grapeseed procyanidin has demonstrated an inhibitory effect on NF-κB activity and MAPK/ERK pathway mediated YB-1 in ovarian cancer cells, suggesting its potential use as a chemo-sensitizer to overcome multidrug resistance in ovarian cancer patients (13).

Based on available reports, grape extract, and its polyphenols such as resveratrol, proanthocyanidin B2 or delphinidin may be considered to influence female reproductive physiological and pathological processes, as well as regulate multiple signaling pathways related to sex hormones, steroid receptors, intracellular regulators of proliferation, oxidative stress, inflammation, and apoptosis (11).

5 Possible application in reproductive biology and medicine

Utilization of grape by-products has attracted increasing attention for the availability of grape skins, their health benefits and pharmacological use. Grape polyphenols can play an important role in the prevention of reproductive disorders due to their ability to mitigate the negative impact of oxidative stress and inflammation on the reproductive processes. Moreover, the beneficial impact on oocyte maturation, cell viability, cell proliferation, as well as steroidogenesis has been reported. Resveratrol from grape stems may have a potential to prevent endometriosis and could serve as a novel dietary supplement. Furthermore, available data suggest the possible use of grapeseed extract to improve oocyte quality, as well as healthy gravidity, embryogenesis, and labour due to its beneficial effect on the endometrium. Moreover, the applicability of grapeseed extract including proanthocyanidin B2 in the prevention and/or management of endometriosis, age-related menopausal reproductive insufficiency and ovarian or cervical cancers has been mentioned. Therefore, grape extract and polyphenols present a promising biostimulator, which can be used as dietary supplement in the improvement of reproduction in the field of animal production, biotechnology, or assisted reproduction. Similarly, phytoestrogenic activity of grape might be used as a potential alternative tool to the hormonal treatment of disorders related to estrogen deficiency, such as menopausal syndrome, and osteoporosis. However, to our knowledge, such potential of grape extract or grape polyphenols has not been examined in depth yet.

6 Conclusions and possible directions of future studies

The present review sheds light on the potential health benefits of grape polyphenols while also emphasizing the need for further research and a more cautious interpretation of the findings. It is evident thatconfirmatory claims about the therapeutic effects of grape polyphenols cannot be made at this stage, given the intricacies of human physiology and the many variables at play. Grape polyphenols exert a wide range of health benefits posing grape extract as an interesting and valuable dietary supplement for natural complementary therapy. This evidence-based study focuses on the

actions of grapeseed extract and grape polyphenols on female reproductive processes at various regulatory levels and multiple signalling pathways by regulating reproductive hormones (GnRH, gonadotropins, prolactin, steroid hormones, IGFBP), steroid receptors, markers of proliferation and apoptosis. Moreover, the role of grapes in various reproductive disorders, including reproductive insufficiency, PCOS, menopausal syndrome, ovarian cancer or ovarian ischemia has been indicated. Studies also demonstrate the impact of grapeseed extracts or their bioactive constituents (proanthocyanidin B2, resveratrol, delphinidin) on steroidogenesis, oocyte quality and maturation, and developmental capacity. However, lack of knowledge of standardized dosage limits the clinical applications of grapeseed extract despite the wide range of biological and therapeutic potential.

On the other hand, it should be remembered that *in vitro* and *in vivo* studies have been performed with far greater quantities of polyphenols than those frequently found in human diets. Hence, the extent of grape polyphenols consumed on a regular basis is an open question and needs to be addressed in future studies. Determining suitable doses for therapeutic applications remains a critical challenge, as highlighted in the previous sections. The appropriate dosage of grape polyphenols is a key factor in achieving the desired health outcomes, and future research should focus on defining these optimal dosage ranges and accounting for potential variations in individual responses. Moreover, the studies have mainly been performed *in vitro* or *in vivo*, whilst clinical studies are lacking and the efficacy of all grape phytosubstances on reproductive processes has not been tested properly yet.

Author contributions

Conceptualization: SR, AK; writing – original draft preparation: LK, AK; writing – review and editing: SB, MM, LB, AS, SR; supervision: AK. All authors contributed to the article and approved the submitted version.

