frontiers | Research Topics

Herbal medicines and their metabolites: Effects on lipid metabolic disorders via modulating oxidative stress

Edited by
Wei Peng, Xiaofei Zhang, Yongmei Xie, Cailu Lin and Adolfo Andrade-Cetto

Coordinated by
Yunhui Chen

Published in
Frontiers in Pharmacology

ISSN 1664-8714
ISBN 978-2-8325-4145-6
DOI 10.3389/978-2-8325-4145-6

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

Herbal medicines and their metabolites: Effects on lipid metabolic disorders via modulating oxidative stress

Topic editors

Wei Peng — Chengdu University of Traditional Chinese Medicine, China
Xiaofei Zhang — Shaanxi University of Chinese Medicine, China
Yongmei Xie — Sichuan University and Collaborative Innovation Center of Biotherapy, China
Cailu Lin — Monell Chemical Senses Center, United States
Adolfo Andrade-Cetto — National Autonomous University of Mexico, Mexico

Topic Coordinator

Yunhui Chen — Chengdu University of Traditional Chinese Medicine, China

Citation

Peng, W., Zhang, X., Xie, Y., Lin, C., Andrade-Cetto, A., Chen, Y., eds. (2023). *Herbal medicines and their metabolites: Effects on lipid metabolic disorders via modulating oxidative stress*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-4145-6

Table of contents

frontiers | Frontiers in Pharmacology

TYPE Editorial
PUBLISHED 15 September 2023
DOI 10.3389/fphar.2023.1279429

OPEN ACCESS

EDITED BY
Javier Echeverria,
University of Santiago, Chile

REVIEWED BY
Luca Rastrelli,
University of Salerno, Italy

*CORRESPONDENCE
Yunhui Chen,
chenyunhui@cdutcm.edu.cn
Wei Peng,
pengwei@cdutcm.edu.cn

RECEIVED 18 August 2023
ACCEPTED 12 September 2023
PUBLISHED 15 September 2023

CITATION
Chen Y, Xie Y, Lin C and Peng W (2023), Editorial: Herbal medicines and their metabolites: effects on lipid metabolic disorders via modulating oxidative stress. *Front. Pharmacol.* 14:1279429. doi: 10.3389/fphar.2023.1279429

Editorial: Herbal medicines and their metabolites: effects on lipid metabolic disorders via modulating oxidative stress

Yunhui Chen[1]*, Yongmei Xie[2], Cailu Lin[3] and Wei Peng[1]*

[1]School of Basic Medical Sciences, CDUTCM-KEELE Joint Health and Medical Sciences Institute, School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, China, [3]Monell Chemical Senses Center, Philadelphia, United States

KEYWORDS

herbal medicines, natural metabolites, oxidative stress, lipids, lipid metabolic disorders

Editorial on the Research Topic
Herbal medicines and their metabolites: effects on lipid metabolic disorders via modulating oxidative stress

Lipids, predominantly triglycerides, phospholipids, and sterols, play pivotal roles in human physiology, particularly nutrient regulation. However, imbalances in lipid metabolism may precipitate a range of severe health conditions, such as non-alcoholic fatty liver diseases, hyperlipidemia, diabetes mellitus, and atherosclerosis. An emerging concern is oxidative stress, a detrimental state arising from an imbalance between oxidation and antioxidation, primarily due to the surge in reactive oxygen species. Mounting evidence associates oxidative stress and mitochondrial dysfunction with lipid metabolic disorders. Intriguingly, recent studies have spotlighted the potential of herbal medicines and their metabolites in modulating oxidative stress and countering these disorders. This Research Topic, "*Herbal Medicines and Their Metabolites: Effects on Lipid Metabolic Disorders via Modulating Oxidative Stress*", has provided an academic platform for scholars to delve into the potential of various herbal interventions in addressing metabolic challenges, and 11 good quality works are collected in our Research Topic.

The original research by Zhang et al. explored the antidiabetic effects of hydroxy-α-sanshool (HAS) from *Zanthoxylum bungeanum* Maxim on streptozotocin-induced Type 2 diabetes mellitus (T2DM) mice and glucosamine (GlcN)-induced HepG2 cells, and the results indicated its mechanism of actions might attribute to the increase of hepatic glycogen synthesis by activating PI3K/Akt/GSK-3β/GS signaling pathway. Another experimental research by Cen et al. reported the ameliorative effects of anti-malarial artesunate, a succinate derivative of artemisinin, on atherogenic diet and lipopolysaccharide-induced atherosclerosis rat by mitigating arterial inflammatory responses via the inhibition of the NF-κB-NLRP3 inflammasome pathway.

Furthermore, a series of insightful and valuable reviews have been included to present the recent advances in the field. He et al. comprehensively summarized natural polysaccharides with promising therapeutic efficacy against T2DM and elaborated on their pharmacological mechanisms relevant to the oxidative stress network. Li et al. illustrated sixteen natural flavonoids as potential anti-atherogenic agents that inhibited oxidative stress in endothelial

cells, accentuating the importance of further research on quercetin and naringenin. Sheng et al. further enriched the preventive and therapeutic roles of catechins, a major group of flavonoids, against atherosclerosis via regulating antioxidant stress and improving abnormal lipid metabolism. Duan et al. reviewed the herbal medicines against low-density lipoprotein (LDL) oxidation and foam cell formation in lipid metabolism disorder, highlighting that herbal interventions could inhibit LDL oxidation and regulate cholesterol homeostasis via downregulating CD36 and SR-A, whereas upregulating ABCA1 and ABCG1. Luo et al. reported that botanical drugs could regulate radical oxygen species via multiple signaling pathways to improve oxidative stress and manage glucolipid metabolic diseases. Jin et al. elucidated the multifaceted molecular mechanisms through which ginsenosides regulate oxidative stress and lower blood lipids in treating hyperlipidemia and its associated diseases.

In addition, Xie et al. presented meta-analyses of animal studies to summarize the hypoglycemic effects of ginsenoside rg1 and provided evidence-based support for its efficacy in reducing MDA levels and restoring SOD activity to exert its antioxidant activity and had a positive effect on the reduction of IL-6 and TNF-α levels. Li et al. also conducted a meta-analysis to provide preclinical and clinical evidence for the treatment of NAFLD with soybean, and the results verified that soybean could protect the liver in NAFLD by regulating lipid metabolism and oxidative stress factors via the Akt/AMPK/PPARα signaling pathway. Moreover, an empirical study by Dai et al. testified that no substantial difference was noted in the effects between randomized controlled trials (RCTs) and non-randomized studies of interventions (NRSIs) in safety assessment when they have similar sample sizes, indicating evidence from NRSIs might be considered a supplement to RCTs for safety outcomes.

This research compendium has illuminated the profound potential of modulating oxidative stress with herbal medicines in treating various lipid metabolic disorders. These *in vivo* and *in vitro* experiments, reviews, and meta-analyses collectively accentuate the promise of targeting oxidative stress with herbal remedies against health challenges of lipid dysmetabolism. Nevertheless, several facets remain uncharted as we delve deeper into this area. More original research is warranted in the future to explore novel pharmacological insights into herbal medicines for managing lipid metabolic disorders; elucidate the intricate molecular mechanisms underlying the therapeutic effects of herbal medicines; identify herbal medicines that may potentially mitigate the side effects of contemporary lipid metabolic disorder drugs; and integrate such strategies as network pharmacology, artificial intelligence, and computer-aided design to discover herbal interventions targeting oxidative stress.

Author contributions

YC: Data curation, Writing–original draft. YX: Data curation, Writing–review and editing. CL: Formal Analysis, Writing–review and editing. WP: Conceptualization, Writing–review and editing, Writing–original draft.

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

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 13 December 2022
DOI 10.3389/fphar.2022.1089558

OPEN ACCESS

EDITED BY
Xiaofei Zhang,
Shaanxi University of Chinese Medicine, China

REVIEWED BY
Zhenzhen Zhang,
Naval Medical Research Institute, China
Jianguo Wu,
Fujian University of Traditional Chinese Medicine, China

*CORRESPONDENCE
Chun-Jie Wu,
wucjcdtcm@163.com
Li Ai,
aili@cdutcm.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Ethnopharmacology, a section of the journal Frontiers in Pharmacology

RECEIVED 04 November 2022
ACCEPTED 25 November 2022
PUBLISHED 13 December 2022

CITATION
Zhang Q, Li R-L, Wang L-Y, Zhang T, Qian D, Tang D-D, He C-X, Wu C-J and Ai L (2022), Hydroxy-α-sanshool isolated from *Zanthoxylum bungeanum* Maxim. has antidiabetic effects on high-fat-fed and streptozotocin-treated mice *via* increasing glycogen synthesis by regulation of PI3K/Akt/GSK-3β/GS signaling.
Front. Pharmacol. 13:1089558.
doi: 10.3389/fphar.2022.1089558

Hydroxy-α-sanshool isolated from *Zanthoxylum bungeanum* Maxim. has antidiabetic effects on high-fat-fed and streptozotocin-treated mice *via* increasing glycogen synthesis by regulation of PI3K/Akt/GSK-3β/GS signaling

Qing Zhang[1†], Ruo-Lan Li[1†], Ling-Yu Wang[1], Ting Zhang[1], Die Qian[1], Dan-Dan Tang[1], Cheng-Xun He[1], Chun-Jie Wu[2*] and Li Ai[3*]

[1]State Key Laboratory of Southwestern Chinese Medicine Resources, School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]Innovative Institute of Chinese Medicine and Pharmacy, Academy for Interdiscipline, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [3]School of Ethnic Medicine, Chengdu University of Traditional Chinese Medicine, Chengdu, China

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease characterized by hyperglycemia. The fruits of *Zanthoxylum bungeanum* Maxim. is a common spice and herbal medicine in China, and hydroxy-α-sanshool (HAS) is the most abundant amide in *Z. bungeanum* and reported to have significant hypoglycemic effects. The purpose of this study was to evaluate the ameliorative effects of HAS on T2DM and the potential mechanisms responsible for those effects. An acute toxicity test revealed the median lethal dose (LD50) of HAS is 73 mg/kg. C57BL/6 J mice were fed a high-fat diet and given an intraperitoneal injection of streptozotocin (STZ) to induce T2DM in mice to evaluate the hypoglycemic effects of HAS. The results showed that HAS significantly reduced fasting blood glucose, reduced pathological changes in the liver and pancreas, and increased liver glycogen content. In addition, glucosamine (GlcN)-induced HepG2 cells were used to establish an insulin resistance cell model and explore the molecular mechanisms of HAS activity. The results demonstrated that HAS significantly increases glucose uptake and glycogen synthesis in HepG2 cells and activates the PI3K/Akt pathway in GlcN-induced cells, as well as increases GSK-3β phosphorylation, suppresses phosphorylation of glycogen synthase (GS) and increases glycogen synthesis in liver cells. Furthermore, these effects of HAS were blocked by the PI3K inhibitor LY294002. The results of our study suggest that HAS reduces hepatic insulin resistance and increases hepatic glycogen synthesis by activating the PI3K/Akt/GSK-3β/GS signaling pathway.

KEYWORDS

type 2 diabetes mellitus, hydroxy-α-sanshool, *Zanthoxylum bungeanum* Maxim., glycogen synthesis, PI3K/Akt/GSK-3β pathway

Introduction

Increasing epidemiological evidence has revealed that type 2 diabetes mellitus (T2DM) is emerging as a considerable socioeconomic pressure on individuals and society due to the huge cost of associated healthcare (Yu et al., 2015). Patients with T2DM commonly have insulin deficiency due to insulin resistance and pancreatic β-cell dysfunction (Vishvanath and Gupta, 2019; Blahova et al., 2021). Insulin resistance is considered the predominant contributing factor in T2DM, which is mainly caused by obesity, sedentary lifestyle, and increasing life span (DeFronzo and Tripathy, 2009). In addition, T2DM is often accompanied by many complications, such as cardiovascular, kidney, and retinal diseases, which are becoming a serious challenge to the improvement and maintenance of human health. Currently, metformin is the first-line drug for treating T2DM. Other available drugs include α-glucosidase inhibitors (such as acarbose), sulfonylureas (such as glimepiride), and DPP-4 inhibitors (such as sitagliptin). However, all available drugs have serious side effects, such as renal impairment, gastrointestinal disorders, and hypoglycemia, among others (Deng et al., 2017). Therefore, the identification of more candidate antidiabetic monomers from natural herbal medicines, with relatively fewer and less serious side effects, will be beneficial in the prevention and clinical treatment of T2DM.

The fruit of *Zanthoxylum bungeanum* Maxim. (Rutaceae family) has been a popular spice and herbal medicine in China for thousands of years. Hydroxy-α-sanshool ($C_{16}H_{25}NO_2$, HAS) is the primary active amide isolated from the fruit of *Z. bungeanum*, which has versatile bioactivities, such as anti-Alzheimer's disease, anti-obesity, and lipid-lowering effects (Wang et al., 2019; Li et al., 2020). In addition, previous studies have reported that amides in the fruit of *Z. bungeanum* have potential hypoglycemic effects (You et al., 2015; Ren et al., 2017a; Ren et al., 2017b). As part of our continuing work in exploring the medical value of the fruit of *Z. bungeanum*, we evaluated the antidiabetic effects of HAS on high-fat-fed and streptozotocin-treated mice, and further explored the associated molecular mechanisms. Our study results suggest that the development of HAS as a clinical treatment for T2DM would be beneficial.

Materials and methods

Animals

Male C57BL/6 J mice (20 ± 2 g) and ICR mice (20 ± 2 g) were obtained from Si Pei Fu Biotech (http://www.spf-tsinghua.com/, Beijing, China). This study was carried out following international guidelines for animal experiments and approved by the Animal Care and Use Committee of Chengdu University of Traditional Chinese Medicine (Ethical approval: No. 2020-27). The animals were adaptively reared in an SPF-grade environment for 1 week on a 12 h light/12 h dark cycle. The indoor temperature was 22°C and the relative humidity was 50%–65%. Animals were allowed to eat and drink freely during this period.

Cells

The human hepatocellular cancer cell line HepG2 was purchased from the Guangzhou Genio Biological Co., Ltd. (Guangzhou, China, https://www.jennio-bio.com/). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), a high sugar medium containing 10% FBS, at 37°C in a humidified, 5% CO_2 atmosphere.

Chemicals and reagents

High-fat diet (HFD, NutriPhenomics®) was supplied by Research Diets, Inc. (New Brunswick, NJ, United States). Blood glucose meters and test strips were obtained from Roche (Shanghai, China). Serum ALT, AST, TC, and TG biochemical assay kits were purchased from Mindray Bio-Medical Electronics Co., Ltd. (Shenzhen, China). Glycated serum protein (GSP) detection kits were obtained from Shanghai Enzyme Linked Biotech (Shanghai, China). Insulin detection kits were obtained from Shenzhen Highcreation Technology Co., Ltd. (Shenzhen, China). Catalase (CAT), malonaldehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH) detection kits were purchased from Suzhou Michy Biomedical Technology Co., Ltd. (Suzhou, China). Glycogen detection kits were obtained from Suzhou Comin Biotechnology Co., Ltd. (Suzhou, China), and the PAS staining kits were purchased from Beijing Solarbio Technology Co., Ltd. (Beijing, China). Streptozotocin (STZ) and 2-NBDG were obtained from Shanghai Maokang Biotechnology Co., Ltd. (Shanghai, China). The EdU staining kit was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Glucosamine was purchased from Beyotime Biotechnology (Shanghai, China). LY294002 was purchased from MedChemExpress (Shanghai, China). Enhanced chemiluminescence (ECL) reagent was obtained from Beijing 4A Biotech Co. (Beijing, China). Primary antibodies for glycogen synthase (GS), phosphorylated (p)-GS, p-GSK-3β, and β-tubulin

were obtained from Abmart Medical Technology Co., Ltd. (Shanghai, China). Primary antibodies for p-PI3K, PI3K, p-Akt, and Akt were purchased from Abclonal Biotechnology Co., Ltd. (Wuhan, China). The GSK-3β primary antibody was supplied from Santa Cruz Biotechnology, Inc. (CA, United States).

Extraction and isolation

The fruit of *Z. bungeanum* was collected from Hanyuan, Sichuan Province, China in August 2020, and identification was confirmed by Prof. Chunjie Wu (School of Pharmacy, CDUTCM). A voucher specimen (#20200822) was deposited in our laboratory. HAS was extracted from the fruit following our previously established method (Zhang et al., 2019). Briefly, the fruit of *Z. bungeanum* (50 g) was crushed into powder and extracted three times, using methanol (1000 ml) and an ultrasonic extractor, for 60 min each time at 40°C. The extracts were then filtered and the filtrates were dried *via* vacuum evaporation. Next, the extract was placed in water and extracted further with ethyl acetate (EtOAc) to obtain the EtOAc fraction. The EtOAc fraction was subjected to repeated silica gel (200–300 mesh) column chromatography and eluted with petroleum ether–EtOAc (v/v, 2:1–1:2). Combination of similar fractions based on TLC analysis resulted in three fractions (I-III). By using prepared high-performance liquid chromatography (HPLC), crude HAS was isolated from fraction II. After recrystallization with complex solvents (EtOAC: n-hexane, v/v, 1: 1), HAS (200 mg) with a purity of over 98% was obtained. The identification of HAS was confirmed by comparing its HPLC chromatographic analysis data with a standard reference sample of HAS (purity ≥ 98%, PUSH Bio-Technology, Chengdu, China).

HAS Toxicity evaluation

For the acute toxicity test, 60 ICR mice were randomly divided into six groups (n = 10). Mice from each group were given an oral dose of HAS at 40.96, 51.20, 64.00, 80.00, 100.00, or 125.00 mg/kg, respectively. The mortality rates of the mice within a 24 h period were recorded, and the median lethal dose (LD50) value was calculated using the Bliss method.

Type 2 diabetic mouse model and HAS treatment

Male C57BL/6 J mice (20 ± 2 g) were prepared as a model of T2DM. After adaptive feeding for 1 week, except for the mice in the normal control (NC) and NC + HAS (5 mg/kg) groups (n = 10), mice were administered HFD. After 1 month of continued feeding, mice in the HFD group were intraperitoneally injected with 40 mg/kg/d STZ (prepared in citrate buffer, pH 4.0) for 7 days, and the mice that were fed a normal diet received the same procedure but with the injection of citrate buffer only. The HFD + STZ mice with fasting blood glucose (FBG) levels higher than 11.1 mmol/L were then selected for the following experiments. The mice were randomly divided into five groups, including HFD/STZ (HFD feeding plus STZ treatment), HFD/STZ + Metformin (Met 150 mg/kg), HFD/STZ + HAS-L (HAS 1.25 mg/kg), HFD/STZ + HAS-M (HAS 2.5 mg/kg), and HFD/STZ + HAS-H (HAS 5 mg/kg), with 10 mice in each experimental group (Gao et al., 2016). Subsequently, HAS was dissolved in 0.5% CMC-Na, and mice in the NC + HAS (5 mg/kg) and HFD/STZ + HAS (1.25, 2.5, 5 mg/kg) groups were administered HAS orally at doses of 5 mg/kg, 1.25 mg/kg, 2.5 mg/kg, or 5 mg/kg for 4 weeks, respectively. The positive control treatment used in this study was Met, which was administered daily by gavage at 150 mg/kg. The mice in the NC and HFD/STZ groups received vehicle solvent. During the experiment, changes in blood glucose, body weight, feed intake, and water intake were recorded.

After 4 weeks of treatment, FBG was assessed by tail vein blood sampling (Roche et al., 2020) after 12 h of fasting. Following body weight measurement, blood samples were collected from the mice by orbital blood sampling. Blood samples were processed by centrifugation at 3000 rpm for 10 min, and serum was separated and stored at -80°C for subsequent biochemical analysis. Changes in liver morphology and color were noted, and weight was recorded. The liver and pancreas were then fixed and frozen for subsequent analysis.

Oral glucose tolerance test

One week before the end of the experiment, mice were fasted without water for 12 h. Mice in each group received an intragastric glucose solution (2 g/kg) to initiate an oral glucose tolerance test (OGTT). Blood glucose was measured by tail tip blood sampling at 0, 30, 60, and 120 min after injection, and the area under the blood glucose curve (AUC) was calculated.

Insulin tolerance tests

One week before the end of the experiment, mice were fasted without water for 12 h, and intraperitoneal injection of 0.75 U/kg insulin was performed to initiate an insulin tolerance test (ITT). Blood glucose was measured by tail tip blood sampling at 0, 30, 60, and 120 min after injection, and the AUC was calculated.

Serum biochemical tests

The levels of alanine transaminase (ALT), glutamic-oxalacetic transaminase (AST), triglyceride (TG), and total

cholesterol (TC) in the serum of mice were detected using an automatic biochemical instrument system. The levels of insulin and GSP in serum were detected according to kit instructions.

Histopathological examinations

Liver and pancreas tissues were fixed in 10% neutral buffered formalin and subsequently embedded in paraffin, sectioned at 5 μm, de-paraffinized, and stained with hematoxylin-eosin (H and E). In addition, PAS staining was carried out to assess liver glycogen. Finally, histopathological changes and the location and abundance of glycogen in liver tissues were observed using an optical microscope.

Determination of the oxidative stress indices in liver

Frozen liver tissue was ground with a cryogenic tissue grinder. Supernatant was collected by centrifugation and the protein concentration of the liver tissue fluid was detected using a BCA kit. The levels of SOD, CAT, MDA, and GSH in the liver were detected according to commercial kit instructions.

Determination of glycogen levels in the liver

Frozen liver tissue was ground with a cryogenic tissue grinder. The supernatant was collected and the glycogen content in liver tissue was determined according to the instructions of the tissue glycogen detection kit.

Cell culture and treatment

HepG2 cells were seeded into 96-well plates (1×10^4 cells/well). After cell adherence, different concentrations of HAS (0–60 μg/ml) were added to the culture, and the cells were incubated for another 24 h. The CCK-8 kit was then used to detect cell viability. The cell survival rate after HAS treatment was calculated to determine the drug action concentration. Glucosamine was used to induce insulin resistance, as described previously (Sun et al., 2007). Briefly, HepG2 cells were incubated with 18 mM glucosamine (GlcN) in serum-free medium for 18 h to induce insulin resistance, followed by treatment with 2.5, 5, or 10 μg/ml HAS for 24 h. At the end of the treatment period, the CCK-8 kit was used to detect the survival rate of cells in each group. The PI3K/Akt inhibitor LY294002 (10 μM) and 10 μg/ml HAS were also used as a treatment for some cells as part of the experiment.