Funding

The work was supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic projects APVV-18-0312, APVV-21-0206 VEGA 1/0266/20, and KEGA 033SPU-4/2021.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Ma ZY, Nie ZL, Ren C, Liu XQ, Zimmer EA, Wen J. Phylogenomic relationships and character evolution of the grape family (Vitaceae). *Mol Phylogenet Evol* (2021) 154:106948. doi: 10.1016/j.ympev.2020.106948

2. Olejar KJ, Ricci A, Swift S, Zujovic Z, Gordon KC, Fedrizzi B, et al. Characterization of an antioxidant and antimicrobial extract from cool climate, white grape marc. *Antioxidants* (2019) 8(7):232. doi: 10.3390/antiox8070232

3. Averilla JN, Oh J, Kim HJ, Kim JS, Kim JS. Potential health benefits of phenolic compounds in grape processing by-products. *Food Sci Biotechnol* (2019) 28:1607–15. doi: 10.1007/s10068-019-00628-2

4. Faria A, Calhau C, de Freitas V, Mateus N. Procyanidins as antioxidants and tumor cell growth modulators. *J Agric Food Chem* (2006) 54(6):2392–7. doi: 10.1021/jf0526487

5. Mantena SK, Baliga MS, Katiyar SK. Grape seed proanthocyanidins induce apoptosis and inhibit metastasis of highly metastatic breast carcinoma cells. *Carcinogenesis* (2006) 27(8):1682–91. doi: 10.1093/carcin/bgl030

6. Xia EQ, Deng GF, Guo YJ, Li HB. Biological activities of polyphenols from grapes. *Int J Mol Sci* (2010) 11(2):622–46. doi: 10.3390/ijms11020622

7. Dwibedi V, Jain S, Singhal D, Mittal A, Rath SK, Saxena S. Inhibitory activities of grape bioactive compounds against enzymes linked with human diseases. *Appl J Microbiol Biotechnol* (2022) 106(4):1399–417. doi: 10.1007/s00253-022-11801-9

8. Li C, Zhang L, Liu C, He X, Chen M, Chen J. Lipophilic grape seed proanthocyanidin exerts anti-Cervical cancer effects in hela cells and a hela-Derived xenograft zebrafish model. *Antioxidants* (2022) 11:2. doi: 10.3390/antiox11020422

9. Yang C, Han Y, Tian X, Sajid M, Mehmood S, Wang H, et al. Phenolic composition of grape pomace and its metabolism. *Crit Rev Food Sci Nutr* (2022) 1:17. doi: 10.1080/10408398.2022.2146048

10. Laurindo LF, Direito R, Bueno Otoboni AMM, Goulart RA, Quesada K, Barbalho SM. Grape processing waste: effects on inflammatory bowel disease and colorectal cancer. *Food Rev Int* (2023) 0(0):1–34. doi: 10.1080/87559129.2023.2168281

11. Sirotkin AV, Kolesarova A. *Environmental contaminants and medicinal plants action on female reproduction*. London: Academic Press (2022).

12. Lim W, Song G. Inhibitory effects of delphinidin on the proliferation of ovarian cancer cells via PI3K/AKT and ERK 1/2 MAPK signal transduction. *Oncol Lett* (2017) 14(1):810–8. doi: 10.3892/ol.2017.6232

13. Zhao BX, Sun YB, Wang SQ, Duan L, Huo QL, Ren F, et al. Grape seed procyanidin reversal of p-glycoprotein associated multi-drug resistance via down-regulation of NF-κB and MAPK/ERK mediated YB-1 activity in A2780/T cells. *PloS One* (2013) 8(8):e71071. doi: 10.1371/journal.pone.0071071

14. Glampedaki P, Dutschk V. Stability studies of cosmetic emulsions prepared from natural products such as wine, grape seed oil and mastic resin. *Colloids Surf A Physicochem Eng Asp* (2014) 460:306–11. doi: 10.1016/j.colsurfa.2014.02.048

15. Nassiri-Asl M, Hosseinzadeh H. Review of the pharmacological effects of Vitis vinifera (Grape) and its bioactive constituents: an update. *Phytother Res* (2016) 30 (9):1392–403. doi: 10.1002/ptr.5644

16. Fernandes L, Casal S, Cruz R, Pereira JA, Ramalhosa E. Seed oils of ten traditional Portuguese grape varieties with interesting chemical and antioxidant properties. *Food Res Int* (2013) 50(1):161–6. doi: 10.1016/j.foodres.2012.09.039

17. Buljeta I, Pichler A, Šimunović J, Kopjar M. Beneficial effects of red wine polyphenols on human health: comprehensive review. *Curr Issues Mol Biol* (2023) 45 (2):782–798. doi: 10.3390/cimb45020052

18. Teixeira A, Baenas N, Dominguez-Perles R, Barros A, Rosa E, Moreno DA, et al. Natural bioactive compounds from winery by-products as health promoters: a review. *Int J Mol Sci* (2014) 15(9):15638–78. doi: 10.3390/ijms150915638

19. Huang Q, Liu X, Zhao G, Hu T, Wang Y. Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. *Anim Nutr* (2018) 4 (2):137–50. doi: 10.1016/j.aninu.2017.09.004

20. Gouvinhas I, Pinto R, Santos R, Saavedra MJ, Barros AI. Enhanced phytochemical composition and biological activities of grape (Vitis vinifera L.) Stems

growing in low altitude regions. *Sci Hortic* (2020) 265:109248. doi: 10.1016/j.scienta.2020.109248