EdU staining

HepG2 cells were treated with 18 mM GlcN for 18 h. HepG2 cells were then incubated with 2.5, 5, or 10 μg/ml HAS for 24 h in the presence of GlcN. The cells were subsequently incubated with EdU staining solution for 2.5 h in the dark. Next, the cell samples were fixed using 4% paraformaldehyde, and cell nuclei were stained with Hoechst dye. The EdU-positive cells were then observed and photographed using a Leica SP8SR confocal laser microscope (Wetzlar, Germany).

Glycogen determination in HepG2 cells

After HAS treatment, the glycogen in the cells was stained *via* PAS staining solution and photographed. In addition, glycogen level was measured using a glycogen detection kit.

Glucose intake

The fluorescent labeled analogue of 2-deoxyglucose, 2-NBDG, can be used as a tracer to evaluate cellular glycogen metabolism and simulate glucose uptake by living cells. After drug treatment, the cells were starved for 12 h and then incubated with 2-NBDG (100 uM) for 30 min; the fluorescence intensity was observed or detected by laser confocal microscopy or flow cytometry.

Immunofluorescence assay

HepG2 cells were seeded into small glass-bottom dishes and received treatment with HAS. After HAS treatment, the cells were incubated with serum blocking solution and 0.3% Triton x-100 for 1 h at room temperature. The corresponding antibodies (GS, 1:300; p-GS, 1:300; GSK-3β, 1:300; p-GSK-3β, 1:300) were then added to the small dishes and incubated overnight at 4°C. The next day, the primary antibody was discarded, the corresponding fluorescent secondary antibody (1:500) was added, and incubation was continued for 1 h at room temperature. After incubation, cellular fluorescence was observed using a laser confocal microscope.

Western blotting

After HAS treatment, cells were harvested, total protein was extracted, and protein concentration was determined using a BCA kit. Denaturing reagents were subsequently added to the protein and samples were heated in preparation for western blotting (WB) experiments. The proteins were then fractionated by SDS/PAGE and transferred to a PVDF membrane. The PVDF

TABLE 1 LD50 value of HAS in mice.

	Dose (mg/kg)	Logarithmic dose	*n*	Death number	Death ratio (%)
1	125.00	2.097	10	10	100
2	100.00	2.000	10	9	90
3	80.00	1.903	10	6	60
4	64.00	1.806	10	3	30
5	51.20	1.709	10	1	10
6	40.96	1.612	10	0	0

LD50 = 73 mg/kg, 95% confidence interval 64.9–82.2 mg/kg.

membrane was incubated in blocking solution for 1 h and then incubated overnight with the corresponding primary antibody (GS, 1:800; p-GS, 1:800; GSK-3β, 1:800; p-GSK-3β, 1:800; AKT, 1:800; p-AKT, 1:800; PI3K, 1:800; p-PI3K, 1:800) at 4°C. The next day, the PVDF membrane was incubated with horseradish peroxidase-labeled secondary antibody (1:1000) for 1 h. Finally, an ECL solution was used to detect the target protein on the PVDF membrane. Expression of the target protein was normalized using β-tubulin as an internal reference standard, and the expression level of the target protein was calculated relative to β-tubulin expression.

Statistical analysis

Results were expressed as mean ± SD and the two-tailed Student's t-test, with a significance level of $p < 0.05$, was used in the statistical analysis of study data.

Results

HAS has a promising safety profile

We investigated the possible toxic effects of HAS. As shown in Table 1, we evaluated the acute toxicity of HAS *via* assessment of the median lethal dose (LD50). The LD50 value of orally administered HAS was 73 mg/kg. In addition, our results suggested HAS had an even better safety profile at doses less than 40.96 mg/kg. In our subsequent experiments, we used orally administered HAS at doses of 1.25, 2.50, and 5.00 mg/kg, which were less than 1/10 of the LD50 value.

HAS reduces body weight and blood glucose in T2DM mice

As shown in Figure 1A, changes in body weight were recorded during the experiment. Although HFD led to weight gain, the body weight of mice decreased significantly following continuous STZ injection. During the 4 weeks, mice in the HFD + STZ groups (T2DM mice) continued to lose weight, a characteristic feature of T2DM. Fortunately, weight loss in T2DM mice could be attenuated by HAS treatment. We found that, similar to the effects of Met treatment, the body weight of HAS-treated T2DM mice (HFD/STZ + HAS-treatment) was significantly higher than that of T2DM mice (Figure 1B). In addition, results shown in Supplementary Figure S1 also suggest that HAS treatments decrease water intake (Supplementary Figure S1A) and food intake (Supplementary Figure S1B) of T2DM mice. The blood glucose of the mice was also monitored during the observation period. After continuous treatment with HFD/STZ, the FBG of T2DM mice increased sharply in the first week and then decreased gradually from 2 weeks to 4 weeks. Importantly, like Met, HAS treatment decreased FBG in T2DM mice at all observation time points (Figure 1C). In fact, after 1 week of treatment with a high dose of HAS, the FBG of the T2DM mice decreased significantly ($p \leq 0.01$ vs. HFD + STZ, Supplementary Figure S1C). At week 3, 2.5 mg/kg HAS treatment also significantly reduced FBG in T2DM mice (Supplementary Figure S1C). At week 4, all HAS-treated groups showed significant improvement in FBG compared with the HFD + STZ group (Figure 1D). Furthermore, pathological changes in liver tissues from T2DM mice were observed. Liver tissue from normal mice was smooth and dark red in color, whereas the liver from the T2DM mice was rough and pale or pink in color. Additionally, the liver index of T2DM mice was significantly increased compared to that of the normal mice (Figure 1E). Interestingly, HAS treatment significantly attenuated the pathological changes induced by HFD + STZ (Figure 1E).

HAS improves liver function and reduces insulin resistance in T2DM mice

As shown in Figure 2A, compared with the NC mice, no obvious difference was observed in ALT or AST in NC + HAS

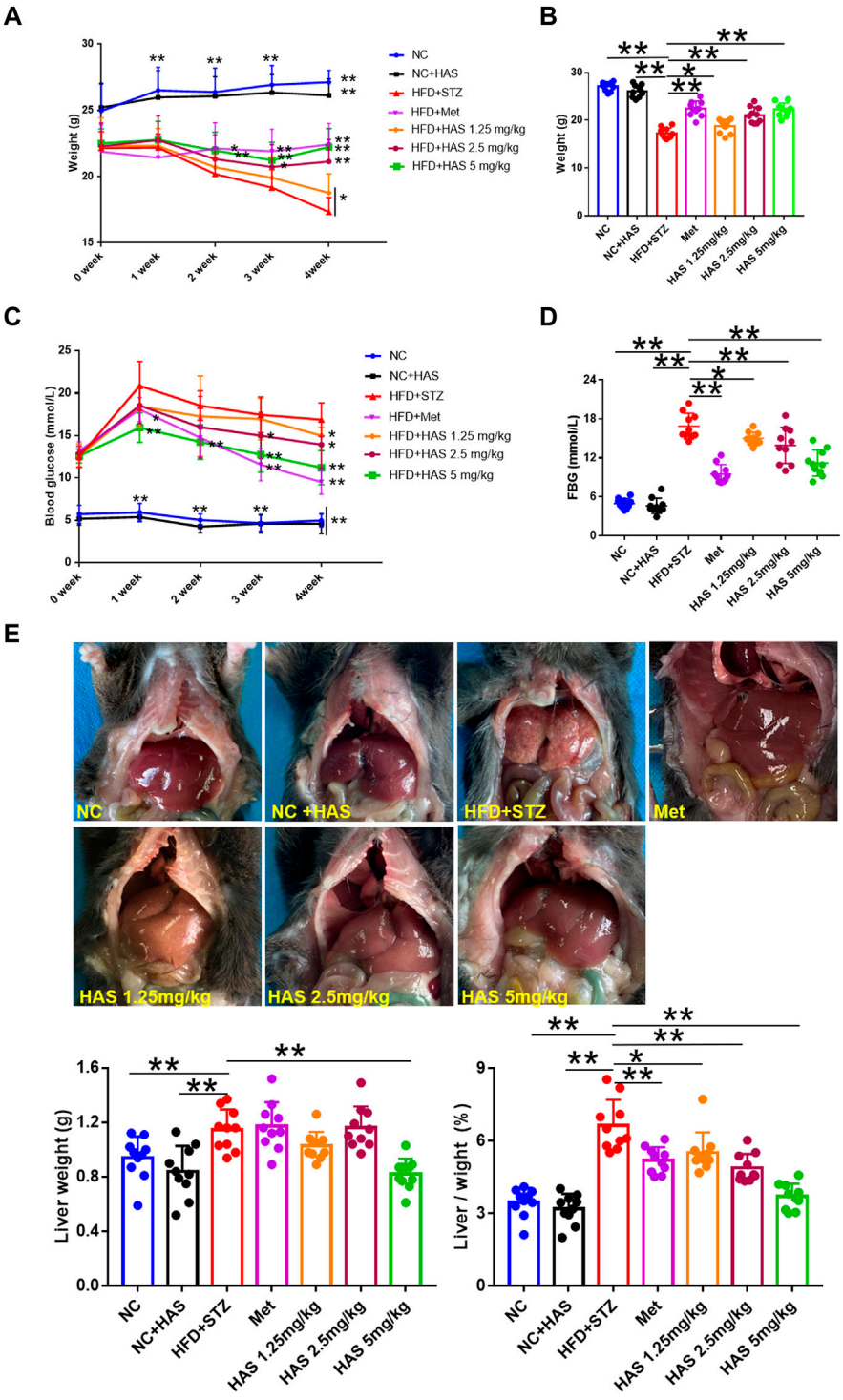


FIGURE 1
Effects of HAS on HFD + STZ mice. **(A,B)** HAS treatment resulted in increased body weight of HFD + STZ mice. **(C,D)** HAS treatment resulted in decreased fasting blood glucose in HFD + STZ mice. **(E)** Representative image of the liver of mice in different treatment groups; HAS treatment reduced liver weight and liver index in HFD + STZ mice. The doses of 2.5, 5.0, and 10 mg/kg indicate low, middle, and high doses of HAS, respectively, and the dose of Met was 150 mg/kg. Data are expressed as mean ± SD (n = 10), $^{**}p < 0.01$ vs. HFD + STZ group.

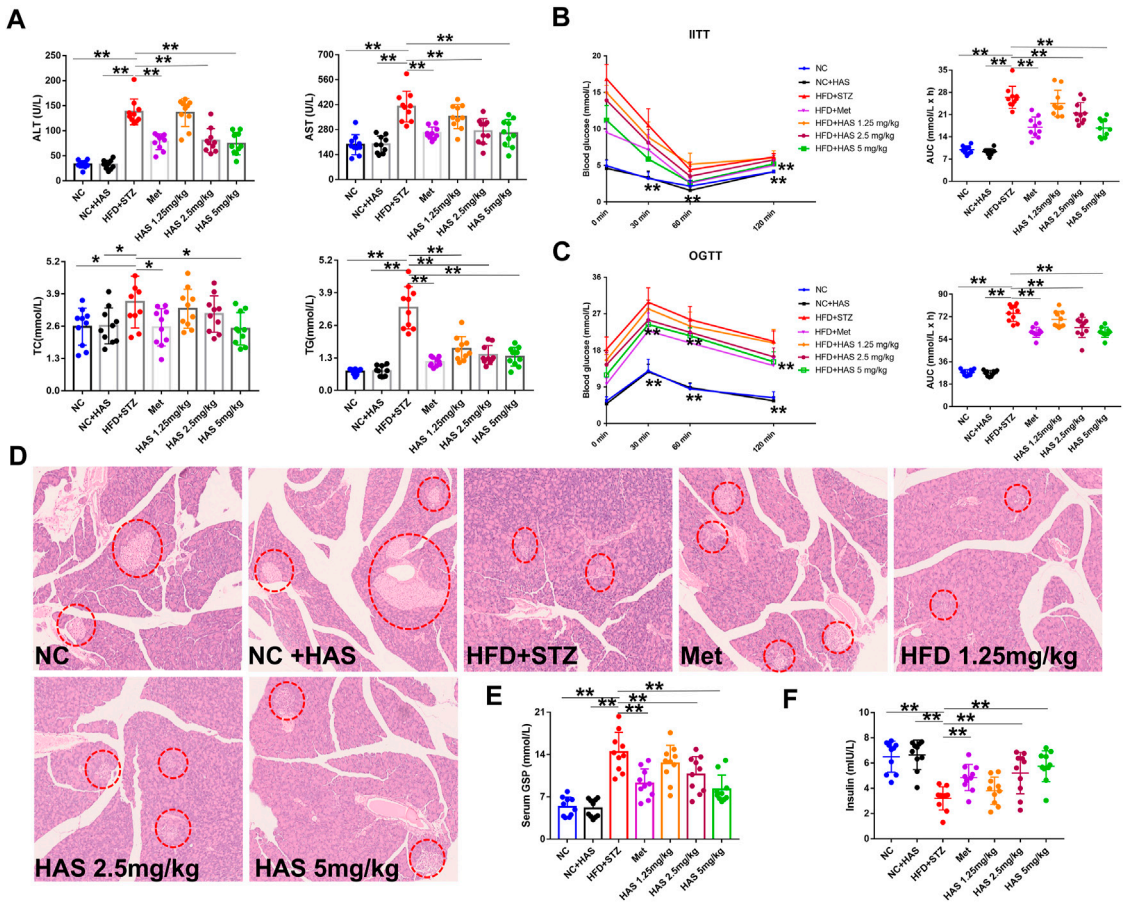


FIGURE 2
Effects of HAS on serum biochemistry and insulin resistance in T2DM mice. **(A)** Effects of HAS on ALT, AST, TC, and TG in T2DM mice. **(B)** Effects of HAS on ITT in T2DM mice. **(C)** Effects of HAS on OGTT in T2DM mice. **(D)** Effects of HAS on histopathology of the pancreas of T2DM mice; islet B cells in pancreatic tissue are marked with red circles. **(E,F)** Effects of HAS on GSP and insulin content in T2DM mice. The doses of 2.5, 5.0, and 10 mg/kg indicate low, middle, and high doses of HAS, respectively, and the dose of Met was 150 mg/kg. Data are expressed as mean ± SD (n = 10), $^{**}p < 0.01$ vs. HFD + STZ group.

treated mice, suggesting HAS has no effect on the liver function of normal mice. However, serum ALT and AST in T2DM mice were significantly increased, indicating HFD + STZ treatment caused serious disruption of liver function. Fortunately, like Met, HAS treatment reduced the levels of ALT and AST of T2DM mice, suggesting HAS could reduce liver injury of T2DM mice induced by HFD + STZ. In addition, like Met, HAS also reduced the serum levels of TG and TC of T2DM mice, indicating HAS has the capacity to improve the lipid levels of T2DM mice.

Glucose tolerance and insulin tolerance of HAS-treated mice were measured by OGTT and ITT, respectively. In the ITT experiment, the blood glucose of the NC group and NC + HAS mice decreased gradually after the intraperitoneal injection of insulin. At 60 min, the blood glucose of the mice decreased to the lowest level, then gradually increased, and recovered to the baseline level within 120 min. Similar results were observed in the other treatment groups, but none returned to baseline within 120 min. Statistical analysis of the AUC of blood glucose changes showed that STZ treatment induced a significant increase in AUC in the ITT compared with NC and NC + HFD, while both HAS and Met treatment reduced that increase in AUC (Figure 2B). The OGTT assesses the function of islet β cells and reflects the body's ability to regulate blood sugar. As shown in Figure 2C, after gavage of glucose, the blood glucose of normal mice reached the maximum value after 30 min, and returned to the baseline blood glucose level within 120 min T2DM mice experienced a rapid increase in blood glucose after receiving glucose gavage, and the blood glucose reached its peak at 30 min, followed by a gradual decrease in blood glucose. However, the blood glucose did not fully recover within 120 min, indicating STZ + HFD treatment causes dysfunction in insulin secretion, blood glucose regulation, and glucose tolerance (Figure 2C). In addition, at the end of the experiment, the serum insulin content of T2DM mice was

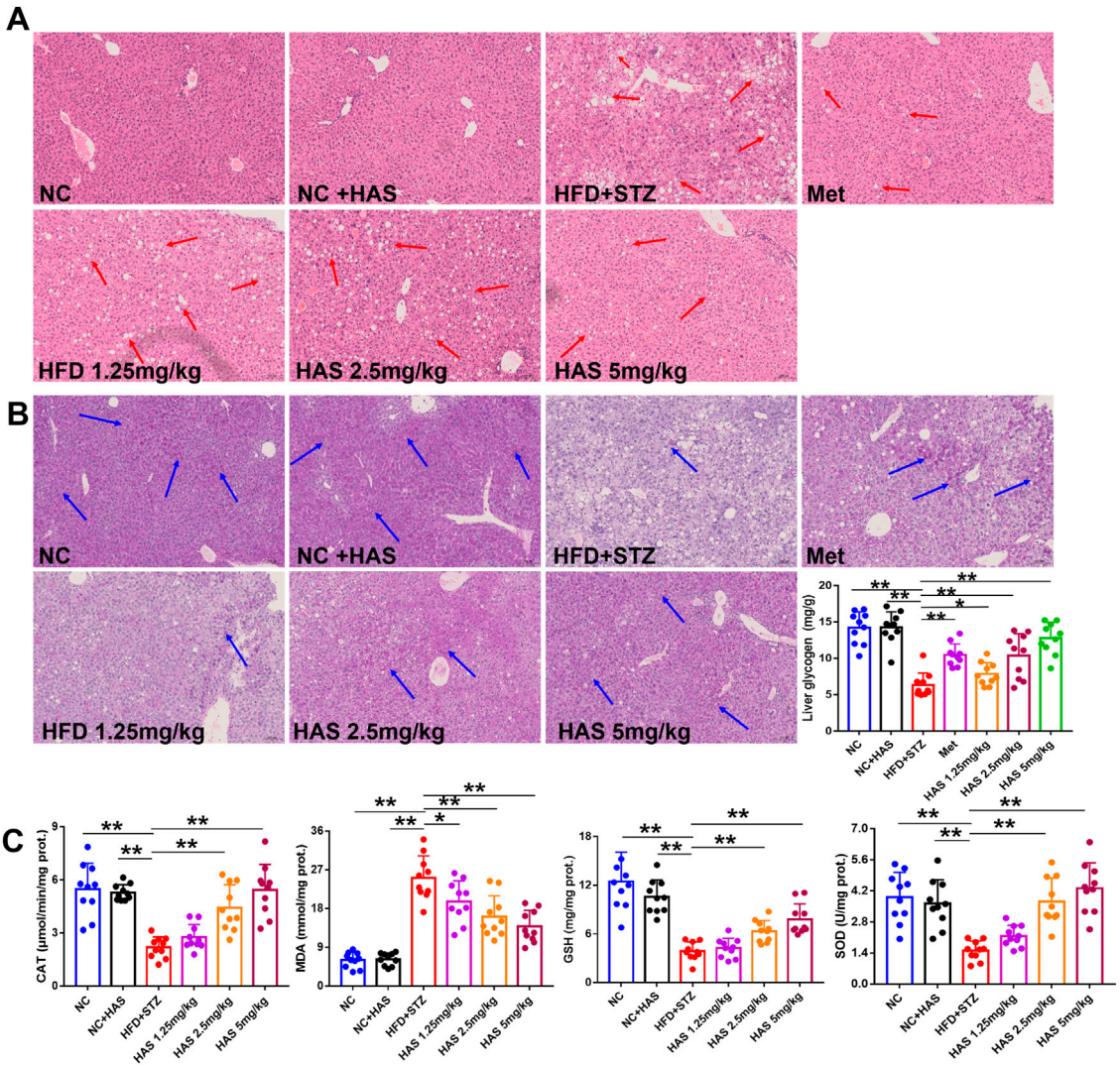


FIGURE 3
Effects of HAS on histopathology, oxidative stress, and liver glycogen of T2DM mice. Liver sections were stained with HE **(A)** and PAS **(B)** to determine the histopathological changes and liver glycogen content, respectively, in HFD + STZ mice. **(C)** Effects of HAS on oxidative stress in liver tissues of T2DM mice. The doses of 2.5, 5.0, and 10 mg/kg indicate low, middle, and high doses of HAS, and the dose of Met was 150 mg/kg. Data are expressed as mean ± SD (n = 10), $^{**}p < 0.01$ vs. HFD + STZ group.

significantly lower than that of normal mice, indicating treatment with STZ + HFD reduced insulin secretion in mice. However, HAS and Met treatment significantly increased insulin levels in T2DM mice (Figure 2E). Similarly, the serum GSP content of T2DM mice increased sharply at the end of the experiment, suggesting blood glucose was at a high level in the most recent 1–2 weeks, which was consistent with the results of blood glucose detection. Interestingly, like Met, HAS reduced GSP levels in T2DM mice at the end of the experiment (Figure 2F). Compared with T2DM mice without HAS treatment, HAS-treated T2DM mice had significantly improved islet function and glucose tolerance. Pathological examination of the pancreas was also carried out. The pancreas of normal mice was soft and grayish red in color, and islet cells were round or oval and distributed in the pancreatic lobules, showing complete cell structure, regular arrangement, clear nucleoli, and no swelling or congestion. After treatment with HFD + STZ, the pancreas of T2DM mice appeared yellow and white in color, and the islet cells showed structural atrophy, and were small in size, reduced in number, loose in distribution, and irregular in shape, suggesting that HFD + STZ treatment damaged the pancreatic tissue. Compared with T2DM mice without HAS treatment, HAS treatment (like Met) significantly improved the physical

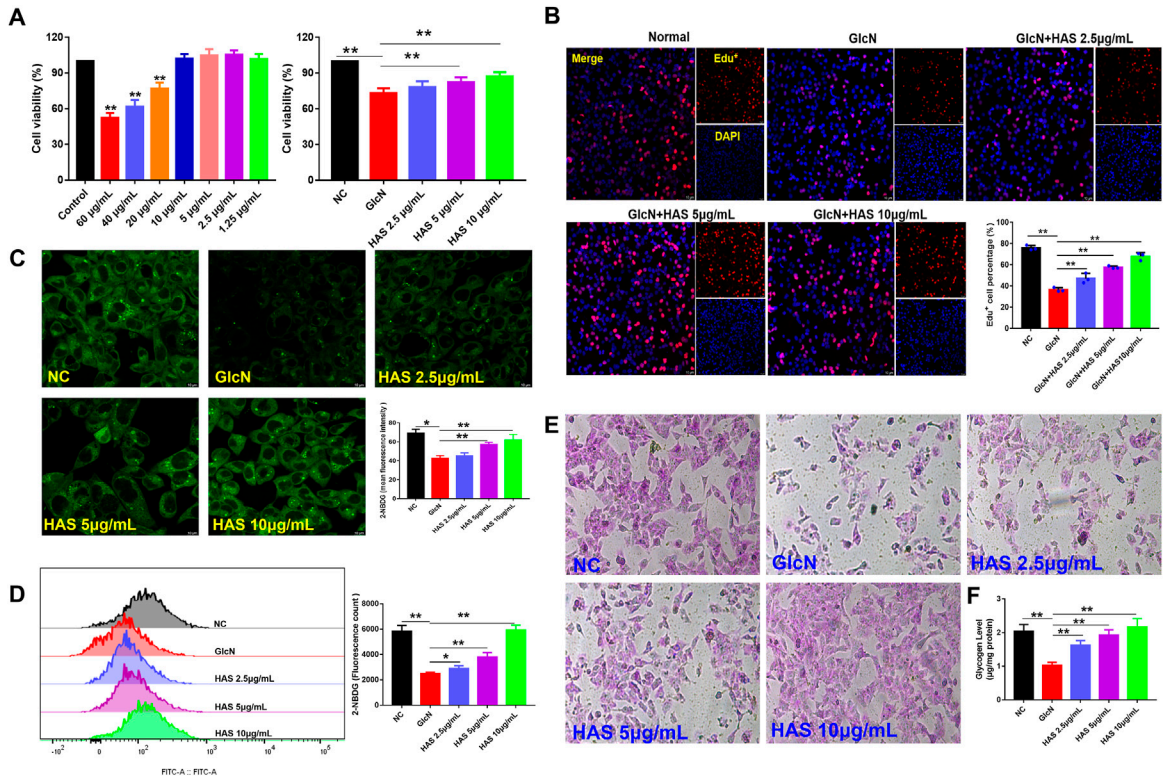


FIGURE 4
Effects of HAS on cell viability and glycogen content of GlcN-induced HepG2 cells. **(A,B)** HAS increased the viability of GlcN-induced HepG2 cells. **(C,D)** HAS increased the glucose uptake of GlcN-induced HepG2 cells, as visualized using the fluorescent glucose analogue 2-NBDG. **(E,F)** HAS increased the glycogen content in GlcN-induced HepG2 cells. Data are expressed as mean ± SD (n = 3), *p < 0.05, **p < 0.01 vs. GlcN-induced HepG2 cells.

aspects of the pancreas of T2DM mice as demonstrated by an increased number of islet cells, clearer structure, and reduced islet atrophy (Figure 2D).