21. Spinei M, Oroian M. The potential of grape pomace varieties as a dietary source of pectic substances. *Foods* (2021) 10:4. doi: 10.3390/foods10040867

22. Georgiev V, Ananga A, Tsolova V. Recent advances and uses of grape flavonoids as nutraceuticals. *Nutrients* (2014) 6(1):391–415. doi: 10.3390/nu6010391

23. Gerardi C, D'amico L, Migoni D, Santino A, Salomone A, Carluccio MA, et al. Strategies for reuse of skins separated from grape pomace as ingredient of functional beverages. *Front Bioeng Biotechnol* (2020) 8:645. doi: 10.3389/fbioe.2020.00645

24. Gerardi C, Pinto L, Baruzzi F, Giovinazzo G. Comparison of antibacterial and antioxidant properties of red (cv. Negramaro) and white (cv. Fiano) skin pomace extracts. *Molecules* (2021) 26(19):5918. doi: 10.3390/molecules26195918

25. Kuršvietienė L, Stanevičienė I, Mongirdienė A, Bernatonienė J. Multiplicity of effects and health benefits of resveratrol. *Medicina* (2016) 52(3):148–55. doi: 10.1016/j.medici.2016.03.003

26. Tabeshpour J, Mehri S, Shaebani Behbahani F, Hosseinzadeh H. Protective effects of Vitis vinifera (grapes) and one of its biologically active constituents, resveratrol, against natural and chemical toxicities: A comprehensive review. *Phytother Res* (2018) 32(11):2164–90. doi: 10.1002/ptr.6168

27. Nashine S, Nesburn AB, Kuppermann BD, Kenney MC. Role of resveratrol in transmitochondrial AMD RPE cells. *Nutrients* (2020) 12(1):159. doi: 10.3390/nu12010159

28. Wallace TC, Giusti MM. Anthocyanins. *Adv Nutr* (2015) 6(5):620–2. doi: 10.3945/an.115.009233

29. Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, et al. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. *Toxicology* (2000) 148:187–97. doi: 10.1016/s0300-483x(00)00210-9

30. Bagchi D, Swaroop A, Preuss HG, Bagchi M. Free radical scavenging, antioxidant and cancer chemoprevention by grape seed proanthocyanidin: an overview. *Mutat Res-Fund Mol M* (2014) 768:69–73. doi: 10.1016/j.mrfmmm.2014.04.004

31. Zerbib M, Cazals G, Enjalbal C, Saucier C. Identification and quantification of flavanol glycosides in Vitis vinifera grape seeds and skins during ripening. *Molecules* (2018) 23(11):2745. doi: 10.3390/molecules23112745

32. Pastrana-Bonilla E, Akoh CC, Sellappan S, Krewer G. Phenolic content and antioxidant capacity of muscadine grapes. *J Agric Food Chem* (2003) 51(18):5497–4503. doi: 10.1021/jf030113c

33. Hernandez-Jimenez A, Gomez-Plaza E, Martinez-Cutillas A, Kennedy JA. Grape skin and seed proanthocyanidins from Monastrell× Syrah grapes. *J Agric Food Chem* (2009) 57(22):10798–803. doi: 10.1021/jf903465p

34. Anna Malinowska M, Billet K, Drouet S, Munsch T, Unlubayir M, Tungmunnithum D, et al. Grape cane extracts as multifunctional rejuvenating cosmetic ingredient: Evaluation of sirtuin activity, tyrosinase inhibition and bioavailability potential. *Molecules* (2020) 25(9):2203. doi: 10.3390/molecules25092203

35. Yu F, Li BY, Yin M, Lu WD, Li XL, Cheng M, et al. Proteomic analysis of liver mitochondria of db/db mice treated with grape seed procyanidin B2. *J Food Biochem* (2020) 44(11):e13443. doi: 10.1111/jfbc.13443

36. Suc L, Rigou P, Mouls L. Detection and identification of oxidation markers of the reaction of grape tannins with volatile thiols commonly found in wine. *J Agric Food Chem* (2021) 69(10):3199–208. doi: 10.1021/acs.jafc.0c07163

37. Yilmazer-Musa M, Griffith AM, Michels AJ, Schneider E, Frei B. Grape seed and tea extracts and catechin 3-gallates are potent inhibitors of α-amylase and α-glucosidase activity. *J Agric Food Chem* (2012) 0:36. doi: 10.1021/jf301147n

38. Luo L, Cui Y, Zhang S, Li L, Li Y, Zhou P, et al. Preparative separation of grape skin polyphenols by high-speed counter-current chromatography. *Food Chem* (2016) 212:712–21. doi: 10.1016/j.foodchem.2016.06.009

39. Fia G, Bucalossi G, Gori C, Borghini F, Zanoni B. Recovery of bioactive compounds from unripe red grapes (cv. Sangiovese) through a green extraction. *Foods* (2020) 9:5. doi: 10.3390/foods9050566

40. Viana-Mattioli S, Cinegaglia N, Bertozzi-Matheus M, Bueno-Pereira TO, Caldeira-Dias M, Cavalli RC, et al. SIRT1-dependent effects of resveratrol and grape juice in an in *vitro* model of preeclampsia. *Biomed Pharmacother* (2020) 131:110659. doi: 10.1016/j.biopha.2020.110659

41. Gašić U, Ćirić I, Pejčić T, Radenković D, Djordjević V, Radulović S, et al. Polyphenols as possible agents for pancreatic diseases. *Antioxidants* (2020) 9:6. doi: 10.3390/antiox9060547

42. Makris DP, Boskou G, Andrikopoulos NK, Kefalas P. Characterization of certain major polyphenolic antioxidants in grape (Vitis vinifera cv. Roditis) stems by liquid chromatography-mass spectrometry. *Eur Food Res Technol* (2008) 226:1075–9. doi: 10.1007/s00217-007-0633-9

43. Ostberg-Potthof JJ, Berger K, Richling E, Winterhalter P. Activity-guided fractionation of red fruit extracts for the identification of compounds influencing glucose metabolism. *Nutrients* (2019) 11:5. doi: 10.3390/nu11051166

44. Karadeniz F, Durst RW, Wrolstad RE. Polyphenolic composition of raisins. *J Agric Food Chem* (2000) 48(11):5343–50. doi: 10.1021/jf0009753

45. Zhang BW, Xing Y, Wen C, Yu XX, Sun WL, Xiu ZL, et al. Pentacyclic triterpenes as α-glucosidase and α-amylase inhibitors: structure-activity relationships and the synergism with acarbose. *Bioorg Med Chem Lett* (2017) 27(22):5065–70. doi: 10.1016/j.bmcl.2017.09.027

46. Rivero-Pérez MD, Muniz P, González-Sanjosé ML. Contribution of anthocyanin fraction to the antioxidant properties of wine. *Food Chem Toxicol* (2008) 46(8):2815–22. doi: 10.1016/j.fct.2008.05.014

47. Zdunić G, Gođevac D, Šavikin K, Krivokuća D, Mihailović M, Pržić Z, et al. Grape seed polyphenols and fatty acids of autochthonous Prokupac vine variety from Serbia. *Chem Biodivers* (2019) 16:7. doi: 10.1002/cbdv.20190.0053

48. Recinella L, Chiavaroli A, Veschi S, Cama A, Acquaviva A, Libero ML, et al. A grape (Vitis vinifera L.) pomace water extract modulates inflammatory and immune response in SW-480 cells and isolated mouse colon. *Phytother Res* (2022) 36:12. doi: 10.1002/ptr.7581

49. Mancini M, Cerny MEV, Cardoso NS, Verissimo G, Maluf SW. Grape seed components as protectors of inflammation, DNA damage, and cancer. *Curr Nutr Rep* (2023) 12:141–150. doi: 10.1007/s13668-023-00460-5

50. Copetti C, Franco FW, MaChado EDR, Soquetta MB, Quatrin A, Ramos VDM, et al. Acute consumption of bordo grape juice and wine improves serum antioxidant status in healthy individuals and inhibits reactive oxygen species production in human neuron-like cells. *J Nutr Metab* (2018) 2018:4384012. doi: 10.1155/2018/4384012

51. Mahdipour R, Ebrahimzadeh-Bideskan A, Hosseini M, Shahba S, Lombardi G, Malvandi AM, et al. The benefits of grape seed extract in neurological disorders and brain aging. *Nutr Neurosci* (2022) 26(5):369–83. doi: 10.1080/1028415X.2022.2051954

52. Leal C, Santos RA, Pinto R, Queiroz M, Rodrigues M, Saavedra MJ, et al. Recovery of bioactive compounds from white grape (Vitis vinifera L.) stems as potential antimicrobial agents for human health. *Saudi J Biol Sci* (2020) 27(4):1009–15. doi: 10.1016/j.sjbs.2020.02.013

53. Elgizawy HA, Ali AA, Hussein MA. Resveratrol: isolation, and its nanostructured lipid carriers, inhibits cell proliferation, induces cell apoptosis in certain human cell lines carcinoma and exerts protective effect against paraquat-induced hepatotoxicity. *J Med Food* (2021) 24(1):89–100. doi: 10.1089/jmf.2019.0286

54. Calabriso N, Massaro M, Scoditti E, Verri T, Barca A, Gerardi C, et al. Grape pomace extract attenuates inflammatory response in intestinal epithelial and endothelial cells: Potential health-promoting properties in bowel inflammation. *Nutrients* (2022) 14(6):1175. doi: 10.3390/nu14061175