HAS treatment reduced pathological changes and oxidative stress in the liver of T2DM mice

As demonstrated in Figure 3A, liver cells of normal mice were neatly arranged and compact, with similar cell size and morphology throughout the tissue, clear nuclear contour, and no pathological damage. However, after treatment with HFD + STZ, the liver of T2DM mice showed obvious steatosis, and many vacuoles of different sizes were scattered in the liver cell cytoplasm. The volume of liver cells increased, the cell shape was irregular, and the cytoplasm was fused. Interestingly, both HAS and Met treatment reduced the pathological changes in liver tissues, as the cells in liver tissue from treated mice appeared closely arranged, and vacuoles were significantly reduced.

Oxidative stress is generally believed to be involved in the pathogenesis of diabetes (Kaneto et al., 2007). In this study, the levels of CAT, MDA, GSH, and SOD in the liver were determined. HFD + STZ treatment significantly reduced the levels of CAT, GSH, and SOD, and significantly increased MDA, indicating high levels of oxidative stress in the liver of T2DM mice; importantly, our results showed that HAS treatment reduced oxidative stress in the liver of T2DM mice compared to mice without HAS treatment (Figure 3C).

HAS increases hepatic glycogen storage in T2DM mice

Glucose metabolism disorder is the most important characteristic of T2DM, and liver glycogen plays an important role in regulating glucose metabolism. PAS staining was used to visualize liver glycogen, and it was found that glycogen-positive staining in the liver of mice was significantly reduced after HFD and STZ treatment, and that glycogen content in the liver gradually recovered after HAS treatment (Figure 3B). Similar

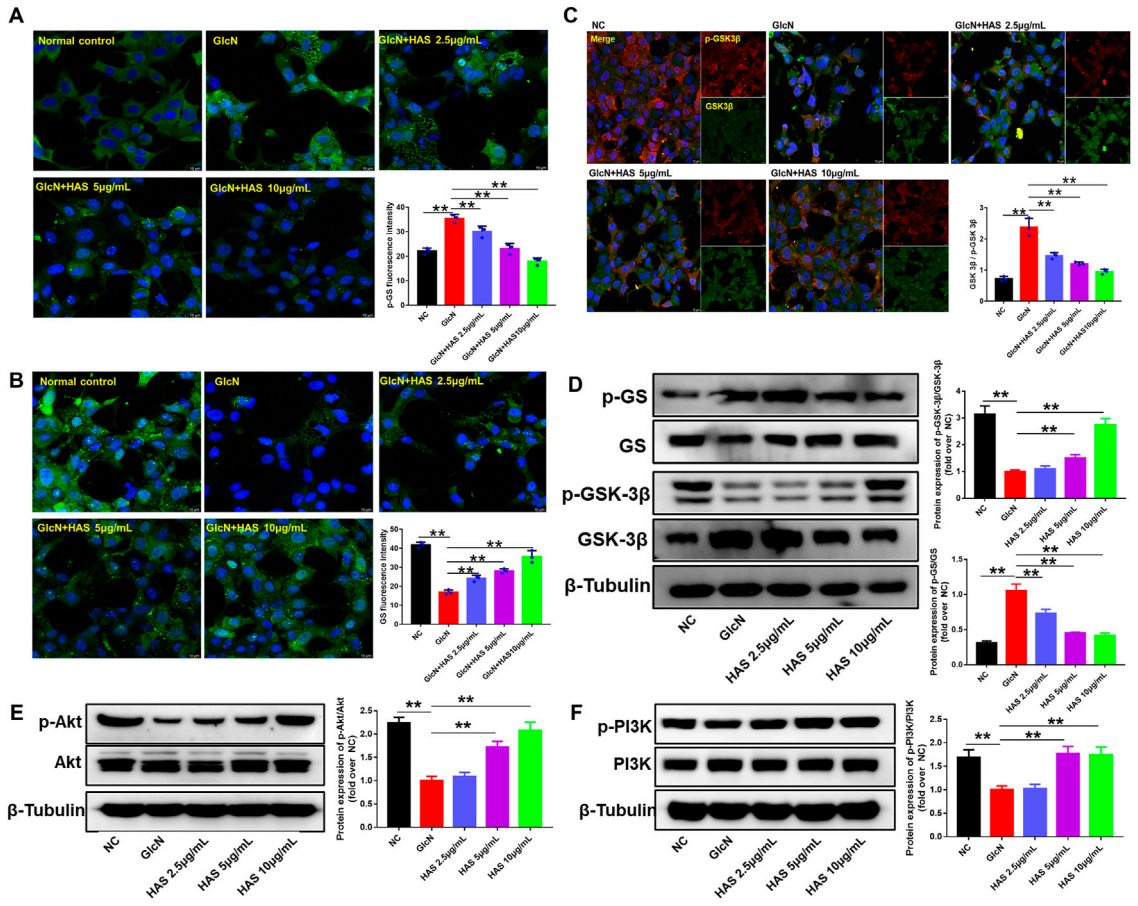


FIGURE 5
Effects of HAS on expression of PI3K/Akt/GSK-3β signaling pathway proteins in GlcN-induced HepG2 cells. **(A,B)** HAS decreased the phosphorylation of GS in GlcN-induced HepG2 cells. **(C)** HAS increased the expression of p-GSK-3β/GSK-3β in GlcN-induced HepG2 cells. **(D)** Western blotting assays of p-GS, GS, p-GSK-3β, and GSK-3β in GlcN-induced HepG2 cells. **(E,F)** HAS increased the phosphorylation of PI3K and Akt in GlcN-induced HepG2 cells. Data are expressed as mean ± SD (n = 3), $^{*}p < 0.05$, $^{**}p < 0.01$ vs. GlcN-induced HepG2 cells.

results were obtained by quantitative assessment of liver glycogen content. HAS significantly increased liver glycogen content at all doses tested, suggesting that the hypoglycemic effect of HAS on T2DM mice was related to the increase in liver glycogen content (Figure 3B). Similar results were observed with the positive control treatment (Met).

HAS increases glucose uptake in HepG2 cells induced by glucosamine

The viability of HepG2 cells after HAS treatment was evaluated by CCK-8 assay (Figure 4A). HAS had no obvious inhibitory effect on normal HepG2 cells at doses under 20 μg/ml and HAS treatment at doses of 2.5–10 μg/ml increased the viability of GlcN-induced HepG2 cells (Figure 4A). Furthermore, EdU staining also demonstrated that HAS reduced the inhibitory effect of GlcN on the proliferation of HepG2 cells (Figure 4B). *In vivo*, we observed that HAS increased glycogen content in the liver of T2DM mice (Figure 4B). We also detected glycogen content in GlcN-induced HepG2 cells. GlcN can induce insulin resistance in HepG2 cells and reduce the efficiency of glucose uptake and utilization in hepatocytes (Xiao et al., 2011; Sun et al., 2020). The fluorescent glucose analogue 2-NBDG is used to visualize glucose uptake by living cells and is often used to evaluate the glucose uptake capacity of cells. As shown in Figures 4C,D, confocal laser microscopy and flow cytometry results demonstrated that GlcN-induced HepG2 cell uptake of 2-NBDG was significantly reduced compared with the NC cells, but HAS treatment reduced GlcN-induced inhibition of glucose uptake in HepG2 cells. Additionally, our results indicate that HAS increased PAS-positive staining and cell glycogen content in GlcN-induced HepG2 cells (Figures 4E,F).

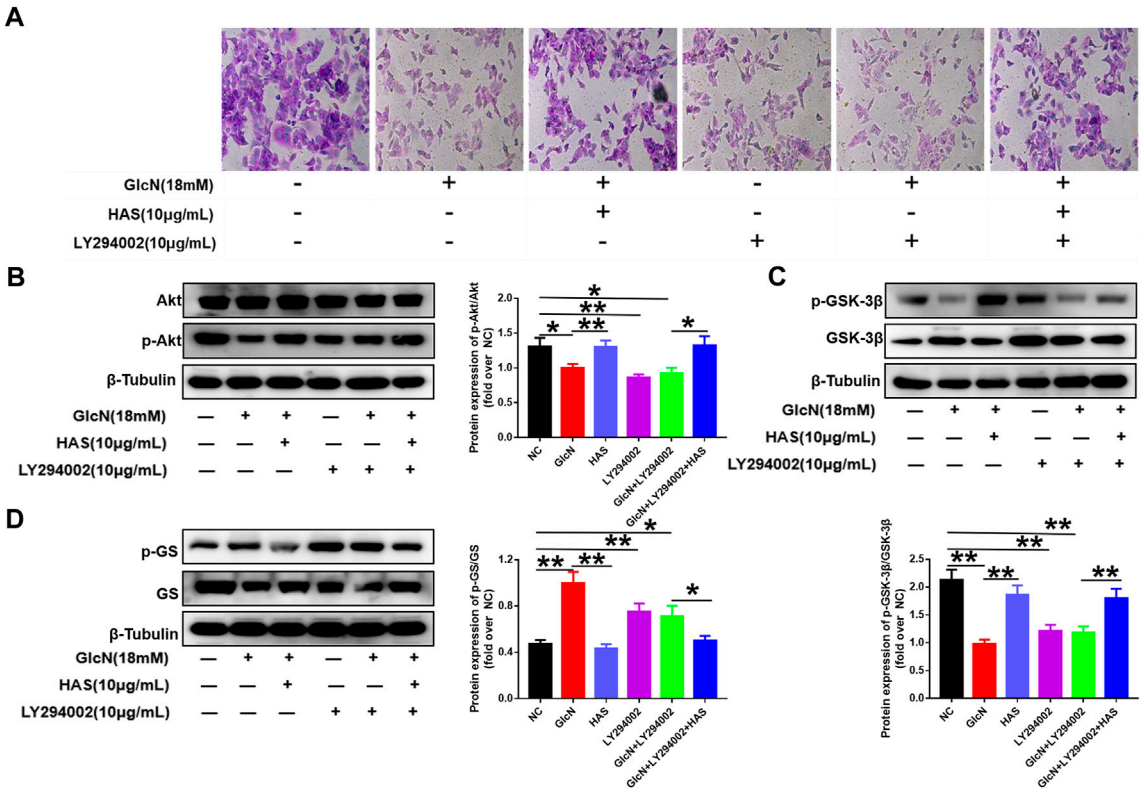


FIGURE 6
Effects of the PI3K inhibitor LY294002 on glycogen content and PI3K/Akt/GSK-3β signaling in GlcN-induced HepG2 cells. **(A)** The effect of HAS in promoting glycogen increase in GlcN-induced HepG2 cells was inhibited by the PI3K inhibitor LY294002. **(B–D)** The activation of the PI3K/Akt/GSK-3β pathway by HAS in GlcN-induced HepG2 cells was inhibited by the PI3K inhibitor LY294002. Data are expressed as mean ± SD ($n = 3$), $^{*}p < 0.05$, $^{**}p < 0.01$ vs. GlcN-induced HepG2 cells.

HAS increases hepatocyte glycogen accumulation *via* the PI3K/Akt/GSK-3β pathway

To investigate the possible mechanisms of HAS antidiabetic bioactivity, we studied the effect of HAS treatment on the PI3K/Akt/GSK-3β pathway in GlcN-induced HepG2 cells. As shown in Figure 5, results of western blotting and immunofluorescence staining indicate that GlcN stimulation significantly reduced the phosphorylation of PI3K, Akt, and GSK-3β in HepG2 cells, whereas GlcN stimulation increased the phosphorylation of GS. Interestingly, HAS effectively up-regulated the phosphorylation of PI3K, Akt, and GSK-3β in GlcN-induced HepG2 cells, whereas HAS down-regulated the phosphorylation of GS.

Furthermore, the PI3K/Akt pathway inhibitor LY294002 was used to confirm the involvement of the PI3K/Akt/GSK-3β pathway in the effects of HAS on T2DM. As shown in Figure 6, GlcN or LY294002 treatment reduced glycogen accumulation in HepG2 cells, and the addition of LY294002 further inhibited glycogen accumulation in GlcN-induced HepG2 cells. Furthermore, blocking PI3K with LY294002 suppressed HAS-mediated up-regulation of PI3K, Akt, and GSK-3β phosphorylation in GlcN-induced HepG2 cells, as well as suppressed HAS-mediated down-regulation of GS phosphorylation.

Conclusion

T2DM is a common chronic endocrine and glucometabolic disease, accounting for more than 90% of all cases of diabetes. Due to changes in dietary habits, T2DM incidence is increasing year by year worldwide, especially in developed and developing countries, which show trends toward epidemic levels of the disease (Sun et al., 2018; Kim et al., 2019). Diabetes has become one of the most prevalent non-infectious diseases threatening human health and life and is ranked third after cardiovascular disease and cancer. T2DM is primarily caused by the relative insufficiency of insulin secretion or the insulin resistance of various organs. Characterized by a significantly increased blood glucose level, T2DM can lead to damage to

multiple organ systems in the body. T2DM patients often endure a series of complications caused by hyperglycemia. Currently, metformin, α-glucosidase inhibitors, sulfonylureas, and DPP-4 inhibitors are clinical drugs commonly used for treating T2DM. However, all the currently available drugs come with potentially serious side effects, such as renal impairment, gastrointestinal disorders, and hypoglycemia. Therefore, finding new antidiabetic drugs is necessary for the prevention and clinical treatment of T2DM. In our study, we investigated the antidiabetic effects of HAS against T2DM and explored the potential related molecular mechanisms. Previous studies have reported that zanthoxylamides extracted from *Z. bungeanum* have potential antidiabetic effects in mice *via* activation of the AMPK/PI3K/Akt signaling pathway (Xu et al., 2022; Zhang et al., 2022). In our study, we found that HAS, which is the most important zanthoxylamide in *Z. bungeanum*, has potential antidiabetic effects against T2DM based on investigation using a high-fat-fed and streptozotocin-induced T2DM mouse model.

Hepatic glycogen synthesis and storage is an important biochemical process to ensure the survival of the human body. Insulin resistance induced by T2DM can inhibit hepatic glycogen synthesis and lead to hyperglycemia (Liu et al., 2015; Flannick et al., 2016). GS is a key enzyme in the process of glycogen synthesis, and its activity is regulated by cycles of phosphorylation (inactive) and dephosphorylation (active). It is known that the enzyme activity of GS is negatively regulated by its phosphorylation, which is primarily regulated by GSK-3, a serine/threonine protein kinase with two isomers, namely GSK-3α and GSK-3β. GSK-3 inhibits glycogen synthesis by catalyzing GS phosphorylation to inactivate it. Furthermore, previous studies have shown that hepatic insulin resistance is usually related to the inhibition of the PI3K/Akt pathway (Whiteman et al., 2002; Yan et al., 2016; Jiang et al., 2021). Activation of PI3K/Akt controls glucose homeostasis through a variety of pathways, including *via* increasing glucose uptake in muscle and adipose tissue (Sano et al., 2007), promoting glycogenesis in liver and muscle (Cross et al., 1995; Irimia et al., 2010), and inhibiting hepatic gluconeogenesis (Puigserver et al., 2003; Matsumoto et al., 2007). The PI3K/Akt pathway is the main upstream regulatory link with GSK-3 and is widely recognized as a crucial step in gluconeogenesis and glycogen synthesis in T2DM (Wang et al., 2014). Generally, activated Akt phosphorylates and inactivates the downstream target of GSK-3 in response to insulin stimulation. In this way, the negative regulatory effects of GSK-3β on GS are removed, and the activity of GS is increased, thus promoting glycogen synthesis. In our study, we confirmed that HAS increases the phosphorylation levels of PI3K and Akt and induces the phosphorylation and inactivation of GSK-3β, thereby removing its inhibitory effect on its target enzyme (GS) and promoting liver glycogen synthesis. As confirmation of PI3K/Akt involvement, the PI3K inhibitor LY294002 blocked PI3K/Akt signaling, which led to reduced accumulation of liver glycogen in insulin-resistant cell models treated with HAS.

In conclusion, our results suggest that HAS reduces blood glucose in the T2DM mouse model by regulating glycogen metabolism through a mechanism involving the regulation of the PI3K/Akt/GSK3β/GS signaling pathway. These data support the need for further exploration of HAS as a safe natural supplement or additional therapeutic option in the prevention or treatment of T2DM.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by the Animal Care and Use Committee of Chengdu University of Traditional Chinese Medicine (No. 2020-27).

Author contributions

QZ and R-LL wrote the draft of this article. C-JW and LA supervised the experiments. QZ and D-DT edited the draft. QZ, TR, R-LL, L-YW, DQ, D-DT, and C-XH performed the experiments. All authors agree to be accountable for all aspects of the work, ensuring integrity and accuracy.

Acknowledgments

The authors thank Jiayi Sun (Innovative Institute of Chinese Medicine and Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, China) for his technical assistance in fluorescence microscopy and analysis. This work was supported by the State Administration of Traditional Chinese Medicine of Sichuan Province of China (No. 2020HJZX001), Project of Science and Technology Department of Sichuan Province (No. 2021YJ0112), and Project of Sichuan Provincial Administration of Traditional Chinese Medicine (No. 2021MS017).

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/fphar.2022.1089558/full#supplementary-material

SUPPLEMENTARY FIGURE S1
(A) HAS reduced the water intake of HFD + STZ mice. **(B)** HAS reduced the food intake of HFD + STZ mice. **(C)** Fasting blood glucose (FBG) of T2DM mice per week.

References

Blahova, J., Martiniakova, M., Babikova, M., Kovacova, V., Mondockova, V., and Radoslav, Omelka. (2021). Pharmaceutical drugs and natural therapeutic products for the treatment of type 2 diabetes Mellitus. *Pharmaceuticals* 14 (8), 806.

Cross, D. A., Alessi, D. R., Cohen, P., Andjelkovich, M., and Hemmings, B. A. (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 378 (6559), 785–789.

DeFronzo, R. A., and Tripathy, D. (2009). Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* 32, S157–S163. doi:10.2337/dc09-S302

Deng, J. J., Liu, Y., Duan, Z. G., Zhu, C. H., Hui, J. F., Yu, M., et al. (2017). Protopanaxadiol and protopanaxatriol-type saponins ameliorate glucose and lipid metabolism in type 2 diabetes mellitus in high-fat diet/streptozocin-induced mice. *Front. Pharmacol.* 8, 506. doi:10.3389/fphar.2017.00506

Flannick, J., Stefan, J., and Njølstad, P. (2016). Common and rare forms of diabetes mellitus: Towards a continuum of diabetes subtypes. *Nat. Rev. Endocrinol.* 12 (7), 394–406. doi:10.1038/nrendo.2016.50

Gao, Y. F., Zhang, M. N., Wang, T. X., Wu, T. C., Ai, R. D., and Zhang, Z. S. (2016). Hypoglycemic effect of D-chiro-inositol in type 2 diabetes mellitus rats through the PI3K/Akt signaling pathway. *Mol. Cell. Endocrinol.* 433, 26–34. doi:10.1016/j.mce.2016.05.013

Irimia, J. M., Meyer, C. M., Peper, C. L., Zhai, L., Bock, C. B., Previs, S. F., et al. (2010). Impaired glucose tolerance and predisposition to the fasted state in liver glycogen synthase knock-out mice. *J. Biol. Chem.* 285 (17), 12851–12861. doi:10.1074/jbc.M110.106534

Jiang, Z., Zhao, M., Voilquin, L., Jung, Y. S., Aikio, M. A., Tanushi, S. H., et al. (2021). Isthmin-1 is an adipokine that promotes glucose uptake and improves glucose tolerance and hepatic steatosis. *Cell. Metab.* 33 (9), 1836–1852.e11. doi:10.1016/j.cmet.2021.07.010

Kaneto, H., Katakami, N., Kawamori, D., Miyatsuka, T., Sakamoto, K., Matsuoka, T. A., et al. (2007). Involvement of oxidative stress in the pathogenesis of diabetes. *Antioxid. Redox Signal.* 9 (3), 355–366. doi:10.1089/ars.2006.1465

Kim, J. Y., Ku, Y. S., Kim, H. J., Trinh, N. T., Kim, W., Jeong, B., et al. (2019). Oral diabetes medication and risk of dementia in elderly patients with type 2 diabetes. *Diabetes Res. Clin. Pract.* 154, 116–123. doi:10.1016/j.diabres.2019.07.004

Li, R. L., Zhang, Q., Liu, J., Sun, J. Y., He, L. Y., Duan, H. X. Y., et al. (2020). Hydroxy-α-sanshool possesses protective potentials on H2O2 stimulated PC12 cells by suppression of oxidative stress induced apoptosis through regulation of PI3K/Akt signal pathway. *Oxid. Med. Cell. Longev.* 2020, 3481758. doi:10.1155/2020/3481758

Liu, T. Y., Shi, C. X., Gao, R., Sun, H. J., Xiong, X. Q., Ding, L., et al. (2015). Irisin inhibits hepatic gluconeogenesis and increases glycogen synthesis via the PI3K/Akt pathway in type 2 diabetic mice and hepatocytes. *Clin. Sci.* 129 (10), 839–850. doi:10.1042/CS20150009

Matsumoto, M., Pocai, A., Rossetti, L., Depinho, R. A., and Accili, D. (2007). Impaired regulation of hepatic glucose production in mice lacking the forkhead transcription factor Foxo1 in liver. *Cell. Metab.* 6 (3), 208–216. doi:10.1016/j.cmet.2007.08.006

Puigserver, P., Rhee, J., Donovan, J., Walkey, C. J., Yoon, J. C., Oriente, F., et al. (2003). Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. *Nature* 423 (6939), 550–555. doi:10.1038/nature01667

Ren, T. Y., Zhu, Y. P., and Kan, J. Q. (2017a). Zanthoxylum alkylamides activate phosphorylated AMPK and ameliorate glycolipid metabolism in the streptozotocin-induced diabetic rats. *Clin. Exp. Hypertens.* 39 (4), 330–338. doi:10.1080/10641963.2016.1259332

Ren, T. Y., Zhu, Y. P., Xia, X. J., Ding, Y. B., Guo, J., and Kan, J. Q. (2017b). Zanthoxylum alkylamides ameliorate protein metabolism disorder in STZ-induced diabetic rats. *J. Mol. Endocrinol.* 58 (3), 113–125. doi:10.1530/JME-16-0218

Roche, O., Trube, G., Zuegge, J., Pflimlin, P., Alanine, A., and Schneider, G. (2020). A virtual screening method for prediction of the hERG potassium channel liability of compound libraries. *Chembiochem* 3 (5), 455–459. doi:10.1002/1439-7633(20020503)3:5<455::AID-CBIC455>3.0.CO;2-L

Sano, H. Y., Eguez, L., Teruel, M. N., Fukuda, M., Chuang, T. D., Chavez, J. A., et al. (2007). Rab10, a target of the AS160 Rab GAP, is required for insulin-stimulated translocation of GLUT4 to the adipocyte plasma membrane. *Cell. Metab.* 5 (4), 293–303. doi:10.1016/j.cmet.2007.03.001

Sun, C., Zhang, F., Ge, X. J., Yan, T. T., Chen, X. M., Shi, X. L., et al. (2007). SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. *Cell. Metab.* 6 (4), 307–319. doi:10.1016/j.cmet.2007.08.014

Sun, H. J., Cao, L., Zhu, M. Y., Wu, Z. Y., Shen, C. Y., Nie, X. W., et al. (2020). DR-region of Na+/K+-ATPase is a target to ameliorate hepatic insulin resistance in obese diabetic mice. *Theranostics* 10 (14), 6149–6166. doi:10.7150/thno.46053

Sun, Y. X., Shi, H., Yin, S. Q., Ji, C., Zhang, X., Zhang, B., et al. (2018). Human mesenchymal stem cell derived exosomes alleviate type 2 diabetes mellitus by reversing peripheral insulin resistance and relieving β-cell destruction. *ACS Nano* 12 (8), 7613–7628. doi:10.1021/acsnano.7b07643

Vishvanath, L., and Gupta, R. K. (2019). Contribution of adipogenesis to healthy adipose tissue expansion in obesity. *J. Clin. Invest.* 129 (10), 4022–4031. doi:10.1172/JCI129191

Wang, C., Chi, Y., Li, J., Miao, Y., Li, S., Su, W., et al. (2014). FAM3A activates PI3K p110α/Akt signaling to ameliorate hepatic gluconeogenesis and lipogenesis. *Hepatology* 59 (5), 1779–1790. doi:10.1002/hep.26945

Wang, L., Fan, W. X., Zhang, M. M., Zhang, Q., Li, L., Wang, J. L., et al. (2019). Antiobesity, regulation of lipid metabolism, and attenuation of liver oxidative stress effects of hydroxy-α-sanshool isolated from Zanthoxylum bungeanum on high-fat diet-induced hyperlipidemic rats. *Oxid. Med. Cell. Longev.* 2019, 5852494. doi:10.1155/2019/5852494

Whiteman, E. L., Cho, H., and Birnbaum, M. J. (2002). Role of Akt/protein kinase B in metabolism. *Trends Endocrinol. Metab.* 13 (10), 444–451. doi:10.1016/s1043-2760(02)00662-8

Xiao, F., Huang, Z. Y., Li, H. K., Yu, J. J., Wang, C. X., Chen, S. H., et al. (2011). Leucine deprivation increases hepatic insulin sensitivity via GCN2/mTOR/S6K1 and AMPK pathways. *Diabetes* 60 (3), 746–756. doi:10.2337/db10-1246

Xu, F., Zhu, Y., Lu, M., Qin, L., Zhao, D., and Ren, T. (2022). Effects of hydroxy-alpha-sanshool on intestinal metabolism in insulin-resistant mice. *Foods* 11, 2040. doi:10.3390/foods11142040

Yan, F. J., Dai, G. H., and Zheng, X. D. (2016). Mulberry anthocyanin extract ameliorates insulin resistance by regulating PI3K/AKT pathway in HepG2 cells and db/db mice. *J. Nutr. Biochem.* 36, 68–80. doi:10.1016/j.jnutbio.2016.07.004

You, Y. M., Ren, T., Zhang, S. Q., Shirima, G. G., Cheng, Y. J., and Liu, X. (2015). Hypoglycemic effects of Zanthoxylum alkylamides by enhancing glucose metabolism and ameliorating pancreatic dysfunction in streptozo-tocin-induced diabetic rats. *Food Funct.* 6 (9), 3144–3154. doi:10.1039/c5fo00432b

Yu, H., Zheng, L., Xu, L., Yin, L. H., Lin, Y., Li, H., et al. (2015). Potent effects of the total saponins from Dioscorea nipponica Makino against streptozotocin-induced type 2 diabetes mellitus in rats. *Phytother. Res.* 29 (2), 228–240. doi:10.1002/ptr.5243

Zhang, M. M., Xie, M. G., Wei, D. N., Wang, L., Hu, M. B., Zhang, Q., et al. (2019). Hydroxy-α-sanshool isolated from Zanthoxylum bungeanum attenuates learning and memory impairments in scopolamine-treated mice. *Food Funct.* 10 (11), 7315–7324. doi:10.1039/c9fo00045c

Zhang, T., Zhang, Q., Zheng, W., Tao, T., Li, R. L., Wang, L. Y., et al. (2022). Fructus Zanthoxyli extract improves glycolipid metabolism disorder of type 2 diabetes mellitus via activation of AMPK/PI3K/Akt pathway: Network pharmacology and experimental validation. *J. Integr. Med.* 20 (6), 543–560. doi:10.1016/j.joim.2022.07.004

frontiers | Frontiers in Pharmacology

TYPE Systematic Review
PUBLISHED 25 January 2023
DOI 10.3389/fphar.2023.1088614

OPEN ACCESS

EDITED BY
Xiaofei Zhang,
Shaanxi University of Chinese Medicine, China

REVIEWED BY
Wei Zhou,
Beijing Institute of Radiation Medicine, Beijing, China
Mei Wang,
Liaoning University of Traditional Chinese Medicine, China

*CORRESPONDENCE
Xuhua Qin,
1978537820@qq.com
Xiao Ma,
tobymaxiao@cdutcm.edu.cn

SPECIALTY SECTION
This article was submitted to Ethnopharmacology, a section of the journal *Frontiers in Pharmacology*

RECEIVED 03 November 2022
ACCEPTED 10 January 2023
PUBLISHED 25 January 2023

CITATION
Li Y, Deng X, Guo X, Zhang F, Wu H, Qin X and Ma X (2023), Preclinical and clinical evidence for the treatment of non-alcoholic fatty liver disease with soybean: A systematic review and meta-analysis. *Front. Pharmacol.* 14:1088614. doi: 10.3389/fphar.2023.1088614

Preclinical and clinical evidence for the treatment of non-alcoholic fatty liver disease with soybean: A systematic review and meta-analysis

Yubing Li[1], Xinyu Deng[1], Xiaochuan Guo[2], Fangling Zhang[1], Hefei Wu[1], Xuhua Qin[1]* and Xiao Ma[1]*

[1]State Key Laboratory of Southwestern Chinese Medicine Resources, School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]Hospital of Chengdu University of Traditional Chinese Medicine, School of Clinical Medicine, Chengdu University of Traditional Chinese Medicine, Chengdu, China

Non-alcoholic fatty liver disease (NAFLD), a prevalent public health issue, involves the accumulation of triglycerides in hepatocytes, which is generally considered to be an early lesion of liver fibrosis and cirrhosis. Thus, the development of treatments for NAFLD is urgently needed. This study explored the preclinical and clinical evidence of soybeans to alleviate NAFLD. Studies indexed in three relevant databases—Web of Science, PubMed, and Embase—between January 2002 and August 2022 were retrieved. A total of 13 preclinical studies and five RCTs that included 212 animals and 260 patients were included in the present analysis. The preclinical analysis showed that liver function indices (AST, SMD = −1.41, $p < 0.0001$ and ALT, SMD = −1.47, $p < 0.0001$) were significantly improved in the soybean group compared to the model group, and fatty liver indicators (TG, SMD = −0.78, $p < 0.0001$; TC, SMD = −1.38, $p < 0.0001$) and that oxidative stress indices (MDA, SMD = −1.09, $p < 0.0001$; SOD, SMD = 1.74, $p = 0.022$) were improved in the soybean group. However, the five RCTs were not entirely consistent with the preclinical results; however, the results confirmed the protective effect on the liver. The results of the clinical RCTs showed that soybean significantly affected liver function, fatty liver, and oxidative stress indicators (ALT, SMD = −0.42, $p = 0.006$; TG, SMD = −0.31, $p = 0.039$; MDA, SMD = −0.76, $p = 0.007$). The current meta-analysis combined preclinical and clinical studies and verified that soybean could protect the liver in NAFLD by regulating lipid metabolism and oxidative stress factors *via* the Akt/AMPK/PPARα signaling pathway. Soybean might be a promising therapeutic agent for treating non-alcoholic fatty liver disease.

Systematic Review Registration: (https://www.crd.york.ac.uk/prospero/#myprospero), identifier (CRD42022335822).

KEYWORDS

soybean, NAFLD, meta-analysis, clinical, preclinical

Highlights

1. This study systematically evaluated soybean as a treatment for NAFLD based on meta-analyses of preclinical and clinical data.

2. The hepatoprotective effect of activating AMPK through P13K/AKT to improve insulin resistance, lipid metabolism, and the oxidative stress signaling pathway was the key process by which soybean alleviated NAFLD.
3. Soybean showed consistent therapeutic effects on NAFLD in clinical studies and preclinical trials.

1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is a prevalent public health issue, especially in developed countries (Angulo, 2002). In recent years, NAFLD has become the most common chronic liver disease worldwide, seriously affecting human health, and has been recognized as a common cause of cirrhosis, with an increasing global prevalence of approximately 25% in the adult population (Powell et al., 2021). The increasing prevalence of chronic liver disease is associated with NAFLD and is proportional to the increase in obesity worldwide (Sherif et al., 2016). Generally, 10–20 years after confirming fatty liver, approximately 10% of patients with non-alcoholic fatty liver will develop cirrhosis (Ekstedt et al., 2015). Fatty liver is generally considered an early lesion of liver fibrosis and cirrhosis. Non-alcoholic fatty liver gradually develops into liver fibrosis and cirrhosis, with consequent large burdens on society. The development of treatments for NAFLD is urgently needed. Thus, the prevention of non-alcoholic fatty liver is critical for reducing liver fibrosis and cirrhosis in patients. The main causes of non-alcoholic fatty liver disease are the excessive accumulation of triglycerides and fatty infiltration of the liver (Mathiesen et al., 1999). PPAR-α activation can reduce fat synthesis and promote fat metabolism by regulating fatty acid transport and promoting FFA entry into mitochondria for β-oxidation to regulate lipid absorption in the liver. Therefore, PPAR-α agonists reduce triglyceride levels in the blood and increase the catabolism of triglyceride-rich lipoproteins. Although several PPAR-α agonists have been developed, only a few have been successfully applied in the clinical setting. Overall, there remains a lack of specific drugs for the treatment of NAFLD (Pan et al., 2014; Lin et al., 2016). At present, NAFLD is typically treated with pioglitazone and glutathione. However, compared to conventional drugs, soybean is considered a medicine and homologous food substance that can be easily obtained in daily life and has fewer adverse effects than conventional drugs. Various components in soybean can reduce blood lipid, total cholesterol, low-density lipoprotein, and triglyceride levels through different pathways.

"Diseases enter by the mouth" is a Chinese idiom, and many studies have shown that poor eating habits are an important cause of non-alcoholic fatty liver disease (Zhu et al., 2022). In contrast, clinical results have shown that good eating habits are an effective way to improve NAFLD. For instance, a Mediterranean diet, which is characterized by large amounts of fiber, polyunsaturated fat, and antioxidants, represents a healthy dietary pattern. The "Seven Countries Study" by Keys and White showed that a Mediterranean diet could ameliorate steatosis and fatty liver disease (Abenavoli et al., 2018). Soybeans are the main component of the Mediterranean diet and are considered an effective treatment for non-alcoholic fatty liver disease. The value of soybean as a treatment for non-alcoholic fatty liver disease has not been examined; however, in recent years, interest has been increasing regarding healthy diets to prevent and treat chronic diseases. Naturally, soybean can be used as a treatment for NAFLD. In recent years, a study indicated that soybean and soybean products may serve as potential treatments to cure NAFLD (Khoury et al., 2015). Soybean has a long history of use as food in China and throughout Asia and is popular with consumers. Soybeans can provide healthy nutrients and are generally recognized by the public; moreover, they have also been approved by the FDA to reduce the risk of complex diseases such as chronic heart disease (Huang et al., 2016). Previous studies demonstrated that the main active ingredients of soybean were various isoflavones (Chatterjee et al., 2018). Phytochemicals belonging to the genus flavonoids effectively reduced fatty liver disease induced by a high-fat diet in rats (Xu et al., 2019).

However, due to the limited research on soybean, particularly regarding the use of soybean for the treatment of NAFLD, there is no clear evidence for its use. Therefore, we conducted a meta-analysis of preclinical and clinical studies to provide further ideas for the use of soybean as a treatment for NAFLD. Furthermore, the mechanism by which soybean products treat non-alcoholic fatty liver disease is unclear and requires further research.

2 Methods

Based on the PRISMA 2020 guidelines, we searched the Web of Science, PubMed, and Embase databases for studies on the use of soybean for the treatment of NAFLD. The retrieval time was set from January 2002 to August 2022. The search term was the treatment combined with the disease. The treatment search terms were "soybean," "soy bean," and "soybean," while the disease search terms were "non-alcoholic fatty liver disease," "NAFLD," and "NASH" (Supplementary Table S1). This study was registered in the International Prospective Register of Systematic Reviews. The PROSPERO register ID is CRD42022335822 (https://www.crd.york.ac.uk/prospero/#myprospero).

2.1 Inclusion criteria

The inclusion criteria for preclinical studies were 1) experimental animal models only for NAFLD/NASH, 2) only soybean and soybean products included in the treatment group, with no other drugs used in combination; 3) outcome indicators of TC, TG, ALT, AST, HDL-C, LDL-C, MDA, SOD, FFA, TNF-α, and insulin; and 4) histomorphology to determine the degeneration of hepatocyte adipose tissue.

The inclusion criteria for clinical studies were 1) participants were patients with NAFLD; 2) interventions of soybean and soybean products, with no other drugs were used in combination; 3) control group administered active therapy, placebo, or no therapy; 4) outcome indicators of TC, TG, ALT, AST, HDL-C, LDL-C, MDA, BMI, body weight, and insulin; and 5) randomized clinical trials.

2.2 Exclusion criteria

Preclinical studies meeting the following criteria were excluded: 1) experimental group not administered soybean or soybean products; 2) experimental group administered drugs other than soybean or soybean products; 3) other chronic liver diseases or other diseases that could cause fatty liver disease in animals; 4) animal liver fat degeneration caused by alcohol; 5) missing data on primary and secondary outcomes; 6) unpublished data or duplicated literature; 7) reviews and conference reports; 8) non-English language.

RCTs meeting the following criteria were excluded: 1) experimental group not administered soybean or soybean products;

2) experimental group administered drugs other than soybean or the control group did not receive a placebo or active treatment; 3) other chronic liver diseases or other diseases that could cause fatty liver disease in patients; 4) missing data on primary and secondary outcomes; and 5) non-English language.

2.3 Data extraction

We extracted the following information from the included literature according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standard: 1) the first author and the year of publication; 2) the method of non-alcoholic fatty liver disease induction in animals; 3) animal species and numbers of animals in the experimental and model groups; 4) intervention methods in the experimental groups and the model groups; and 5) primary and secondary outcome measures in the included literature.

For the five RCTs, we extracted the following information: 1) the first author and the year of publication; 2) sex and age of patients with non-alcoholic fatty liver disease; 3) intervention methods in the control and experimental groups; 4) sample size and treatment duration; and 5) outcome indicators. For studies requiring data extraction from images, we used universal Desktop Ruler software to extract the corresponding outcome indicators.

2.4 Quality assessment

We used the SYstematic Review Center for Laboratory Animal Experimentation (SYRCLE) animal laboratory bias risk assessment tool in Review Manager 5.3 software to evaluate the quality of the preclinical literature on soybean treatment of NAFLD and the methodological quality. The SYRCLE tool comprises 10 items and the final evaluation results are "Yes," "No," and "uncertain". "Yes" indicates a low risk of bias, "No" indicates a high risk of bias, and "uncertain" indicates an uncertain risk of bias. The quality evaluation for preclinical studies included the following 10: 1) the allocation sequence was generated or applied adequately; 2) the baselines of each group were the same; 3) the allocation concealment was sufficient; 4) the animals were randomly placed during the experiment; 5) the researchers were blinded; 6) the animals were selected at random; 7) the evaluator was blinded; 8) incomplete data were processed completely; 9) no selective results were reported for these studies; and (10) no other issues in the study that might lead to a high risk of bias.

For RCTs, the evaluation checklist of the Cochrane Library was used to evaluate the study quality. This checklist contained the following seven items: 1) random sequence generation; 2) allocation concealment; 3) blinding of participants and personnel; 4) blinding of outcome assessments; 5) incomplete outcome data; 6) selection reporting; and 7) other biases. This evaluation was performed independently by Yubing Li and Xinyu Deng. For any disagreement, the results were submitted to a third researcher, Xiao Ma, for resolution.

2.5 Mechanism analysis

We summarized the mechanistic pathways in the 13 documents and expanded the possible mechanisms. We also referred to the relevant literature to improve and identify the included mechanism pathways.

2.6 Statistical analysis

All outcome indicators were analyzed in Stata 15 because the outcome indicators that we collected were continuous variable data; therefore, the combined-effect size of the standardized mean square difference (SMD) was used for all outcome indicators, with a confidence interval of 95%. Heterogeneity was evaluated using the I-squared (I^2) statistic. When $I^2 < 50\%$, the included studies had good heterogeneity and no source of heterogeneity, and the outcome indicators were analyzed using a fixed-effect model. When $I^2 > 50\%$, heterogeneity was present in the included studies and the outcome indicators were analyzed using a random-effects model. We then applied subgroup analysis to determine the source of the heterogeneity. We used p-values to determine significant differences; with $p < 0.05$ indicating a significant difference between the experimental and control groups and $p > 0.05$ indicating no statistically significant difference. In addition, publication bias was determined using Egger's test, with $|t| < 0.05$ considered potential publication bias.

3 Results

3.1 Eligible studies

A flowchart showing the literature screening process based on the literature retrieval procedures was constructed (Figure 1A). According to our retrieval strategy, a total of 161 articles were obtained through the preliminary retrieval. Then, 82 duplicated articles and 29 unrelated articles were excluded. Finally, the full text of each result was read, and 13 preclinical and 5 RCT articles met the inclusion criteria.

3.2 Characteristics of the included studies

A total of 212 animals were included in 13 articles, including 106 animals in the soybean product group and 106 animals in the model group. The animal species were Sprague–Dawley rats, C57BL/6 mice, and ICR mice. The animal models included high-fat diet induction. Two studies used OLETF rats as a pathological model and reported that soybean did not decrease serum triglycerides (TG) in animals. The administration duration ranged from 4 weeks to 22 weeks. The main reasons were differences in animal species, initial body weight, and drug used. The experimental group was administered different soybean treatments, while the model group was administered normal saline and high-fat diets. The main outcome indicators included TC, TG, ALT, AST, HDL-C, LDL-C, FFA, MDA, SOD, TNF-α, and insulin levels. The therapeutic effects of soybean on non-alcoholic fatty liver were analyzed. Thirteen studies measured TG levels; 13 studies measured TC levels; 6 studies measured AST levels; 7 studies measured ALT levels; 8 studies measured HDL levels; 7 studies measured LDL levels; 8 studies measured FFA levels; 3 studies measured MDA, SOD, and insulin levels; and 5 studies examined TNF-α levels (Supplementary Table S2).