55. Decorde K, Agne A, Lacan D, Ramos J, Fouret G, Ventura E, et al. Preventive effect of a melon extract rich in superoxide scavenging activity on abdominal and liver fat and adipokine imbalance in high-fat-fed hamsters. *J Agric Food Chem* (2009) 57 (14):6461–7. doi: 10.1021/jf900504g

56. González-Abuín N, Martínez-Micaelo N, Margalef M, Blay M, Arola-Arnal A, Muguerza B, et al. A grape seed extract increases active glucagon-like peptide-1 levels after an oral glucose load in rats. *Food Funct* (2014) 5(9):2357–64. doi: 10.1039/c4fo00447g

57. Dulundu E, Ozel Y, Topaloglu U, Toklu H, Ercan F, Gedik N, et al. Grape seed extract reduces oxidative stress and fibrosis in experimental biliary obstruction. *J Gastroenterol Hepatol* (2007) 22(6):885–92. doi: 10.1111/j.1440-1746.2007.04875.x

58. Choi SK, Zhang XH, Seo JS. Suppression of oxidative stress by grape seed supplementation in rats. *Nutr Res Pract* (2012) 6(1):3–8. doi: 10.4162/nrp.2012.6.1.3

59. Zhang JQ, Wang XW, Chen JF, Ren QL, Wang J, Gao BW, et al. Grape seed procyanidin B2 protects porcine ovarian granulosa cells against oxidative stress-induced apoptosis by upregulating let-7a expression. *Oxid Med Cell Longev* (2019) 2019:1076512. doi: 10.1155/2019/1076512

60. Barbe A, Mellouk N, Ramé C, Grandhaye J, Anger K, Chahnamian M, et al. A grape seed extract maternal dietary supplementation improves egg quality and reduces ovarian steroidogenesis without affecting fertility parameters in reproductive hens. *PloS One* (2020) 15(5):e0233169. doi: 10.1371/journal.pone.0233169

61. Rajput SA, Sun L, Zhang NY, Khalil MM, Ling Z, Chong L, et al. Grape seed proanthocyanidin extract alleviates aflatoxinB1-induced immunotoxicity and oxidative stress via modulation of NF-κB and Nrf2 signaling pathways in broilers. *Toxins* (2019) 11:1. doi: 10.3390/toxins11010023

62. Yu F, Li BY, Li XL, Cai Q, Zhang Z, Cheng M, et al. Proteomic analysis of aorta and protective effects of grape seed procyanidin B2 in db/db mice reveal a critical role of milk fat globule epidermal growth factor-8 in diabetic arterial damage. *PloS One* (2012) 7(12):e52541. doi: 10.1371/journal.pone.0052541.e52541

63. Yin W, Li B, Li X, Yu F, Cai Q, Zhang Z, et al. Critical role of prohibition in endothelial cell apoptosis caused by glycated low-density lipoproteins and protective effects of grape seed procyanidin B2. *J Cardiovasc Pharmacol* (2015) 65(1):13–21. doi: 10.1097/fjc.0000000000000157

64. Qian YP, Cai YJ, Fan GJ, Wei QY, Yang J, Zheng LF, et al. Antioxidant-based lead discovery for cancer chemoprevention: the case of resveratrol. *J Med Chem* (2009) 52(7):1963–74. doi: 10.1021/jm8015415

65. Yilmaz Y, Toledo RT. Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. *J Agric Food Chem* (2004) 52(2):255–60. doi: 10.1021/jf030117h

66. Anastasiadi M, Chorianopoulos NG, Nychas GJE, Haroutounian SA. Antilisterial activities of polyphenol-rich extracts of grapes and vinification byproducts. *J Agric Food Chem* (2009) 57(2):457–63. doi: 10.1021/jf8024979

67. Sahin K, Akdemir FATİ. H., Orhan C, Tuzcu M, Hayirli A, Sahin N. Effects of dietary resveratrol supplementation on egg production and antioxidant status. *Poult Sci* (2010) 89(6):1190–8. doi: 10.3382/ps.2010-00635

68. Chen Q, Liu XF, Zheng PS. Grape seed proanthocyanidins (GSPs) inhibit the growth of cervical cancer by inducing apoptosis mediated by the mitochondrial pathway. *PloS One* (2014) 9(9):e107045. doi: 10.1371/journal.pone.0107045

69. Said RS, El-Demerdash E, Nada AS, Kamal MM. Resveratrol inhibits inflammatory signaling implicated in ionizing radiation-induced premature ovarian failure through antagonistic crosstalk between silencing information regulator 1 (SIRT1) and poly (ADP-ribose) polymerase 1 (PARP-1). *Biochem Pharmacol* (2016) 103:140–50. doi: 10.1016/j.bcp.2016.01.019

70. Zhang JQ, Gao BW, Wang J, Ren QL, Chen JF, Ma Q, et al. Critical role of FoxO1 in granulosa cell apoptosis caused by oxidative stress and protective effects of grape seed procyanidin B2. *Oxid Med Cell Longev* (2016) 2016:16. doi: 10.1155/2016/6147345.6147345