The five RCTs contained a total of 260 patients diagnosed with NAFLD, including 130 patients each in the experimental and control groups, respectively. The patients in the experimental group were treated with soybean, whereas the control group was treated with a placebo. The subjects in the included trials ranged from 43 to 57 years of age. The administration duration was 8 weeks, although one study had a duration of 24 weeks with 8–24 weeks of follow-up. The main

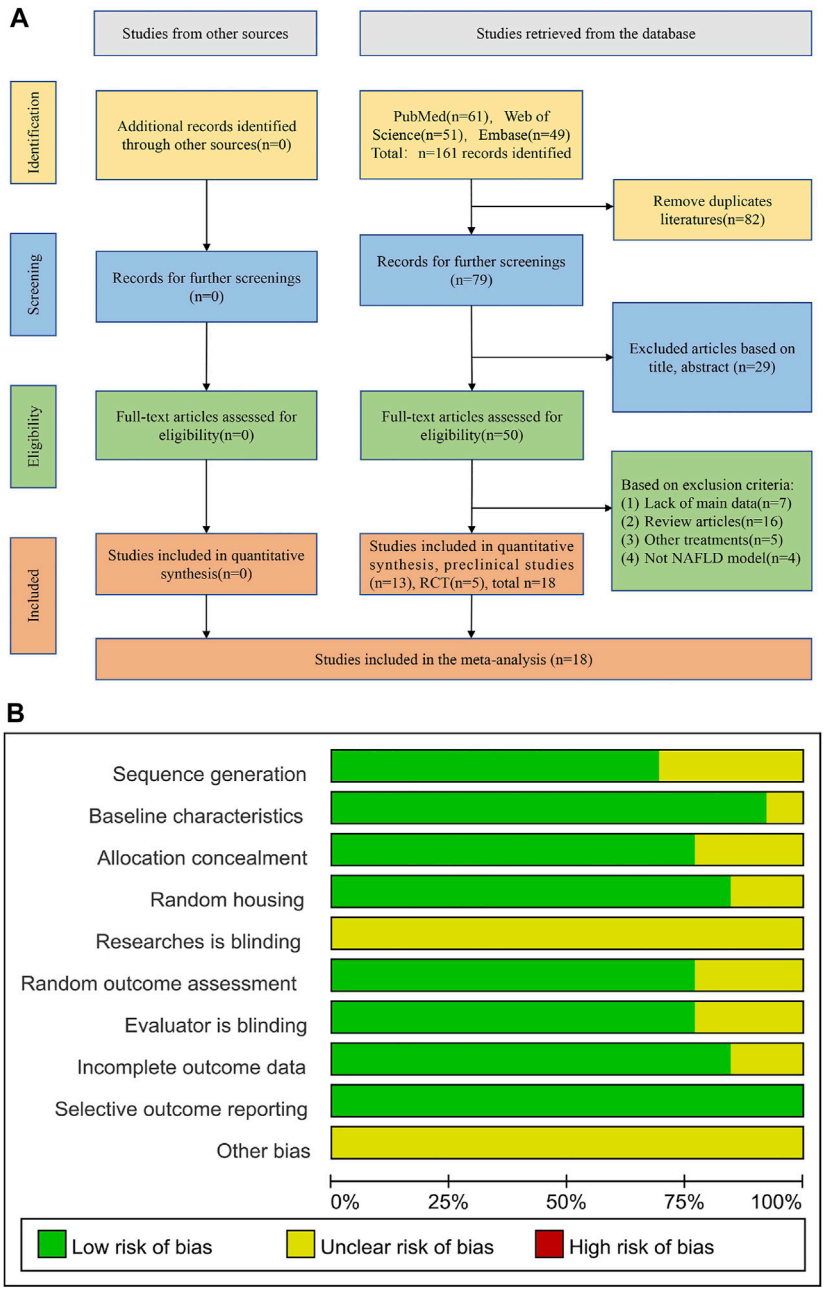


Figure 1: Flowchart and quality assessment of the included studies

(A. Flowchart of the selection process. B. Quality assessment of preclinical studies)

FIGURE 1
Flowchart and quality assessment of the included studies. **(A)** Flowchart of the selection process. **(B)** Quality assessment of the preclinical studies.

outcome indicators included obesity, liver function, and fat indices. Indicators related to insulin resistance, serum insulin, and MDA were also assessed (Supplementary Table S3).

3.3 Study quality

The results of the literature quality evaluation showed an average score of 6.6 points (Supplementary Figure S1). Twelve studies reported that the animals were similar at baseline. Nine studies reported that the distribution sequence was fully generated or applied. Ten studies described sufficient animal allocation concealment. Eleven studies reported random assignment of the animals during the experiment. No studies reported that the researchers were blinded during the experiments. Ten studies reported that the animals were randomly selected to evaluate the results, and the evaluators were blinded. Eleven studies reported incomplete data. No studies reported selective results. However, because none of the studies described the other bias, the other bias was not clear (Figure 1B).

RCTs were assessed according to seven aspects of the methodology. The overall quality indicated a relatively low risk of bias, with an average score of 4.4 points (total of 7 points). All RCTs reported selection and reporting biases. Moreover, only one study described allocation concealment, and four studies described the blinding of outcome assessments and performance bias. In addition, three studies reported incomplete outcome data. However, other biases were absent from the included studies (Supplementary Figure S2).

3.4 Effects of soybean in preclinical studies

Hematoxylin and eosin (H&E) staining was used in 12 of 13 studies. We carefully read the remaining paper and found that histopathological observation was not performed because it applied a classic OLETF congenital obesity rat model and examined all indicators of non-alcoholic fatty liver disease. Nine studies reported intact liver tissue structure in normal rats, with an orderly arrangement of liver cells, clear nuclear structure, and normal hepatic lobule structure, with no pathological changes such as steatosis and hepatocyte swelling. Rats fed a high-fat diet showed more yellow livers; however, soybean reversed the change in liver color to light brown. Moreover, while HFD feeding induced the accumulation of lipid droplets, an effect significantly reversed by soybean treatment (Panasevich et al., 2017). NAFLD rats showed a significant accumulation of liver fat, as well as inflammatory cell infiltration and liver fibrosis. Micrographs of the liver tissue of NAFLD rats showed laminar and diffuse steatosis. Hepatocytes were noticeably swollen. Most of the nuclei were extruded to one side, and some nuclei were deformed and had disappeared. The results showed that soybean significantly inhibited hepatic steatosis caused by NAFLD and that soybean products decreased the levels of outcome indicators such as TG, TC, AST, ALT, LDL-C, FFA, TNF-α, MDA, and insulin and increased the levels of SOD and HDL-C. Liver function indicators, lipid metabolism indicators, oxidative stress indicators, and inflammatory indicators were individually assessed in the meta-analysis of preclinical studies of soybean for the treatment of NAFLD to investigate the potential of soybean to affect these indicators. The results showed that soybean effectively improved the liver function index, lipid metabolism, and inflammatory factor levels and improved non-alcoholic fatty liver through multiple pathways, including antioxidant stress and lipid metabolism pathways.

3.4.1 Effects on liver function

A total of six studies with 98 animals examined AST levels after soybean treatment (I^2 = 26.9% and p = 0.233). Due to the minor heterogeneity, a fixed-effect model was used for further analysis. The results showed that soybean significantly reduced the AST level compared to that in the model group [*SMD* = −1.41, *95% CI* (−1.87, −0.95), p < 0.0001] (Figure 2A). Seven studies with 116 animals examined ALT levels after soybean treatment. However, the results of the heterogeneity analysis suggested minor heterogeneity (I^2 = 2.4%, p = 0.407). Thus, a fixed-effect model was used. The meta-analysis demonstrated that there was a significant difference between the two groups [*SMD* = −1.47, *95% CI* (−1.89, −1.04), p < 0.0001] (Figure 2B).

3.4.2 Effects on fatty liver indicators

Thirteen studies with 212 animals reported blood TG levels after soybean treatment (I^2 = 42.3% and p = 0.054); thus, a random-effects model was used for further meta-analysis. Compared to that in the model group, soybean reduced blood TG levels [*SMD* = −0.78, *95% CI* (−1.07, −0.49), and p < 0.0001] (Figure 3A). A total of 13 studies with 212 animals examined blood TC levels after soybean treatment. Heterogeneity analysis suggested significant heterogeneity (I^2 = 60.7% and p = 0.002); therefore, a random-effects model was used for further analysis. Compared to the model group, soybean significantly reduced blood TC levels [*SMD* = −1.38, *95% CI* (−1.89, −0.87), and p < 0.0001] (Figure 3B). Seven studies with 124 animals examined LDL-C levels after soybean treatment for NAFLD. Heterogeneity analysis suggested obvious heterogeneity (I^2 = 69.8%, p = 0.003); therefore, we used a random-effects model in further meta-analyses. The results showed that soybean significantly reduced LDL-C levels [*SMD* = −1.01, *95% CI* (−1.73, −0.29), and p < 0.0001] (Supplementary Figure S3A) compared to that in the model group. Eight studies with 138 animals examined HDL levels after soybean treatment for NAFLD. Due to minor heterogeneity [I^2 = 29.6% and p = 0.192], we used a fixed-effect model with a combined effect size. A minor increase in HDL-C level was observed compared to that in the model group [*SMD* = 0.08, *95% CI* (−0.26, 0.42), and p = 0.630] (Supplementary Figure S3B), but the differences were not statistically significant. HDL-C is an antiatherosclerotic lipoprotein that is synthesized primarily in the liver and can transport cholesterol from extrahepatic tissue to the liver (Robertson et al., 2004). High serum TC concentrations can stimulate HDL-C transport, leading to transient high cholesterol accumulation in the liver (Yin et al., 2021). Eight studies with 132 animals examined FFA levels after soybean treatment for NAFLD. Due to obvious heterogeneity in FFA levels [I^2 = 74.7% and p < 0.0001], so we used a random-effects model in the meta-analysis. The results indicated that soybean significantly reduced the FFA level compared to that in the model group [*SMD* = −1.55, *95% CI* (−2.38, −0.73), and p < 0.000] (Supplementary Figure S4).

3.4.3 Effects on oxidative stress regulation

Soybean significantly reduced MDA levels and increased SOD levels in the NAFLD model. Three studies with 46 animals were included in the analysis of MDA level, and a fixed-effect model was used to combine the effect sizes (I^2 = 0.0% and p = 0.553). The results showed that the use of soybean significantly reduced the MDA level compared to that in the model group [*SMD* = −1.09, *95% CI* (−1.72, −0.46), and p < 0.0001]. Three studies with 46 animals examined changes in SOD levels. Due to the significant heterogeneity (I^2 = 76.0% and p = 0.016), a random-effect model was used for further meta-analysis. Compared to that in the model group, soybean significantly increased SOD levels [*SMD* = 1.74, *95% CI* (0.25, 3.23), and p = 0.022] (Figure 4).

3.4.4 Effects on TNF-α and insulin

We evaluated the anti-inflammatory effects of soybean by comparing TNF-α levels to those in the model group. Five studies with 82 animals examined the level of TNF-α after soybean treatment (I^2 = 7.7% and p = 0.363); due to minor heterogeneity, a fixed-effects model was used for further meta-analysis. Soybean significantly reduced TNF-α levels [*SMD* = −1.94, *95% CI* (−2.49, −1.40), and p < 0.0001] compared to those in the model group (Supplementary Figure S5A). Three studies with 54 animals examined insulin levels after soybean treatment. However, due to significant heterogeneity

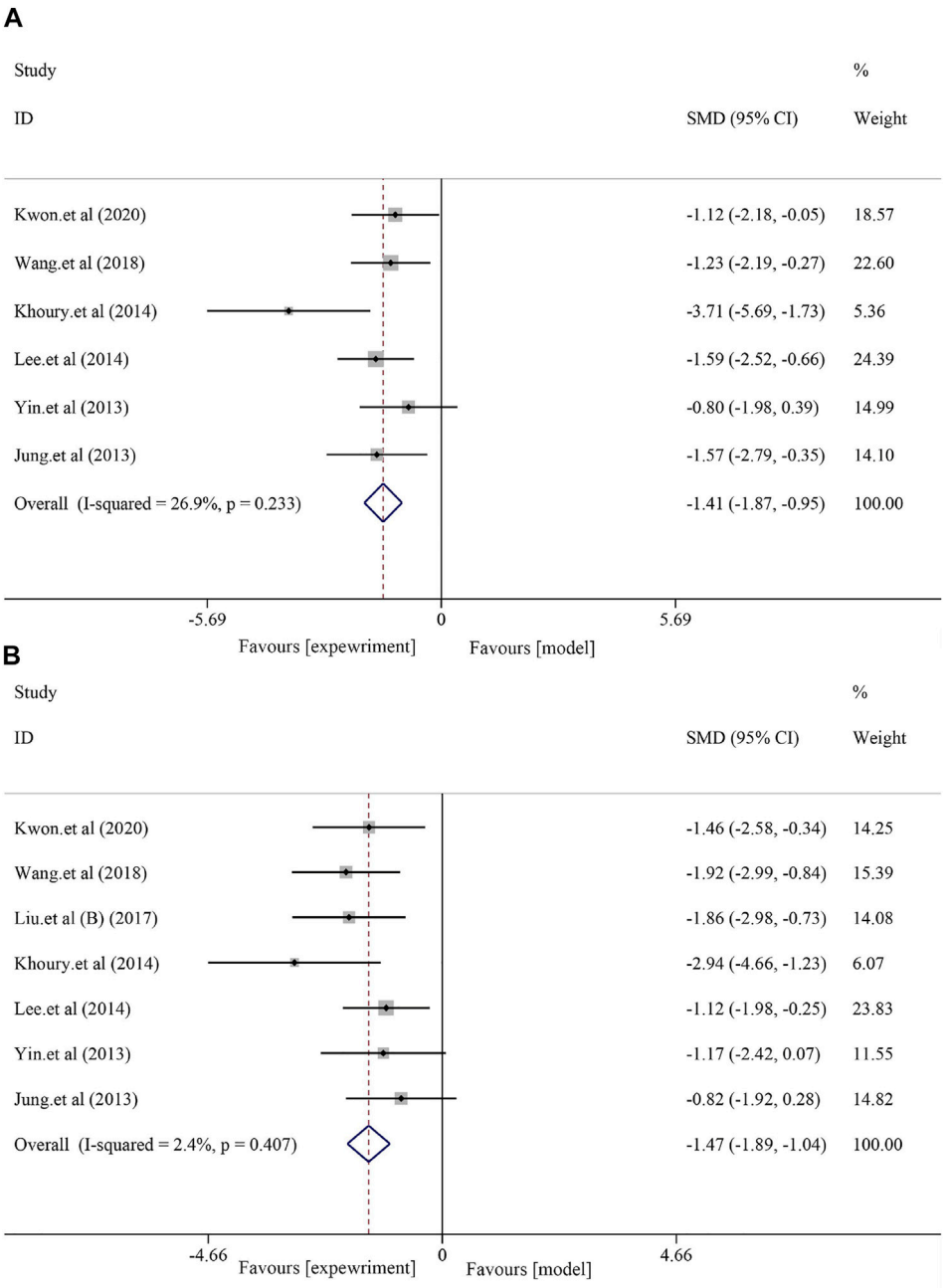


FIGURE 2
Effect of soybean on AST and ALT levels in preclinical studies. **(A)** Pooled effect of AST. **(B)** Pooled effect of ALT.

(I^2 = 88.7% and $p < 0.0001$), a random-effects model was used. The meta-analysis revealed a minor difference between the two groups [*SMD* = −0.18, *95% CI* (−1.94,1.58), $p = 0.842$] (Supplementary Figure S5B).

3.5 Subgroup analysis of related major indicators

3.5.1 Subgroup analysis of blood TG levels

We performed a subgroup analysis of blood TG in the animal models and among animal species (Supplementary Table S4). The results of the subgroup analysis of the animal models (Supplementary Figure S6) showed that soybean reduced the blood TG levels in the HFD model [n = 182, *SM*D = −0.99, 95% CI (−1.30, −0.67), $p < 0.0001$]. In the OLETF model, soybean had no significant effect on blood TG levels [n = 30, SMD = 0.32, 95% CI (−0.40, 1.04), $p = 0.383$]. The results indicated that soybean significantly reduced the blood TG level in the HFD model but not in the OLETF model. Therefore, we preliminarily concluded that the failure of soybean to reduce blood TG levels may be related to OLETF rats. This may be an unexpected result, which we discuss in a later section. Panasevich et al. (2017) and Wanezaki et al. (2015) used OLETF rats as models and found that soybean did not affect blood TG levels.

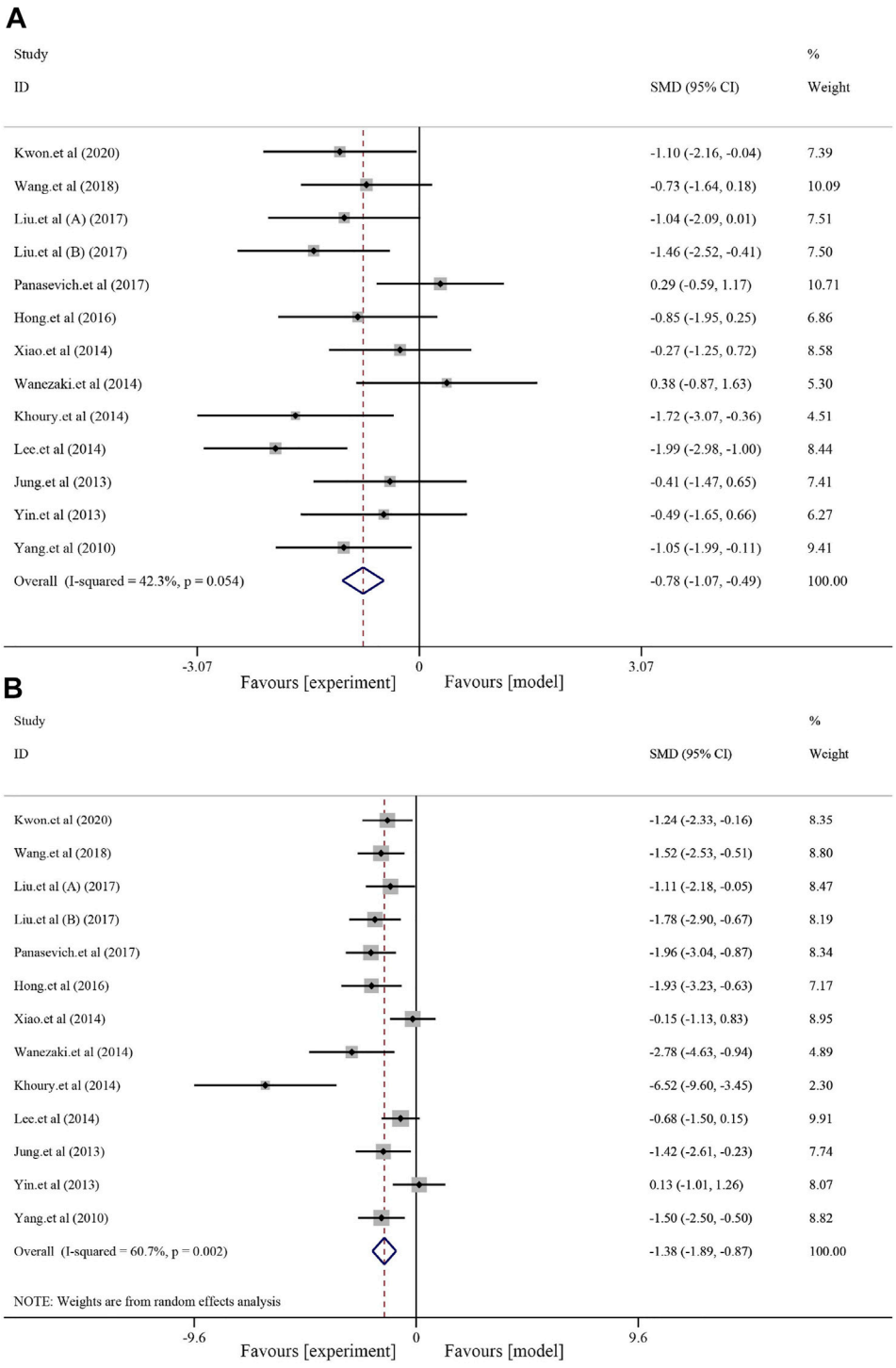


FIGURE 3
Effects of soybean on TG and TC levels in preclinical studies. **(A)** Pooled effect of TG. **(B)** Pooled effect of TC.

The subgroup analysis of mouse species (Supplementary Figure S7) showed that soybean alleviated the blood TG levels in mice [n = 100, SMD = −1.09, 95% CI (−1.53, −0.66), and $p < 0.0001$]. In rat species, although soybean reduced blood TG levels, the change was not statistically significant [Rat, n = 112, SMD = −0.52, *95% CI* (−0.91, −0.13), and $p = 0.008$]. This difference may be related to the use of OLETF rats, which may have affected the analysis results. Therefore, soybean reduced serum TG levels in rats and mice to some extent.

3.5.2 Subgroup analyses of blood TC levels

Thirteen studies with 212 mice examined blood TC levels after soybean treatment for NAFLD. We performed subgroup analyses of blood TC levels in the animal models and among animal species. The

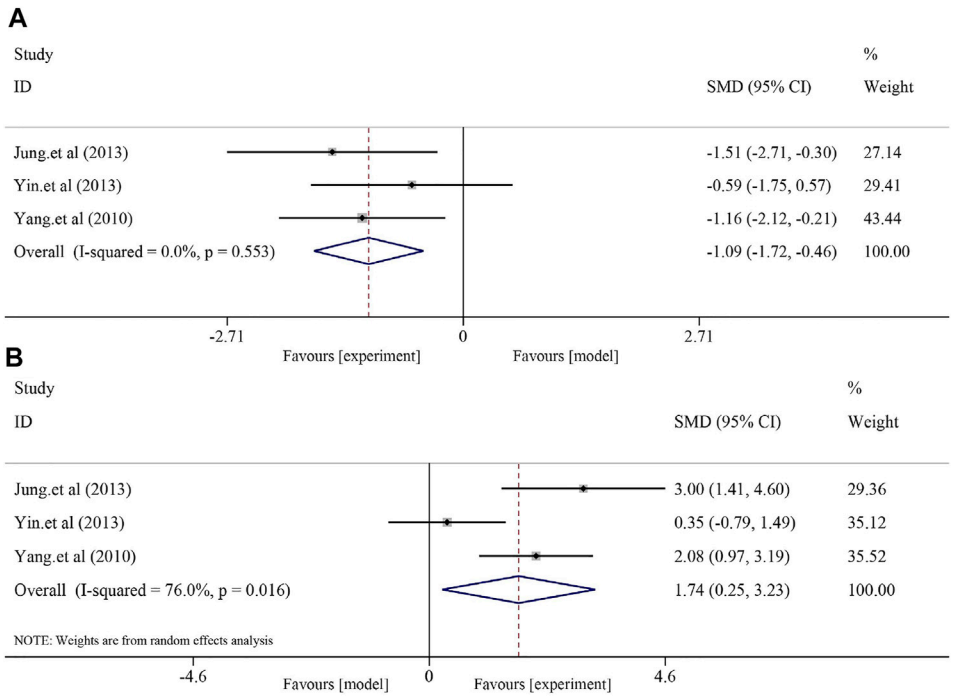


FIGURE 4
Effect of soybean on MDA and SOD levels in preclinical studies. **(A)** Pooled effect of MDA. **(B)** Pooled effect of SOD.

results of the subgroup for animal models (Supplementary Figure S8) showed that soybean reduced the blood TC levels in the HFD model [n = 182, SMD = −1.24, 95% CI (−1.78, −0.70), $p < 0.0001$] and in the OLETF model [n = 30, SMD = −2.17, 95% CI (−3.11, −1.24), $p < 0.0001$]. Therefore, soybean significantly reduced the blood TC levels in the HFD and OLETF models.

Subgroup analysis of animal species (Supplementary Figure S9) showed that soybean alleviated the blood TC levels in mice [n = 100, *SMD* = −1.63, 95% CI (−2.46, −0.79), $p = 0.001$] and rats [n = 112, SMD = −1.21, 95% CI (−1.91, −0.52), $p < 0.0001$]. Therefore, soybean reduced the levels of blood TC associated with NAFLD in rats and mice.