71. Liu X, Lin X, Mi Y, Li J, Zhang C. Grape seed proanthocyanidin extract prevents ovarian aging by inhibiting oxidative stress in the hens. *Oxid Med Cell Longev* (2018) 2018:16. doi: 10.1155/2018/9390810.9390810z

72. Dull AM, Moga MA, Dimienescu OG, Sechel G, Burtea V, Anastasiu CV. Therapeutic approaches of resveratrol on endometriosis via anti-inflammatory and anti-angiogenic pathways. *Molecules* (2019) 24(4):667. doi: 10.3390/molecules24040667

73. Citrinovitz ACM, Langer L, Strowitzki T, Germeyer A. Resveratrol enhances decidualization of human endometrial stromal cells. *Reproduction* (2020) 159(4):453–63. doi: 10.1530/REP-19-0425

74. Luo Y, Zhuan Q, Li J, Du X, Huang Z, Hou Y, et al. Procyanidin B2 improves oocyte maturation and subsequent development in type 1 diabetic mice by promoting mitochondrial function. *Reprod Sci* (2020) 27:2211–22. doi: 10.1007/s43032-020-00241-3

75. Spicer LJ, Schütz LF. Effects of grape phenolics, myricetin and piceatannol, on bovine granulosa and theca cell proliferation and steroid production in *vitro*. *Food Chem Toxicol* (2022) 167:113288. doi: 10.1016/j.fct.2022.113288

76. Chen SE, McMurtry JP, Walzem RL. Overfeeding-induced ovarian dysfunction in broiler breeder hens is associated with lipotoxicity. *Poult Sci* (2006) 85(1):70–81. doi: 10.1093/ps/85.1.70

77. Richards MP, Proszkowiec-Weglarz M. Mechanisms regulating feed intake, energy expenditure, and body weight in poultry. *Poult Sci* (2007) 86(7):1478–90. doi: 10.1093/ps/86.7.1478

78. Predescu DV, Crețoiu SM, Crețoiu D, Alexandra Pavelescu L, Suciu N, Radu BM, et al. G protein-coupled receptors (GPCRs)-mediated calcium signaling in ovarian cancer: Focus on GPCRs activated by neurotransmitters and inflammation-associated molecules. *Int J Mol Sci* (2019) 20(22):5568. doi: 10.3390/ijms20225568

79. Snider AP, Wood JR. Obesity induces ovarian inflammation and reduces oocyte quality. *Reproduction* (2019) 158(3):R79–90. doi: 10.1530/REP-18-0583

80. Wang Y, Nicholes K, Shih IM. The origin and pathogenesis of endometriosis. *Annu Rev Pathol* (2020) 15:71–95. doi: 10.1146/annurev-pathmechdis-012419-032654

81. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnol Adv* (2015) 33(8):1582–614. doi: 10.1016/j.bioteChadv.2015.08.001

82. Forni C, Facchiano F, Bartoli M, Pieretti S, Facchiano A, D'Arcangelo D, et al. Beneficial role of phytochemicals on oxidative stress and age-related diseases. *BioMed Res Int* (2019) 2019:8748253. doi: 10.1155/2019/8748253

83. Wocławek-Potocka I, Mannelli C, Boruszewska D, Kowalczyk-Zieba I, Waśniewski T, Skarżyński DJ. Diverse effects of phytoestrogens on the reproductive performance: cow as a model. *Int J Endocrinol* (2013) 2013:650984. doi: 10.1155/2013/650984

84. Ly C, Yockell-Lelievre J, Ferraro ZM, Arnason JT, Ferrier J, Gruslin A. The effects of dietary polyphenols on reproductive health and early development. *Hum Reprod* (2015) 21(2):228–48. doi: 10.1093/humupd/dmu058

85. Olaku OO, Ojukwu MO, Zia FZ, White JD. The role of grape seed extract in the treatment of chemo/radiotherapy induced toxicity: a systematic review of preclinical studies. *Nutr Cancer* (2015) 67(5):730–40. doi: 10.1080/01635581.2015.1029639

86. Barbe A, Bongrani A, Mellouk N, Estienne A, Kurowska P, Grandhaye J, et al. Mechanisms of adiponectin action in fertility: an overview from gametogenesis to gestation in humans and animal models in normal and pathological conditions. *Int J Mol Sci* (2019) 20(7):1526. doi: 10.3390/ijms20071526

87. Shen M, Lin F, Zhang J, Tang Y, Chen WK, Liu H. Involvement of the up-regulated FoxO1 expression in follicular granulosa cell apoptosis induced by oxidative stress. *J Biol Chem* (2012) 287(31):25727–40. doi: 10.1074/jbc.M112.349902