3.6 Effects of soybean on patients in RCTs

Liver function indicators, anthropometric indices, lipid metabolism indicators, and oxidative stress indicators were individually assessed in the meta-analysis of clinical studies of soybean treatment for NAFLD. The potential for soybean to affect those indicators was investigated. The results showed that soybean improved liver function indices, lipid metabolism, and oxidative stress levels.

3.6.1 Effects on liver function

For liver function indices, a fixed-effects model was used for further analysis due to minor heterogeneity in AST ($I^2 = 43.0\%$, $p = 0.173$) and ALT ($I^2 = 32.4\%$, $p = 0.228$). The results showed no significant effect of soybean on AST levels in patients with NAFLD compared to patients in the control group [*SMD* = −0.23, *95% CI* (−0.53, 0.07), $p = 0.128$] (Figure 5A) and a significant effect on ALT [*SMD* = −0.42, *95% CI* (−0.72, −0.12), $p = 0.006$] (Figure 5B).

3.6.2 Effects on fatty liver indicators

The fatty liver indicators containing TG, TC, LDL-C, and HDL-C were compared between the soybean and control groups. The heterogeneities of TG, TC, LDL, and HDL were generally minor ($I^2 = 0.0\%$, $p = 0.416$; $I^2 = 0.0\%$, $p = 0.981$; and $I^2 = 0.0\%$, respectively, $p = 0.581$; $I^2 = 0.0\%$, $p = 0.655$). Soybean use did not significantly decrease serum TG levels [SMD = −0.31, 95% CI (−0.61, −0.02), $p = 0.039$] and TC levels [SMD = −0.06, 95% CI (−0.35, 0.24), $p = 0.694$]. Similarly, no significant effect was observed in LDL [SMD = −0.02, 95% CI (−0.31, 0.28), $p = 0.911$] or HDL [SMD = 0.17, *95% CI* (−0.13, 0.47), $p = 0.260$] (Figure 6).

3.6.3 Effects on anthropometric indices

Three included RCTs were used to assess anthropometric indices, including body weight (BW) and body mass index (BMI). The results showed decreased BW and BMI after soybean treatment, although the differences were not significant [BW, $I^2 = 94.5\%$, $p < 0.0001$, *SMD* = −1.33, *95% CI* (−3.38, 0.71), $p = 0.202$; BMI, $I^2 = 0.3\%$, $p = 0.367$, *SMD* = −0.17, *95% CI* (−0.47, 0.13), $p = 0.264$] compared to the control group (Figure 7).

3.6.4 Effects on MDA and insulin

Analysis of MDA and insulin showed that soybean had a significant effect on MDA [(I2 = 64.6%, $p = 0.060$), SMD = −0.76, 95% CI (−1.30, −0.21), $p = 0.007$] and decreased insulin levels [($I^2 = 48.5\%$, $p = 0.144$), SMD = −0.59, *95% CI* (−0.89, −0.28), $p < 0.0001$] (Figure 7).

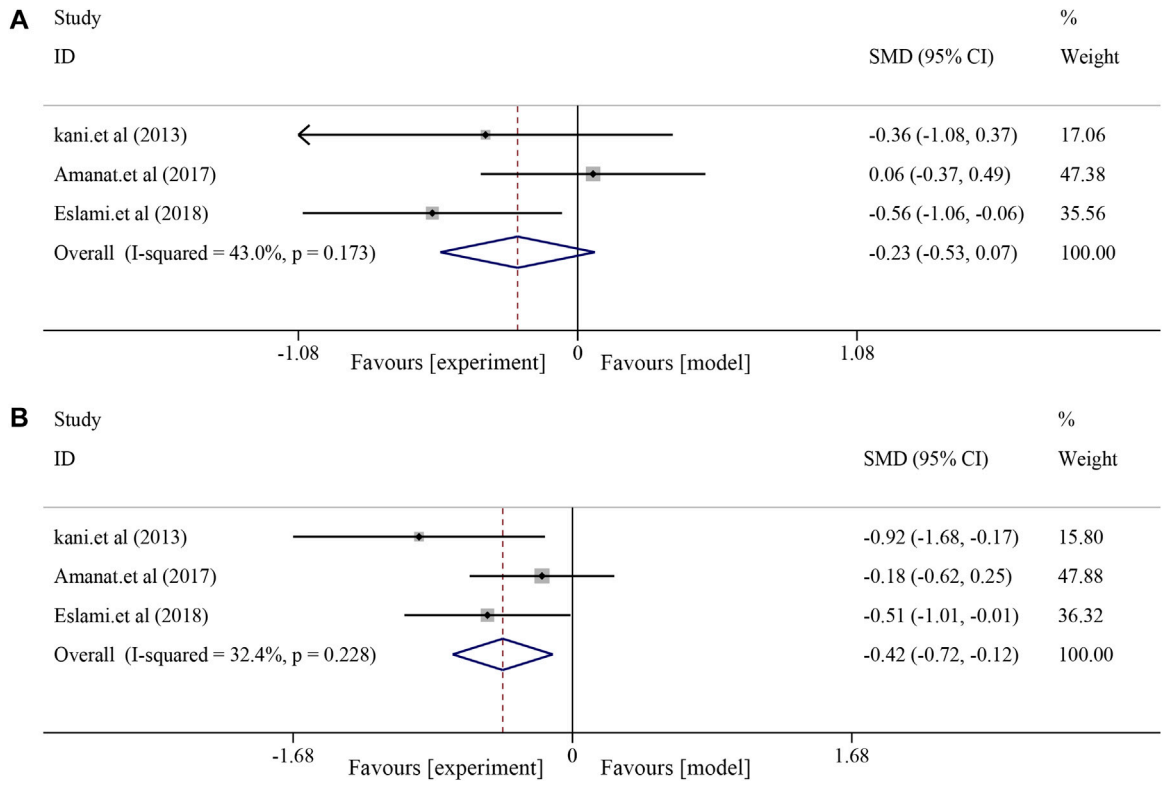


FIGURE 5
Effects of soybean on AST and ALT levels in clinical studies. **(A)** Pooled effect of AST. **(B)** Pooled effect of ALT.

3.7 Publication bias

The main indicators were publication bias (Supplementary Figure S10). The TG outcome of Begg's test index was $p = 0.669$, the absolute value was >0.05, and the Egger's test index was $p = 0.579$, which was >0.05, indicating no publication bias. The TC outcome of Begg's test index was $p = 0.017 < 0.05$, while that of Egger's test index was $p = 0.004 < 0.05$. The results showed some publication bias in serum TC levels and no significant publication bias in serum TG levels.

3.8 Comparison of soybean effects in preclinical studies and clinical trials

We examined a variety of preclinical and clinical indicators to summarize the effect and mechanism of soybean in the treatment of NAFLD. The results strongly suggested that soybean effectively reduced AST and ALT levels to improve NAFLD-induced liver injury and reduce TG, TC, LDL, and FFA and upregulate HDL levels to improve lipid metabolism. In addition, soybean reduced MDA and increased SOD expression levels through oxidative stress pathways, improving NAFLD-induced inflammation by reducing TNF-α levels. Soybean also exerted hepatoprotective effects by improving insulin levels through the insulin resistance pathway. Soybean also showed a therapeutic effect in clinical trials, especially by regulating ALT, TG, insulin, and MDA levels, and showed consistent results in preclinical trials, which may indicate the protective effects of soybean on liver function by improving lipid metabolism, insulin resistance, oxidative stress, and other aspects. In addition, preclinical and clinical studies assessed NAFLD treatment with the soybean extract genistein. The preclinical findings suggested that the continuous administration of 8–64 mg/kg/d of genistein for 12–22 weeks significantly alleviated NAFLD. This finding was consistent with the clinical results; the results of the RCTs demonstrated that the continuous administration of 250 mg/d genistein for 8 weeks was effective in alleviating NAFLD in patients. In the HFD animal model and the OLETF rat model, severe insulin resistance was induced in OLETF rats due to genetic defects. However, soybean improved TG levels in the HFD but not the OLETF model. Patients in clinical trials showed improved TG levels after soybean treatment, which also indirectly demonstrated that soybean could improve lipid metabolism by improving insulin resistance.

3.9 Summary of the mechanism of soybean in the treatment of NAFLD

We summarized the mechanistic pathways in the 13 preclinical studies (Supplementary Table S4). The studies showed that soybean products improved non-alcoholic fatty liver disease through lipid metabolism, insulin resistance, inhibiting inflammation, and other mechanisms. Kwon et al. (2020) suggested that soybean affected lipid metabolism in the liver and adipose tissue and promoted the secretion of lipids in adipose tissue to promote the oxidation of liver fatty acids. Jung and Kim (2013) reported that soybean increased SREBP2 levels

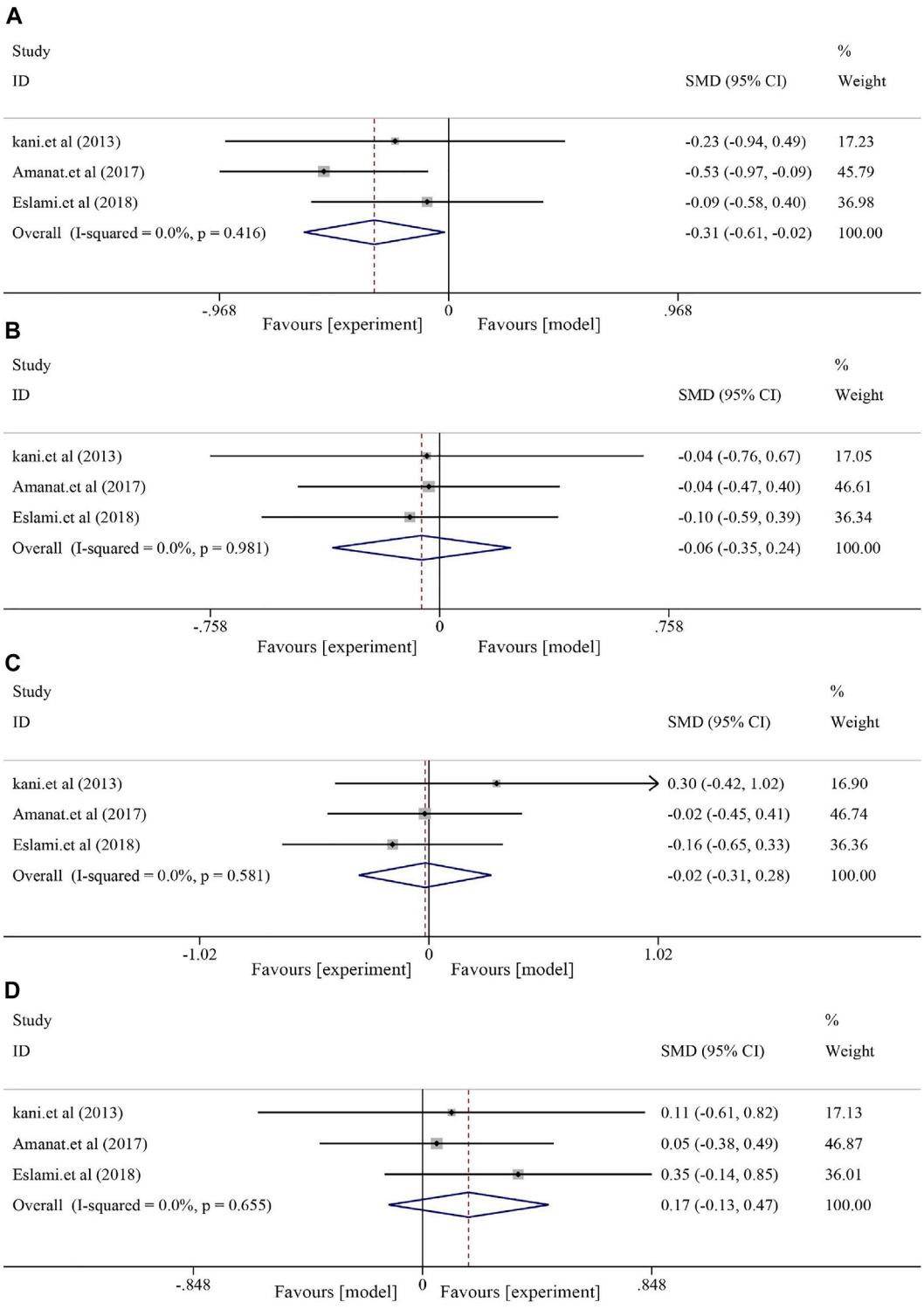


FIGURE 6
Effect of soybean on TG, TC, LDL, and HDL levels in clinical studies. **(A)** Pooled effects of TG, **(B)** TC, **(C)** LDL, and **(D)** HDL.

to suppress ABCA1 expression, increasing cholesterol outflow, reducing cholesterol accumulation in the liver, and improving NAFLD. Khoury et al. (2015) demonstrated that soybean changed the distribution of the T-adjustment factor by using the inherent abilities of the gastrointestinal immune system to induce the T-adjustment factor to inhibit chronic inflammation related to metabolic syndrome, thereby controlling unnecessary systemic immune response. Lee et al. (2014) reported that soybean promoted the expression of fatty factors such as leptin, promoted bile secretion through ApoE, and prevented the accumulation of liver fatty acids, thereby improving NAFLD and providing liver protection. Liu et al. (2017) demonstrated that soybean activated AMPK and

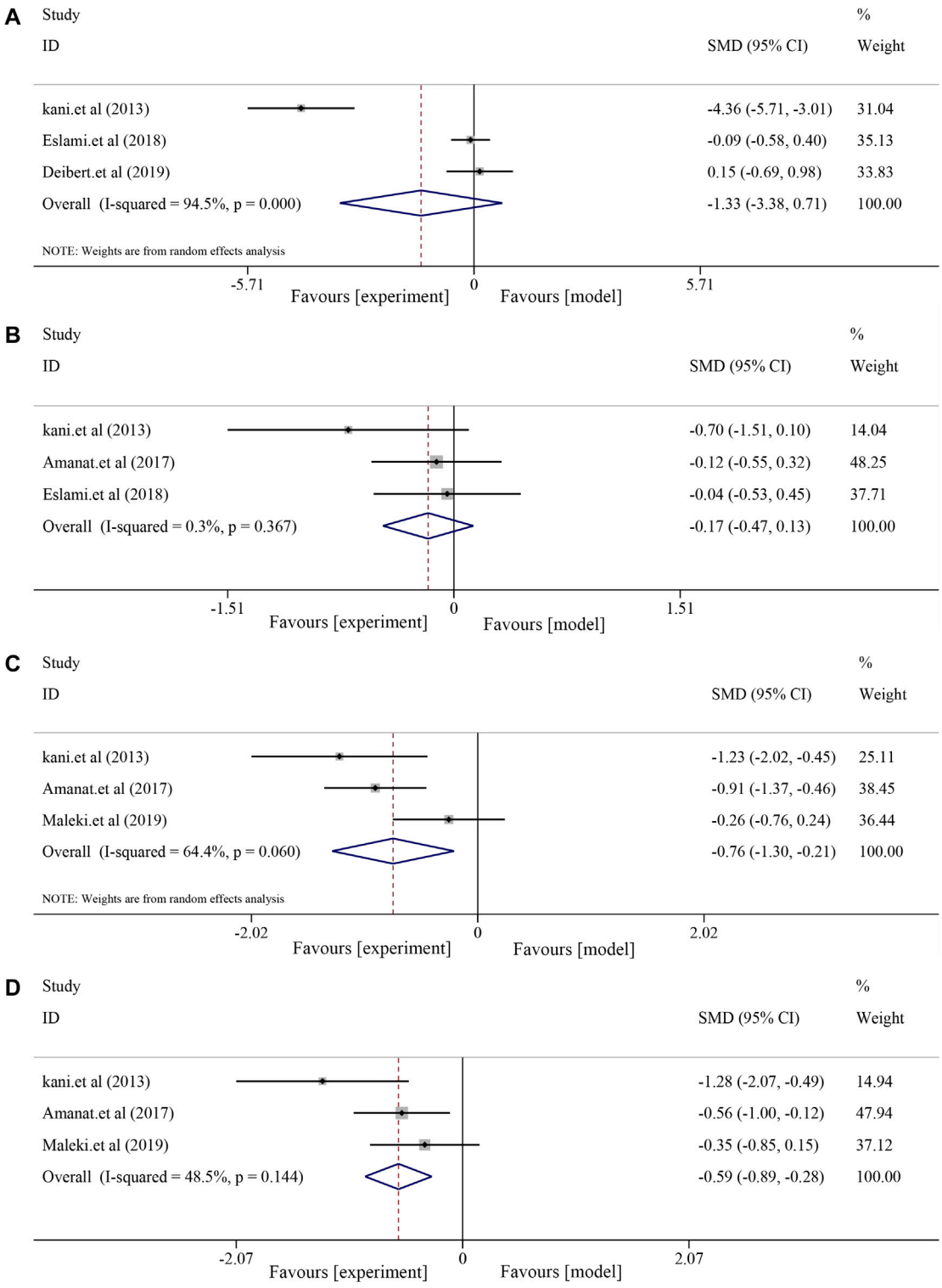


FIGURE 7
Effects of soybean on BW, BMI, MDA, and insulin levels in clinical studies. **(A)** Pooled effect of BW, **(B)** BMI, **(C)** MDA, and **(D)** insulin.

regulated the expression of SREBP-1 and PPARα to reduce lipid synthesis and promote fatty acid oxidation through SREBP-1C and PPARα. Panasevich et al. (2017) suggested that soybean improved liver damage by regulating lipid metabolism and intestinal microorganisms. Wanezaki et al. (2015) showed that soybean regulated lipid metabolism in the liver by inhibiting FAS expression or activity and reducing triglyceride levels in the liver. Wang et al. (2018) demonstrated that soybean improved insulin resistance by directly targeting COX-1 and the downstream TXA2 pathway. Yang et al. (2011) suggested that soybean regulated

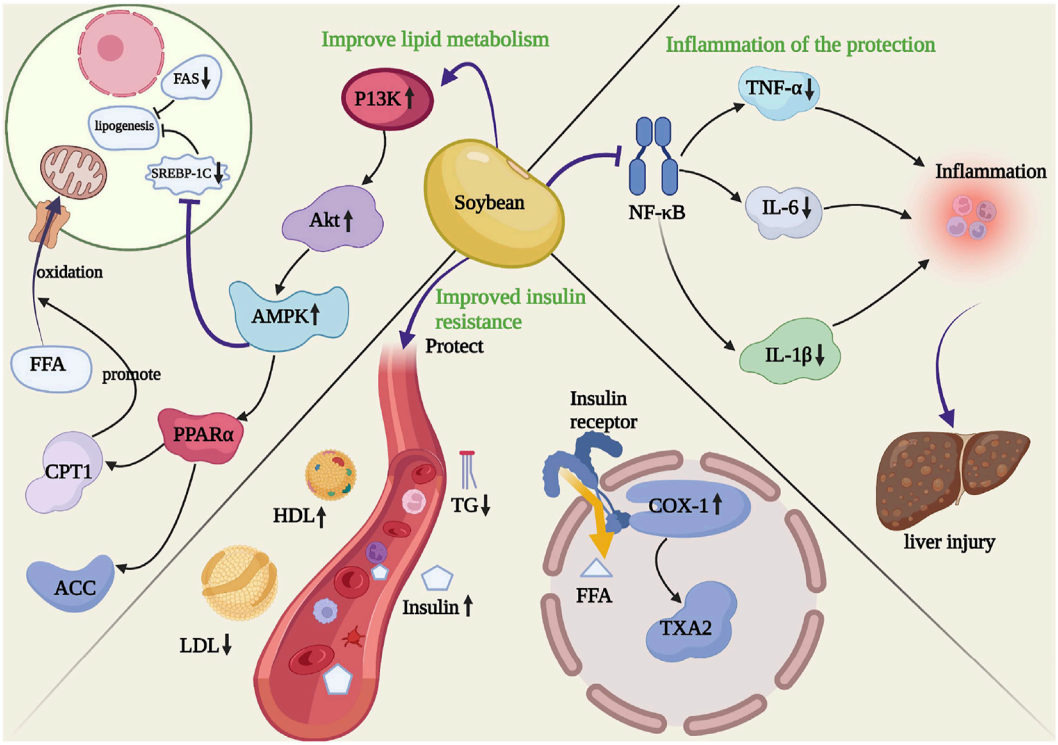


FIGURE 8
Potential molecular mechanisms for the effects of soybean on NAFLD.

the inflammatory response and immune function to improve oxidative stress, thereby reducing liver cell damage.

4 Discussion

This study systematically evaluated soybean treatment of NAFLD based on preclinical and clinical meta-analyses. Thirteen preclinical studies and five RCTs including 212 animals and 260 patients with non-alcoholic fatty liver disease were collected and analyzed. The results of this study showed that soybean alleviated NAFLD, with significant improvements in liver function and fatty liver indicators. Animals with high-fat diet-induced NAFLD exhibited more yellow liver; however, soybean reversed this change in liver color to dark brown. HFD feeding induced a significant accumulation of lipid droplets, which was reduced with soybean treatment. In addition, the results of this meta-analysis showed that soybean effectively improved lipid metabolism and oxidative stress factors. Analysis of the 13 preclinical studies showed that soybean, which contains mainly isoflavones, decreased TG, TC, AST, ALT, and MDA levels and increased SOD levels. Overall, soybean significantly improved insulin resistance, inhibited inflammation, and regulated lipid metabolic and oxidative stress factors to improve non-alcoholic fatty liver. We also identified the relevant mechanistic pathways (Figure 8).

4.1 Regulation of insulin resistance

Soybean did not affect the serum levels of TG in OLETF rats. The pathological manifestations of OLETF rats are insulin resistance and, especially elevated triglyceride levels. Some studies have suggested that serum TG levels are negatively correlated with insulin sensitivity (Yamada et al., 2012). Insulin resistance may cause abnormal triglyceride metabolism through several mechanisms. Insulin is a hormone that regulates adipose tissue and blood flow, significantly reduces blood flow in tissues, and reduces the transport of TG to adipose tissue and skeletal muscle, leading to the accumulation of TG in blood vessels and abnormal lipid metabolism (Shima et al., 1999). Furthermore, insulin resistance increases the release of fatty acids in fat tissue and further increases serum FFA levels. However, increased free fatty acid entry into the liver leads to increased low-density lipoprotein synthesis to increase the endogenous synthesis of TG (Jia et al., 2010). The TXA2 pathway may participate in insulin resistance (Axelsson et al., 2017). The hepatoprotective effect of soybean can be partially explained by the direct targeting of COX-1 and the downstream TXA2 pathway, and some studies have reported that knockout of the *TBXA2R* gene promotes insulin signal transduction (Wang et al., 2018). Thus, we concluded that soybean did not reduce serum TG levels in OLETF rats due to high insulin resistance.

4.2 Regulation of lipid metabolism and oxidative stress

NAFLD patients typically have higher TG, TC, and LDL levels and lower HDL levels. TG is oxidized or transported out of the cell by LDL-C and accumulates in the liver once the balance between input and output is disrupted (Liu et al., 2017). Soybean can reduce SREBP-1C and FAS-mediated liposomes and can activate PPARα expression to promote fatty acid oxidation in the liver. Moreover, soybean enhances AMPK phosphorylation. This finding further suggests that soybean products inhibit oxidative stress by activating AMPK. Moreover, SREBP-1c binds to ACC, FAS, and GPAT (Wang et al., 2015) in the target gene promoter region to regulate liver fat production. In patients with NAFLD, liver expression of SREBP-1C and related fatal genes increases, which may be one explanation for increased lipid production in the liver (Yokozawa et al., 2012) and increased HFD-induced SREBP-1c protein and FAS and GPAT mRNA levels. After soybean treatment, the levels of these fat-generation factors were significantly reduced. Thus, soybean may reduce SREBP1-c and inhibit lipid peroxidation, thereby reducing liver fat degeneration.

Soybean activates AMPK through P13K/AKT, which inhibits the expression of liver sterol regulatory element-binding protein (SREBP-1C) and activates peroxidase (PPARα). AMPK is a "stress indicator" that regulates liver lipid metabolism. AMPK activation results in the inactivation of ACC (Winder and Hardie, 1996), which is a key enzyme that catalyzes malonyl-CoA production, thereby enhancing CPT-1 activity. AMPK/PPARα activation promotes CPT1-mediated fatty acid transport into the mitochondria for β-oxidation (Day et al., 2017). Some studies have suggested that soybean increases the protein expression of PGC1α, ACOX, and PPARα to stimulate the β-oxidation of mitochondrial and peroxisomal fatty acids, thereby increasing the catabolic effect of FFAs and preventing adipogenesis and lipid accumulation. Thus, AMPK/PPARα activation may be an important mechanism by which soybean inhibits lipid accumulation.

PPARα is an important regulatory factor that affects lipid metabolism, plays an important role in the liver, and can directly regulate the expression of target genes that participate in fatty acid oxidation, such as CPT-1 and ACO (Pawlak et al., 2015). PPARα significantly improves NAFLD-related symptoms and slows the degree of liver fat damage, thereby delaying NAFLD progression. Staels et al. (2013) reported that PPARα agonists protected against liver fat degeneration. PPARα activation (Mittelman et al., 2002) increases the oxidation of fatty acids and the expression of the fatty acid oxidation genes, reducing synthetic fatty acids and topical fatty acids, thereby reducing lipid deposition. PPARα can also directly improve liver fatty protein and lipid metabolism genes, and the β-oxidation of liver FFAs increases to inhibit lipid deposition in the liver (Schoonjans et al., 1996). PPARα regulates the encoding of the fatty acid transporter transporters 1 (FATP1) gene and promotes fatty acid absorption (Poirier et al., 2001). PPARα activation can regulate fatty acid dial enzymes (FAT/CD36), increasing fatty acid transfer (Motojima et al., 1998). Studies have shown that PPARα-knockout mice can have severe fat infiltration in hepatic tissue and significantly higher blood TG levels compared to those in normal mice. Therefore, inhibiting PPARα expression can cause lipid deposition in the liver and accelerate fatty liver occurrence and development (Neuschwander-Tetri and Caldwell, 2003). Soybean can effectively reverse changes in PPARα, CPT-1, and ACO mRNA levels caused by high-fat treatment, suggesting that soybean can regulate SREBP-1 and PPARα expression and lipid metabolism, which may be related to AMPK activation. Additionally, the accumulation of FFAs in hepatocytes promotes mitochondrial β-oxidation. Overburdening this metabolic pathway leads to an imbalance in fatty acid metabolism and can cause mitochondrial damage, increased oxidative stress, and steatosis. ROS formation induces lipid peroxidation, which alters mitochondrial DNA. Furthermore, high-sugar or high-cholesterol diets can cause excessive infiltration of cholesterol in the mitochondrial membrane, leading to mitochondrial damage, thus causing oxidative stress. These results showed that soybean can regulate the protein expression of PGC-1α and CYP2E1 to decrease ROS and MDA production, resulting in improved mitochondrial function. Therefore, soybean can improve oxidative stress and maintain mitochondrial homeostasis by inhibiting fat synthesis and reducing fat accumulation. The integrity of mitochondrial function further promotes the β-oxidation of free fatty acids and improves lipid metabolism. However, the levels of FFAs (*SM*D = −1.55, $p < 0.000$) and MDA (SMD = −1.09, $p < 0.0001$) decreased significantly, while that of SOD increased significantly after soybean treatment (SMD = 1.74, $p = 0.022$) in preclinical studies. These results suggested that soybean inhibited fat synthesis and promoted fat decomposition by improving lipid metabolism and maintaining the integrity of mitochondrial function by improving oxidative stress. Thus, β-oxidation was promoted, and fat accumulation was further reduced.

4.3 Regulation of inflammation

The liver is an important organ that regulates body lipid metabolism through the intake of FFAs and the synthesis, storage, and output of lipids (Rui, 2014). The liver transfer cycle of FFAs can impair the insulin sensitivity of this organ (Cusi, 2012), induce SREBP-1C transcription, and lead to liver fat regeneration. Soybean regulates the hepatic NF-κB pathway and Kupffer cells by inhibiting TNF-α, IL-6, and IL-1β generation (Boden et al., 2005; Parker, 2018). Decreasing TNF-α levels can improve the insulin sensitivity of adipocytes, and the consequent decreased release of FFAs can alleviate liver burden (Kern et al., 2001; Zhang et al., 2002). Therefore, soybean improves insulin resistance by improving inflammation, thereby protecting the liver. In this study, the normalization of serum FFA levels after treatment with soybean was explained by decreases in fat production, inflammation, and hepatocyte damage (Kwon et al., 2020). The use of soybean-related products improves hepatic fat degeneration. The cellular mechanisms underlying these improvements have been reported (Wang et al., 2017).

4.4 Limitations

1) Although the effectiveness of soybean in the treatment of NAFLD has been established by examining pathological indicators, the main components that result in the treatment of NAFLD have not been fully determined due to the multiple components in soybean. Thus, further studies are needed to investigate the optimal ingredient for the treatment of NAFLD. 2) In addition, the administration of therapeutic drugs mixed with a high-fat diet cannot be used to determine the specific dose. Therefore, subsequent studies should focus on the specified dose of therapeutic drugs to provide a research basis for clinical studies. 3) The results of the meta-analysis showed some heterogeneity. This heterogeneity was influenced by a combination of factors such as different laboratories using different instruments to determine the active components of soybeans, the duration of administration, the dose administered, and the soybean extraction process. 4) Among the

18 articles included in this study, none described blinding during the experiment, which may lead to selection and implementation biases.

4.5 Implications

Many animals are used for scientific research annually, and the worldwide standards concerning the use of laboratory animals have also become more rigorous. Therefore, the effective use of animal models and research results is an important means to reduce unnecessary animal experiments. A meta-analysis of animal experiments can make full use of animal resources and reduce unnecessary sacrifice of experimental animals, which is more ethical. Our study provides evidence for the research of natural drugs, such as natural herbal and dietary ingredients, and provides examples of methods for the meta-analysis of animal studies. The effect of soybean varies depending on the animal model and the time of administration. Therefore, in an experimental design, different models and administration times are used depending on the experimental purpose. The selection of an appropriate animal model must consider the differences between diseased organs in animals and humans. In addition, the dose range of soybean requires more attention. Therefore, subsequent studies should focus on the dose of therapeutic agents to provide a research basis for clinical studies.

5 Conclusion

The preclinical results showed that animals treated with soybean showed significant improvements. Compared to the model group, soybean effectively reduced major outcome indicators in the experimental group, including AST, ALT, TG, TC, HDL, LDL, MDA, TNF-α, and insulin levels, and increased FFA and SOD levels. The clinical results also indicated the hepatoprotective effect of soybean, especially regarding ALT, TG, MDA, and insulin levels, results that were consistent with those of the preclinical studies. Mechanistically, soybean can significantly ameliorate lipid metabolism and oxidative stress, improve insulin resistance, suppress inflammation, and improve liver function. Therefore, these findings support soybean as a therapeutic agent to prevent and improve NAFLD; these findings also provide a theoretical basis for exploiting NAFLD drugs through evidence-based medical means.

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 authors.

Author contributions

YL was a major contributor to the manuscript. YL conducted a comprehensive summary and finished the work. LY, DX, and GX processed the graphical data. FZ and HW provided constructive opinions and suggestions for the paper. XQ (corresponding author) and XM (corresponding author) conceived and coordinated the review. All authors read and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (82274187), the Major Scientific Research Problems and Key Topics of Medical Technology Problems of China Medical Education Association (2022KTZ016), and the Xinglin Scholar Research Project of Chengdu University of TCM (grant no. QJRC2022028).

Acknowledgments

The authors thank the reviewers and the authors of the references. The reviewer's great comments improved the work.

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/fphar.2023.1088614/full#supplementary-material

References

Abenavoli, L., Di Renzo, L., Boccuto, L., Alwardat, N., Gratteri, S., and De Lorenzo, A. (2018). Health benefits of Mediterranean diet in nonalcoholic fatty liver disease. *Expert Rev. gastroenterology hepatology* 12, 873–881. doi:10.1080/17474124.2018.1503947

Angulo, P. (2002). Nonalcoholic fatty liver disease. *N. Engl. J. Med.* 346, 1221–1231. doi:10.1056/nejmra011775

Axelsson, A. S., Tubbs, E., Mecham, B., Chacko, S., Nenonen, H. A., Tang, Y., et al. (2017). Sulforaphane reduces hepatic glucose production and improves glucose control in patients with type 2 diabetes. *Sci. Transl. Med.* 9, eaah4477. doi:10.1126/scitranslmed.aah4477

Boden, G., She, P., Mozzoli, M., Cheung, P., Gumireddy, K., Reddy, P., et al. (2005). Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-kappaB pathway in rat liver. *Diabetes* 54, 3458–3465. doi:10.2337/diabetes.54.12.3458

Chatterjee, C., Gleddie, S., and Xiao, C. W. (2018). Soybean bioactive peptides and their functional properties. *Nutrients* 10, 1211. doi:10.3390/nu10091211

Cusi, K. (2012). Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: Pathophysiology and clinical implications. *Gastroenterology* 142, 711–725. e6. doi:10.1053/j.gastro.2012.02.003

Day, E. A., Ford, R. J., and Steinberg, G. R. (2017). AMPK as a therapeutic target for treating metabolic diseases. *Trends Endocrinol. metabolism TEM* 28, 545–560. doi:10.1016/j.tem.2017.05.004

Ekstedt, M., Hagström, H., Nasr, P., Fredrikson, M., Stål, P., Kechagias, S., et al. (2015). Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatol. Baltim. Md* 61, 1547–1554. doi:10.1002/hep.27368

Huang, H., Krishnan, H. B., Pham, Q., Yu, L. L., and Wang, T. T. (2016). Soy and gut microbiota: Interaction and implication for human health. *J. Agric. food Chem.* 64, 8695–8709. doi:10.1021/acs.jafc.6b03725

Jia, W., Sun, F., and Hu, M. (2010). Relationship between abnormal triglyceride metabolism and insulin resistance in obese hypertensive patients. *Clin. meta-clin* 25, 1143–1144.

Jung, J. H., and Kim, H. S. (2013). The inhibitory effect of black soybean on hepatic cholesterol accumulation in high cholesterol and high fat diet-induced non-alcoholic fatty liver disease. *Food Chem. Toxicol. Int. J. Publ. Br. Industrial Biol. Res. Assoc.* 60, 404–412. doi:10.1016/j.fct.2013.07.048

Kern, P. A., Ranganathan, S., Li, C., Wood, L., and Ranganathan, G. (2001). Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am. J. physiology Endocrinol. metabolism* 280, E745–E751. doi:10.1152/ajpendo.2001.280.5.E745

Khoury, T., Ben Ya'acov, A., Shabat, Y., Zolotarovya, L., Snir, R., and Ilan, Y. (2015). Altered distribution of regulatory lymphocytes by oral administration of soy-extracts exerts a hepatoprotective effect alleviating immune mediated liver injury, non-alcoholic steatohepatitis and insulin resistance. *World J. gastroenterology* 21, 7443–7456. doi:10.3748/wjg.v21.i24.7443

Kwon, D., Kim, S. H., Son, S. W., Seo, J., Jeong, T. B., Kim, K. M., et al. (2020). Germinated soybean embryo extract ameliorates fatty liver injury in high-fat diet-fed obese mice. *Pharm. (Basel, Switz.* 13, 380. doi:10.3390/ph13110380

Lee, H. S., Nam, Y., Chung, Y. H., Kim, H. R., Park, E. S., Chung, S. J., et al. (2014). Beneficial effects of phosphatidylcholine on high-fat diet-induced obesity, hyperlipidemia and fatty liver in mice. *Life Sci.* 118, 7–14. doi:10.1016/j.lfs.2014.09.027

Lin, Z., Wu, Z. F., Jiang, C. H., Zhang, Q. W., Ouyang, S., Che, C. T., et al. (2016). The chloroform extract of Cyclocarya paliurus attenuates high-fat diet induced non-alcoholic hepatic steatosis in Sprague Dawley rats. *Phytomedicine Int. J. phytotherapy Phytopharm.* 23, 1475–1483. doi:10.1016/j.phymed.2016.08.003

Liu, H., Zhong, H., Leng, L., and Jiang, Z. (2017). Effects of soy isoflavone on hepatic steatosis in high fat-induced rats. *J. Clin. Biochem. Nutr.* 61, 85–90. doi:10.3164/jcbn.16-98

Mathiesen, U. L., Franzén, L. E., Frydén, A., Foberg, U., Bodemar, G., and Fryden, A. (1999). The clinical significance of slightly to moderately increased liver transaminase values in asymptomatic patients. *Scand. J. gastroenterology* 34, 85–91. doi:10.1080/00365529950172880

Mittelman, S. D., Van Citters, G. W., Kirkman, E. L., and Bergman, R. N. (2002). Extreme insulin resistance of the central adipose depot *in vivo*. *Diabetes* 51, 755–761. doi:10.2337/diabetes.51.3.755

Motojima, K., Passilly, P., Peters, J. M., Gonzalez, F. J., and Latruffe, N. (1998). Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor alpha and gamma activators in a tissue- and inducer-specific manner. *J. Biol. Chem.* 273, 16710–16714. doi:10.1074/jbc.273.27.16710

Neuschwander-Tetri, B. A., and Caldwell, S. H. (2003). Nonalcoholic steatohepatitis: Summary of an AASLD single topic conference. *Hepatol. Baltim. Md* 37, 1202–1219. doi:10.1053/jhep.2003.50193

Pan, M. H., Lai, C. S., Tsai, M. L., and Ho, C. T. (2014). Chemoprevention of nonalcoholic fatty liver disease by dietary natural compounds. *Mol. Nutr. food Res.* 58, 147–171. doi:10.1002/mnfr.201300522

Panasevich, M. R., Schuster, C. M., Phillips, K. E., Meers, G. M., Chintapalli, S. V., Wankhade, U. D., et al. (2017). Soy compared with milk protein in a Western diet changes fecal microbiota and decreases hepatic steatosis in obese OLETF rats. *J. Nutr. Biochem.* 46, 125–136. doi:10.1016/j.jnutbio.2017.05.004

Parker, R. (2018). The role of adipose tissue in fatty liver diseases. *Liver Res.* 2, 35–42. doi:10.1016/j.livres.2018.02.002

Pawlak, M., Lefebvre, P., and Staels, B. (2015). Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. *J. hepatology* 62, 720–733. doi:10.1016/j.jhep.2014.10.039

Poirier, H., Niot, I., Monnot, M. C., Braissant, O., Meunier-Durmort, C., Costet, P., et al. (2001). Differential involvement of peroxisome-proliferator-activated receptors alpha and delta in fibrate and fatty-acid-mediated inductions of the gene encoding liver fatty-acid-binding protein in the liver and the small intestine. *Biochem. J.* 355, 481–488. doi:10.1042/0264-6021:3550481

Powell, E. E., Wong, V. W., and Rinella, M. (2021). Non-alcoholic fatty liver disease. *Lancet (London, Engl.* 397, 2212–2224. doi:10.1016/s0140-6736(20)32511-3

Robertson, R. P., Harmon, J., Tran, P. O., and Poitout, V. (2004). Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes* 53, S119–S124. doi:10.2337/diabetes.53.2007.s119

Rui, L. (2014). Energy metabolism in the liver. *Compr. Physiol.* 4, 177–197. doi:10.1002/cphy.c130024

Schoonjans, K., Peinado-Onsurbe, J., Lefebvre, A. M., Heyman, R. A., Briggs, M., Deeb, S., et al. (1996). PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J.* 15, 5336–5348. doi:10.1002/j.1460-2075.1996.tb00918.x

Sherif, Z. A., Saeed, A., Ghavimi, S., Nouraie, S. M., Laiyemo, A. O., Brim, H., et al. (2016). Global epidemiology of nonalcoholic fatty liver disease and perspectives on US minority populations. *Dig. Dis. Sci.* 61, 1214–1225. doi:10.1007/s10620-016-4143-0

Shima, K., Zhu, M., and Mizuno, A. (1999). Pathoetiology and prevention of NIDDM lessons from the OLETF rat. *J. Med. investigation JMI* 46, 121–129.

Staels, B., Rubenstrunk, A., Noel, B., Rigou, G., Delataille, P., Millatt, L. J., et al. (2013). Hepatoprotective effects of the dual peroxisome proliferator-activated receptor alpha/delta agonist, GFT505, in rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Hepatol. Baltim. Md* 58, 1941–1952. doi:10.1002/hep.26461

Wanezaki, S., Tachibana, N., Nagata, M., Saito, S., Nagao, K., Yanagita, T., et al. (2015). Soy β-conglycinin improves obesity-induced metabolic abnormalities in a rat model of nonalcoholic fatty liver disease. *Obes. Res. Clin. Pract.* 9, 168–174. doi:10.1016/j.orcp.2014.03.005

Wang, S., Wang, Y., Pan, M. H., and Ho, C. T. (2017). Anti-obesity molecular mechanism of soy isoflavones: Weaving the way to new therapeutic routes. *Food and Funct.* 8, 3831–3846. doi:10.1039/c7fo01094j

Wang, W., Chen, J., Mao, J., Li, H., Wang, M., Zhang, H., et al. (2018). Genistein ameliorates non-alcoholic fatty liver disease by targeting the thromboxane A2 pathway. *J. Agric. food Chem.* 66 (23), 5853–5859. doi:10.1021/acs.jafc.8b01691

Wang, Y., Viscarra, J., Kim, S. J., and Sul, H. S. (2015). Transcriptional regulation of hepatic lipogenesis. *Nat. Rev. Mol. Cell Biol.* 16, 678–689. doi:10.1038/nrm4074

Winder, W. W., and Hardie, D. G. (1996). Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise. *Am. J. physiology* 270, E299–E304. doi:10.1152/ajpendo.1996.270.2.E299

Xu, Y., Lu, Z., and Luo, J. (2019). Effect of soybean isoflavone on nonalcoholic fatty liver disease in rats. *Mod. Med. health* 35, 188–191.

Yamada, T., Kose, H., Ohta, T., and Matsumoto, K. (2012). Genetic dissection of complex genetic factor involved in NIDDM of OLETF rat. *Exp. diabetes Res.* 2012, 582546. doi:10.1155/2012/582546

Yang, H. Y., Tzeng, Y. H., Chai, C. Y., Hsieh, A. T., Chen, J. R., Chang, L. S., et al. (2011). Soy protein retards the progression of non-alcoholic steatohepatitis via improvement of insulin resistance and steatosis. *Nutr. (Burbank, Los Angel. Cty. Calif.)* 27, 943–948. doi:10.1016/j.nut.2010.09.004

Yin, M., Matsuoka, R., Xi, Y., and Wang, X. (2021). Comparison of egg yolk and soybean phospholipids on hepatic fatty acid profile and liver protection in rats fed a high-fructose diet. *Foods (Basel, Switz.* 10, 1569. doi:10.3390/foods10071569

Yokozawa, T., Cho, E. J., Park, C. H., and Kim, J. H. (2012). Protective effect of proanthocyanidin against diabetic oxidative stress. *Evidence-based complementary Altern. Med. eCAM* 2012, 623879. doi:10.1155/2012/623879

Zhang, H. H., Halbleib, M., Ahmad, F., Manganiello, V. C., and Greenberg, A. S. (2002). Tumor necrosis factor-alpha stimulates lipolysis in differentiated human adipocytes through activation of extracellular signal-related kinase and elevation of intracellular cAMP. *Diabetes* 51, 2929–2935. doi:10.2337/diabetes.51.10.2929

Zhu, H., Bi, D., Zhang, Y., Kong, C., Du, J., Wu, X., et al. (2022). Ketogenic diet for human diseases: The underlying mechanisms and potential for clinical implementations. *Signal Transduct. Target. Ther.* 7, 11. doi:10.1038/s41392-021-00831-w

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 02 February 2023
DOI 10.3389/fphar.2023.1123700

OPEN ACCESS

EDITED BY
Wei Peng,
Chengdu University of Traditional Chinese Medicine, China

REVIEWED BY
Xiaohong Yang,
Chongqing Institute of Green and Intelligent Technology (CAS), China
Zhongwen Qi,
China Academy of Chinese Medical Sciences, China

*CORRESPONDENCE
Xichun Pan,
xichunpan@tmmu.edu.cn

SPECIALTY SECTION
This article was submitted to Ethnopharmacology, a section of the journal Frontiers in Pharmacology

RECEIVED 14 December 2022
ACCEPTED 18 January 2023
PUBLISHED 02 February 2023

CITATION
Cen Y, Xiong Y, Qin R, Tao H, Yang Q and Pan X (2023), Anti-malarial artesunate ameliorates atherosclerosis by modulating arterial inflammatory responses *via* inhibiting the NF-κB–NLRP3 inflammasome pathway.
Front. Pharmacol. 14:1123700.
doi: 10.3389/fphar.2023.1123700

Anti-malarial artesunate ameliorates atherosclerosis by modulating arterial inflammatory responses *via* inhibiting the NF-κB–NLRP3 inflammasome pathway

Yanyan Cen, Yalan Xiong, Rongxin Qin, Hui Tao, Qunfang Yang and Xichun Pan*

Department of Pharmacology, College of Pharmacy, Army Medical University, Chongqing, China

Introduction: Chronic inflammation plays a critical role in the pathogenesis of atherosclerosis (AS), and involves a complex interplay between blood components, macrophages, and arterial wall. Therefore, it is valuable in the development of targeted therapies to treat AS.