88. Sun YL, Tang SB, Shen W, Yin S, Sun QY. Roles of resveratrol in improving the quality of postovulatory aging oocytes in *vitro*. *Cells* (2019) 8(10):1132. doi: 10.3390/cells8101132

89. Karimian M, Zandi M, Sanjabi MR, Masoumian M, Ofoghi H. Effects of grape seed extract, quercetin and vitamin C on ovine oocyte maturation and subsequent embryonic development. *Cell Mol Biol* (2018) 64(4):98–102. doi: 10.14715/cmb/2018.64.4.16

90. Sedighi P, Helli B, Sharhani A, Vatanpur A. Effects of grape seed extract supplementation on fasting blood glucose, insulin resistance, and lipid profile in women with polycystic ovary syndrome. *Anat Sci J* (2020) 17(2):73–82.

91. Cutts JK, Peavy TR, Moore DR, Prasain J, Barnes S, Kim H. Ovariectomy lowers urine levels of unconjugated (+)-catechin,(−)-epicatechin, and their methylated metabolites in rats fed grape seed extract. *Horm Mol Biol Clin Investig* (2013) 16(3):129–38. doi: 10.1515/hmbci-2013-0044

92. Yıldırım Ş., Topaloğlu N, Tekin M, Küçük A, Erdem H, Erbaş M, et al. Protective role of Proanthocyanidin in experimental ovarian torsion. *Med J Islam Repub Iran* (2015) 29:185.

93. Barbe A, Ramé C, Mellouk N, Estienne A, Bongrani A, Brossaud A, et al. Effects of grape seed extract and proanthocyanidin B2 on in *vitro* proliferation, viability, steroidogenesis, oxidative stress, and cell signaling in human granulosa cells. *Int J Mol Sci* (2019) 20(17):4215. doi: 10.3390/ijms20174215

94. Colitti M, Sgorlon S, Stradaioli G, Farinacci M, Gabai G, Stefanon B. Grape polyphenols affect mRNA expression of PGHS-2, TIS11b and FOXO3 in endometrium of heifers under ACTH-induced stress. *Theriogenology* (2007) 68(7):1022–30. doi: 10.1016/j.theriogenology.2007.07.018

95. Bruner-Tran KL, Osteen KG, Taylor HS, Sokalska A, Haines K, Duleba AJ. Resveratrol inhibits development of experimental endometriosis in *vivo* and reduces endometrial stromal cell invasiveness in *vitro*. *Biol Reprod* (2011) 84(1):106–12. doi: 10.1095/biolreprod.110.086744

96. Hashem NM, Gonzalez-Bulnes A, Simal-Gandara J. Polyphenols in farm animals: source of reproductive gain or waste? *Antioxidants* (2020) 9(10):1023. doi: 10.3390/antiox9101023

97. Yildiz HB, Kiralp S, Toppare L, Yagci Y. Immobilization of tyrosinase in poly (ethyleneoxide) electrodes and determination of phenolics in red wines. *React Funct Polym* (2005) 63(2):155–61. doi: 10.1016/j.reactfunctpolym.2005.02.016

98. Meeprom A, Sompong W, Suwannaphet W, Yibchok-anun S, Adisakwattana S. Grape seed extract supplementation prevents high-fructose diet-induced insulin resistance in rats by improving insulin and adiponectin signaling pathways. *Br J Nutr* (2011) 106(8):1173–81. doi: 10.1017/S0007114511001589

99. Eng ET, Ye J, Williams D, Phung S, Moore RE, Young MK, et al. Suppression of estrogen biosynthesis by procyanidin dimers in red wine and grape seeds. *Cancer Res* (2003) 63(23):8516–22.

100. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell* (2007) 129(7):1261–74. doi: 10.1016/j.cell.2007.06.009

101. Homayoun M, Targhi RG, Soleimani M. Anti-proliferative and anti-apoptotic effects of grape seed extract on chemo-resistant OVCAR-3 ovarian cancer cells. *Res Pharm Sci* (2020) 15(4):390. doi: 10.4103/1735-5362.293517

102. Kara K, Kocaoğlu Güçlü B, Baytok E, Şentürk M. Effects of grape pomace supplementation to laying hen diet on performance, egg quality, egg lipid peroxidation and some biochemical parameters. *J Appl Anim Res* (2016) 44(1):303–10. doi: 10.1080/09712119.2015.1031785

103. Galli GM, Da Silva AS, Biazus AH, Reis JH, Boiago MM, Topazio JP, et al. Feed addition of curcumin to laying hens showed anticoccidial effect, and improved egg quality and animal health. *Res Vet Sci* (2018) 118:101–6. doi: 10.1016/j.rvsc.2018.01.022