Methods: AS rat model was induced by atherogenic diet plus with lipopolysaccharide (LPS) and then treated by anti-malarial artesunate (Art), a succinate derivative of artemisinin. The arterial morphology was observed after Oil red O, hematoxylin—eosin, and Masson's staining. The arterial protein level was detected by immunohistochemistry or immunofluorescence. The expression level of mRNA was determined by PCR array or real-time PCR.

Results: Herein, we showed that Art possessed a dose-dependently protective effect on AS rats. In detail, Art showed a comparable inhibitory effect on arterial plaque and serum lipids compared to those of rosuvastatin (RS), and further showed a better inhibition on arterial lipid deposition and arterial remodeling comprised of arterial wall thicken and vascular collagen deposition, than those of RS. The improvement of Art on AS rats was related to inhibit arterial macrophage recruitment, and inhibit nuclear factor κB (NF-κB)-related excessive arterial inflammatory responses. Critically, Art showed significant inhibition on the NLRP3 inflammasome activation in both arterial wall and arterial macrophages, by down-regulating the expression of NOD-like receptor thermal protein domain associated protein 3 (NLRP3) and apoptosis associated speckle-like protein containing CARD (ASC), leading to less production of the NLRP3 inflammasome—derived caspase-1, interleukin-1β (IL-1β), IL-18, and subsequent transforming growth factor β1 (TGF-β1) in AS rats.

Conclusion: We propose that Art is an anti-AS agent acts through modulating the arterial inflammatory responses *via* inhibiting the NF-κB – NLRP3 inflammasome pathway.

KEYWORDS

atherosclerosis, artesunate, atherogenic diet, inflammation, the NF-κB–NLRP3 inflammasome pathway

Highlights

- Artesunate protects atherosclerotic rats induced by an atherogenic diet plus lipopolysaccharide.
- Artesunate modulates arterial inflammatory responses.
- Artesunate inhibits the NF-κB–NLRP3 inflammasome pathway.

Introduction

Atherosclerosis (AS) is a fundamental cause of cardiovascular diseases (CVDs), accounting for about 50% of deaths worldwide (Libby et al., 2019). As a well-studied chronic disease, AS is characterized mainly by the formation and development of lipid and immune-cell containing plaques in the inner lining of the arteries (Libby et al., 2011; Schaftenaar et al., 2016). The pathogenesis of AS is complicated and involves numerous etiological factors, wherein lipid deposition and inflammatory response have been proposed to play key roles in the process of plaque development (Schaftenaar et al., 2016). It is quite attractive to unveil the regulatory mechanism of arterial lipid deposition and inflammatory response and develop promising therapy for AS treatment.

Arterial lipid deposition and inflammation form a complicated regulatory network during AS. Deposited lipids induce a series of arterial responses, including induction of inflammation, intimal accumulation of lipids, and foam cell formation, which play an essential role in AS, especially in the initial stage (Chistiakov et al., 2017). Arterial macrophages, differentiated from circulating monocytes, are responsible for clearance of deposited lipids to prevent cytotoxicity, tissue injury, inflammation, and metabolic disturbances (Chistiakov et al., 2016). Macrophages use pattern recognition receptors (PRRs) and subsequently initiate signaling cascades that lead to the transcription of pro-inflammatory cytokines and chemokines to manage lipid internalization (Grebe and Latz, 2013). However, excessively accumulated macrophages are also associated with chronic inflammation of vascular walls, which is strongly related to the initiation, growth, and rupture of arterial plaques, and vascular remodeling (Xu et al., 2019).

As a well-studied transcription factor, nuclear factor κB (NF-κB) is activated by pathogen-associated molecular patterns (PAMPs) or cytokines and then primes transcription of a series of genes encoding cytokines and chemokines in macrophages (Hu et al., 2021). In the past 3 decades, NF-κB has been regarded as the most critical player in AS because the genes it primed are involved in all phases of AS (Li et al., 2022). In addition, NF-κB also primes mRNA expression of NOD-like receptor thermal protein domain-associated protein 3 (NLRP3) and apoptosis-associated speckle-like protein containing CARD (ASC), which form NLRP3 inflammasome to cleave pro-caspase-1 into caspase-1, by which it converts pro-interleukin 1β (pro-IL-1β) and pro-IL-18 to mature forms (Baldrighi et al., 2017; Grebe et al., 2018). NLRP3 inflammasome has been considered a link between lipid metabolism and inflammation in AS (Li et al., 2016). As a critical pathogenic factor involved in all stages of AS, cholesterol crystals are one of the most potent activators of NLRP3 inflammasome to initiate and exacerbate AS *via* IL-1β and IL-18, which are well-known as the pro-atherogenic cytokines (Sharma and Kanneganti, 2021; Takahashi, 2022). In addition, reports indicated that NLRP3 expression was upregulated in the aorta of patients with coronary AS, and the aortic NLRP3 expression was correlated with the severity of disease (Afrasyab et al., 2016; Paramel Varghese et al., 2016). In various AS animal models, high NLRP3 levels were observed in monocytes and macrophages (Karasawa and Takahashi, 2017). Therefore, numerous reports have suggested that the NF-κB–NLRP3 inflammasome pathway is a possible target for future drug discoveries and clinical settings (Parsamanesh et al., 2019).

Previously, we reported that anti-malarial artesunate (Art) could protect AS in an apolipoprotein E-knockout (*ApoE*$^{-/-}$) mice model *via* inhibition of the release of chemokines including IL-8 and C–C motif chemokine ligand 2 (CCL2) (Jiang et al., 2016). In addition, recent reports also showed that Art possessed similar anti-AS activity in an *ApoE*$^{-/-}$ mice model (He et al., 2020; Wang et al., 2022). However, an ApoE-deficiency model which possesses the APO-dysfunction phenotype cannot fully account for the pathogenic factors, especially complicated inflammatory responses which have been regarded as the critical trigger during the AS process (Ross, 1999). Moreover, the detailed anti-AS mechanism of Art has not been well elucidated yet. In this study, we further evaluated the anti-AS activity of Art in a normal rat model induced by an atherogenic diet supplemented with lipopolysaccharide (LPS), which consisted of both a high-fat pathogenic factor and pro-inflammatory pathogenic factor that are widely used for accelerating the progression of AS (Wiesner et al., 2010; Yin et al., 2013), and the possible mechanism was also discussed from the perspective of inflammation controlled by the NF-κB–NLRP3 inflammasome pathway.

Materials and methods

Animal experiments

All animal experiments were performed in accordance with the National Guidelines for Animal Care and Use (NIH Publication No. 85–23, revised 1996) and approved by the Ethics Committee for Animal Experimentation of Army Medical University (License No. AMUWEC20181680). All surgeries and sacrifices were performed in a manner to minimize animal suffering.

Female Wistar rats (6–8 weeks old; 190 ± 20 g) were supplied by the Experimental Animal Center of our university and were housed under a pathogen-free condition (License No. SCXK-2017002). All rats were raised on a 12-h light/dark cycle with free access to water, and the temperature was 20°C ± 2°C with a humidity of 50 ± 5% following a 7-day acclimatization period. The inflammatory immune method was used for the animal model (Wiesner et al., 2010; Fu et al., 2017). A total of 70 rats were given 70,000 U/kg of vitamin D3 (i.p.; Sigma, St. Louis, MO, United States) three times in the first week, and 0.15 mg/kg of LPS (i.m.; Sigma) was given six times every week. These rats were fed with the atherogenic diet (1.25% cholesterol, 15% fat, and 0.5% cholic acid) for 8 weeks to induce acute AS. The rats used as a control (n = 10) were fed with a standard chow diet. At the end of the 8th week, 10 atherogenic diet rats were used for detecting serum biochemistry and observing histological changes of the aortic arch vessel. The rest were randomly divided into six groups (n = 10) for a further 8-week treatment. These rats were fed with the atherogenic diet containing normal saline (AS group), 4.5 mg/kg of Art (bid, H-bid group; qd, H-qd group; Guilin Pharmaceuticals, Guilin, Guangxi, China), 1.5 mg/kg of Art (bid, L-bid group; qd, L-qd group), or 4.8 mg/kg/day of rosuvastatin (RS group; Pfizer Inc., NY, United States).

Art was dissolved in 5% sodium bicarbonate and was diluted in normal saline. All drug solutions were prepared at room temperature before use.

Samplings

At the end of experiments, all rats were anesthetized with isoflurane and sacrificed. The abdominal aorta, full-length aorta, and peripheral blood were harvested, respectively. Serum samples were prepared using peripheral blood by centrifugation (3000 × g/min for 5 min) and then were used for detecting serum biochemistry. The thoracic aortas were quickly excised and collected for extracting total RNA or fixed with paraformaldehyde (4%; *m*/*v*) for 48 h and then embedded in paraffin for subsequent histological observations.

Serum biochemistry and ELISA

The serum levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were estimated using commercial kits (Boster, Wuhan, Hubei, China). The serum levels of caspase-1 and IL-1β were measured using corresponding enzyme-linked immunosorbent assay (ELISA) kits (Boster).

Histological observations

Oil red O staining was performed to observe atherosclerotic lesions. Briefly, the heart and entire aorta were dissected free from the rats, and photographs were captured. The whole thoracic aortas from the same position of each sample were opened longitudinally and stained with Oil red O (Beyotime, Shanghai, China). The paraffin sections were stained by hematoxylin–eosin (HE; Boster, Wuhan, China), Masson's trichrome (Boster), and Oil red O according to the user manuals. All section images were captured under an Olympus BX51 light microscope (Tokyo, Japan). The lesion-positive staining (%) values and the collagen-positive staining (%) values were analyzed using ImageJ (Schindelin et al., 2015).

Immunohistochemistry

Immunohistochemistry (IHC) was performed using an IHC kit (Boster) according to the user manual. Briefly, the paraffin sections (n = 5) were probed with the primary antibodies (Cell Signaling, Beverly, MA, United States) against NF-κB p65 subunit (1: 200 dilution), NLRP3 (1:200 dilution), ASC (1:200 dilution), collagen I and III (Col-I and Col-III; 1:200 dilution), IL-1β (1: 200 dilution), IL-18 (1:200 dilution), and transforming growth factor β1 (TGF-β1; 1:200 dilution). The IHC images were captured under a light microscope (Olympus, Japan). Images were analyzed using the ImageJ packages (Schindelin et al., 2015).

Immunofluorescence

Arterial immunofluorescence was performed (n = 5) as described previously (Zhuang et al., 2019). In summary, macrophages were probed by the F4/80 (Cell Signaling; 1:200 dilution) antibody, vascular smooth muscle cells were probed by the smooth muscle actin α (α-SMA; Cell Signaling; 1:200 dilution) antibody, and NLRP3 expression was probed by the NLRP3 antibody (Cell Signaling; 1:200 dilution). These proteins were then stained by the secondary antibody conjugated with Alexa Fluor 488 (Sigma; green; 1:400 dilution) or Alexa Fluor 555 (Sigma; red; 1:400 dilution) as indicated, followed by DAPI staining (Sigma; blue) for visualizing nuclear DNA. The images were captured under a Zeiss LSM780 confocal microscope (Jena, Germany) and were analyzed using ImageJ.

PCR array

In accordance with the user manual, total RNA was extracted from the arterial samples from the control group, the AS group, and the Art treatment (H-bid) group (n = 3) using a TRIzol reagent (Takara, Dalian, Liaoning, China) and was reverse-transcribed afterward using PrimeScript™ RT Master Mix (Takara). Then, mRNA expression was detected using Cytokines and Chemokines PCR Array (Wcgene Biotech, Shanghai, China). All primers used are listed in Table 1. Data were analyzed with the comparative Ct method normalizing to *GAPDH* and *β-actin*. The heatmap was analyzed using an online server Heatmapper (www.heatmapper.ca/expression/). Rat peritoneal macrophages (RPMs) were isolated and cultured as described previously (Chen et al., 2020). RPMs grown in 12-well plates (5×10^5 cells/well) were treated by LPS (10 ng/mL) plus soluble cholesterol (CHO; 100 μg/mL) with or without the presence of Art (20 μg/mL) for 1 h (n = 5). Similarly, total RNA of rat PMs was extracted and analyzed by Cytokines and Chemokines PCR Array as described previously.

Real-time PCR

Total RNA extraction and reverse transcription were performed similarly to the aforementioned PCR array procedure. Subsequently, real-time PCR was performed using SsoAdvanced SYBR Green Supermix (Bio-Rad, Hercules, CA, United States) with primer pairs listed in Table 1. The mRNA levels (fold change) were calculated by normalizing to β-actin using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Statistical analysis

Data are presented as the mean ± standard deviation (S.D.). Differences were analyzed using Excel by the one-way ANOVA method for multiple comparisons (weight and feed intake) and by Student's t-test method for comparison between individual groups. $p < 0.05$ was considered to be statistically significant.

Results

Art ameliorates AS in atherogenic diet–LPS-induced rats

During the animal experiment (Figure 1A), no mortality was observed in any group. Compared to the control, the AS group, the

TABLE 1 Primers used in this study.

Gene	GenBank accession no.	Forward primer (5′→3′)	Reverse primer (5′→3′)
NLRP3	NM_001191642	TGGATAGGTTTGCTGGGATA	CCACATCTTAGTCCTGCCAAT
ASC	AB053165	GCACAGCCAGAACAGAACAT	TACAGAGCATCCAGCAAACC
TNF-α	BC107671	ATGAGCACGGAAAGCATGATCCGAG	CTCGGATCATGCTTTCCGTGCTCAT
*IL-1*β	BC091141	GCTTCAAATCTCACAGCAGCAT	GCAGGTCGTCATCATCCCAC
IL-6	RATIL6	TTCTTGGGACTGATGTTGTTG	TACTGGTCTGTTGTGGGTGGT
IL-7	AF367210	GATTGCCCAAATAATGAACC	TGTGCCGTCTGAAACTCTTA
IL-10	RATIL10X	GCACTGCTATGTTGCCTGCTCT	CCCAAGTAACCCTTAAAGTCCTG
*IL-12*α	NM_053390	CTGAAGACCACGGACGACAT	AGATGCTACCAAGGCACAGG
IL-17	NM_001106897	ACCTCAACCGTTCCACTTCA	CACTTCTCAGGCTCCCTCTTC
IL-18	AY258448	GCCATGTCAGAAGAAGGCTCT	GCTCCGTATTACTGCGGTTGT
IL-23	AY055379	TTCTCCGTTCCAAGATCCTTC	CTCAGTCAGAGTTGCTGCTCC
IL-27	XM_039101198	CTGTTGCTGCTACCTTTGCTT	GTGGACATAGCCCTGAACCTC
TGF-β1	BC076380	AACAATTCCTGGCGTTACCTT	GCCCTGTATTCCGTCTCCTT
CCL-1	NM_001191092	TGCTGCTTGAACACCTTGGA	TAGCAGGGCTTCACCTTCTT
CCL-2	XM_039101198	CCCCAATGTTTCCCTGACCT	TGAATCCTACAGCCAGCACC
CCL-5	NM_031116	GACACCACTCCCTGCTGCTT	GTTGATGTATTCTTGAACCCACTT
CCL-11	NM_019205	GCCACTTCCTTCACCTCCCA	TCAGCGTGCATCTGTTGTTG
CCL-17	NM_057151	TGTCACTTCAGATGCTGCTCC	TTCCCTGGACAGTCTCAAACA
CCL-21	NM_001008513	CTACAACATTGTCCGAGGCTAC	CCTTCCTTTCTTCCCAGACTTA
CXCL1	NM_030845	CAGTGGCAGGGATTCACTTCA	GGGACACCCTTTAGCATCTTTT
GM-CSF	FGDFAD	TATACAAGCAGGGTCTACGGG	ATGAAATCCTCAAAGGTGGTG
*IFN-*β	NM_019127	TGCCATTCAAGTGATGCTCC	CACCCAAGTCAATCTTTCCTCT
β-*actin*	NM_031144	CGTAAAGACCTCTATGCCAACA	CGGACTCATCGTACTCCTGCT
GAPDH	NM_017008	CAAGTTCAACGGCACAGTCAA	GATCTCGCTCCTGGAAGATGG

RS-treated group, and all the Art-treated groups showed no significant influence on body weight and food intake of rats (Figures 1B,C). At the end of *in vivo* experiments, the heart and entire aorta were harvested, and the general observations showed that only the AS group demonstrated abnormal aortic morphology (Figure 1D).

Subsequently, Oil red O staining was applied to observe the atherosclerotic lesions of the aorta. Compared to the control group, the AS group possessed large and deep red lesions (75.2 ± 8.9%); however, those were markedly attenuated by Art or RS (Figures 1E,F). Among four groups of Art treatment, low-dose Art (1.5 mg/kg) showed lower positive staining (%) of 30.8 ± 10.6 (bid) and 65.5 ± 6.37% (qd), whereas high-dose Art (5.0 mg/kg) showed further lower positive staining (%) of 16.0 ± 5.8 (bid) and 26.2 ± 6.1 (qd). Critically, twice-daily Art groups, in spite of high or low dose, showed a significant difference compared to the AS group ($p < 0.01$). Moreover, the high-dose Art with twice-daily (H-bid) group showed lower positive staining (%) than the RS group ($p < 0.05$). These results demonstrate that Art attenuates the progression of AS in the rat model in a dose-dependent manner and can better protect the aorta AS than RS.

Hyperlipidemia is widely recognized as the most important risk factor for AS development (Libby et al., 2019). Therefore, the blood samples harvested at the end of *in vivo* experiments were used to detect serum lipids. The levels of serum Tch, TG, HDL-C, and LDL-C markedly increased in the AS group compared with those in the control group (Figure 1G). However, Art or RS treatment decreased all of these variables including Tch, TG, and LDL-C. In addition, the lipid-reducing capacity of Art showed no significant difference compared with that of RS. Therefore, our data suggest that Art possesses a strong antihyperlipidemic effect. Moreover, arterial paraffin sections were used to observe whether Art affected lipid deposition within plaques using Oil red O staining. Similar to the results of serum lipids, lipid deposition remarkably increased in the AS group but decreased by the use of RS or Art (Figures 1H,I). Interestingly, lipid deposition in the H-bid group was much lower than that in the RS group, which was very similar to the data from aortas (Figure 1F). Taken together, these findings indicate that Art shows similar inhibition on hyperlipidemia compared to RS and better inhibition on lipid deposition within plaques in the AS rat model.

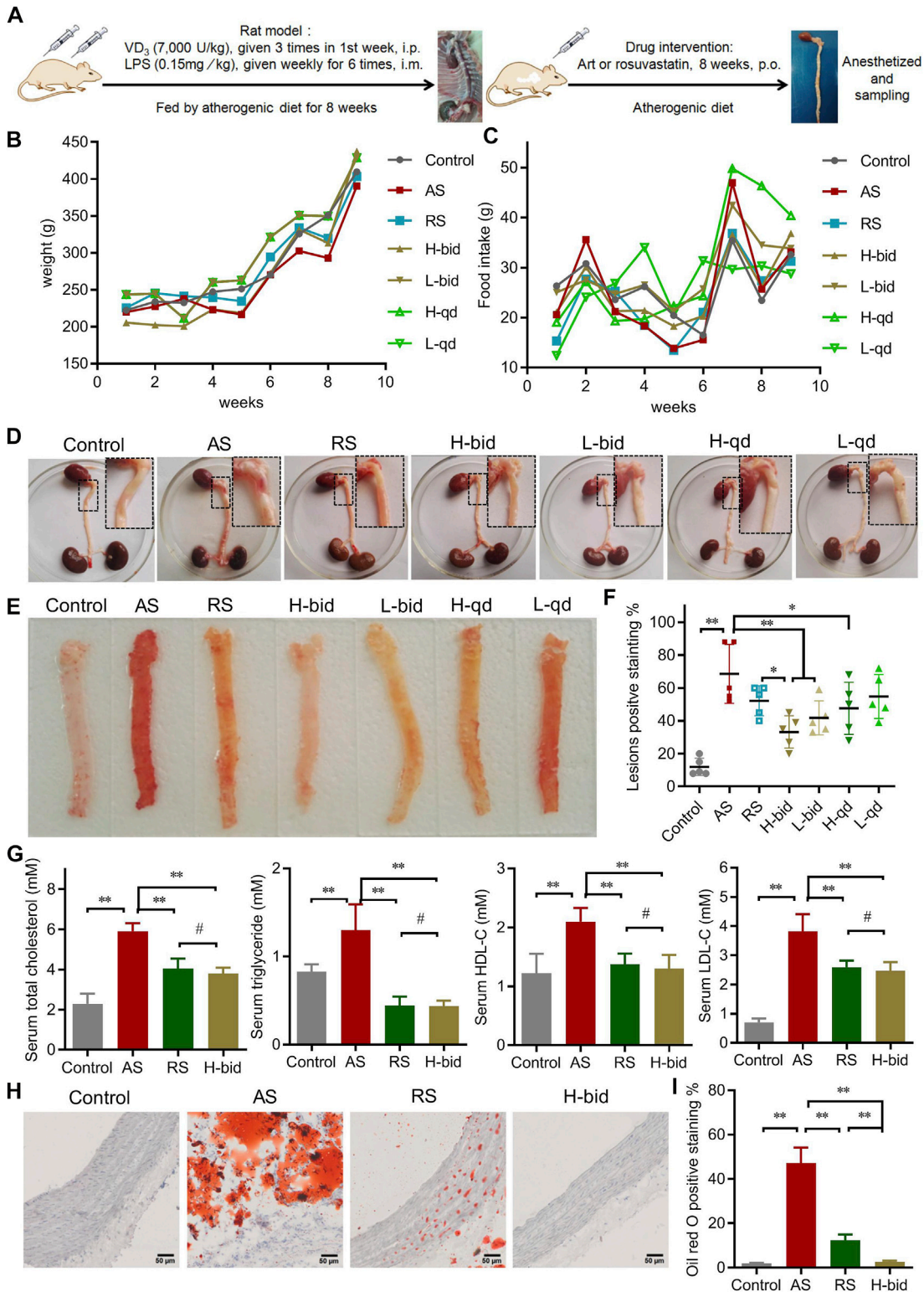


FIGURE 1
Art ameliorates AS in atherogenic diet–LPS-induced rats. **(A)** Procedure of the animal experiments. **(B)** Body weight of AS rats (n = 10). **(C)** Feed intake of AS rats (n = 10). **(D)** General observation of the heart and entire aorta. **(E–F)** Oil red O staining of aortas (n = 5). **(G)** Serum level of lipids (n = 5). **(H–I)** Oil red O staining of arterial sections. Paraffin sections were stained using Oil red O and then observed under a light microscope (Bar = 50 μm). The bar graph shows lesion-positive staining % (n = 5). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{\#}p > 0.05$.