104. Reis JH, Gebert RR, Barreta M, Boiago MM, Souza CF, Baldissera MD, et al. Addition of grape pomace flour in the diet on laying hens in heat stress: Impacts on health and performance as well as the fatty acid profile and total antioxidant capacity in the egg. *J Therm Biol* (2019) 80:141–9. doi: 10.1016/j.jtherbio.2019.01.003

105. Mikuła-Pietrasik J, Sosińska P, Murias M, Wierzchowski M, Brewińska-Olchowik M, Piwocka K, et al. High potency of a novel resveratrol derivative, 3, 3′, 4, 4′-tetrahydroxy-trans-stilbene, against ovarian cancer is associated with an oxidative stress-mediated imbalance between DNA damage accumulation and repair. *Oxid Med Cell Longev* (2015) 2015:135691. doi: 10.1155/2015/135691

106. Tousson E, Elgharabawy RM, Elmasry TA. Grape seed proanthocyanidin ameliorates cardiac toxicity induced by boldenone undecylenate through inhibition of NADPH oxidase and reduction in the expression of NOX2 and NOX4. *Oxid Med Cell Longev* (2018) 2018:9434385. doi: 10.1155/2018/9434385

107. Salmabadi Z, Kouchesfahani HM, Parivar K, Karimzadeh L. Effect of grape seed extract on lipid profile and expression of interleukin-6 in polycystic ovarian syndrome wistar rat model. *Int J Fertil Steril* (2017) 11(3):176–93. doi: 10.22074/ijfs.2017.5007

10.3389/fendo.2023.1245512

Glossary

ADP	adenosine diphosphate
AMH	anti-Müllerian hormone
BAD	Bcl-2-associated death promoter protein
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma protein 2
CDKI	cyclin-dependent kinase inhibitor
CREB	cyclic adenosine monophosphate response element-binding protein
ERK1/2	extracellular signal-regulated kinases ½
ERα	estrogen receptors alpha
ERß	estrogen receptors beta
ESC	endometrial stromal cell
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
H_2O_2	hydrogen peroxide
HeLa	human cervical cancer cells
hGC	primary luteinized human granulosa cells
IGF1	insulin-like growth factor 1
IGFBP	insulin-like growth factor binding protein 1
KGN	human ovarian granulosa-like tumor cell line
let-7a	a member of the let-7 miRNA family
LH	luteinizing hormone
MAP	mitogen-activated protein
MAPK	mitogen-activated protein kinase
MCF-7	human breast cancer cells
MUFA	monounsaturated fatty acids
NF-κB	nuclear factor kappa B
NOX	NADPH oxidase
PARP	poly(ADP-ribose) polymerase
PARP-1	poly(ADP-ribose)polymerase-1
PCOS	polycystic ovary syndrome
PI3K	phosphoinositide 3-kinase
PPAR-γ	peroxisome proliferator-activated receptor γ
PUFAs	polyunsaturated fatty acids
ROS	reactive oxygen species
SFA	saturated fatty acids
SIRT1	sirtuin 1
SOD	superoxide dismutase
StAR	steroidogenic acute regulatory protein
YB-1	Y-box binding protein 1

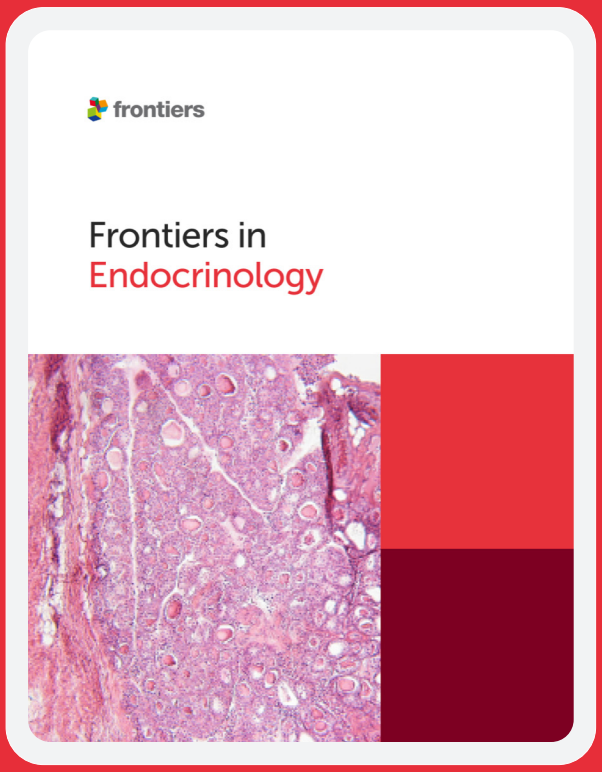
Frontiers in Endocrinology

Explores the endocrine system to find new therapies for key health issues

The second most-cited endocrinology and metabolism journal, which advances our understanding of the endocrine system. It uncovers new therapies for prevalent health issues such as obesity, diabetes, reproduction, and aging.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact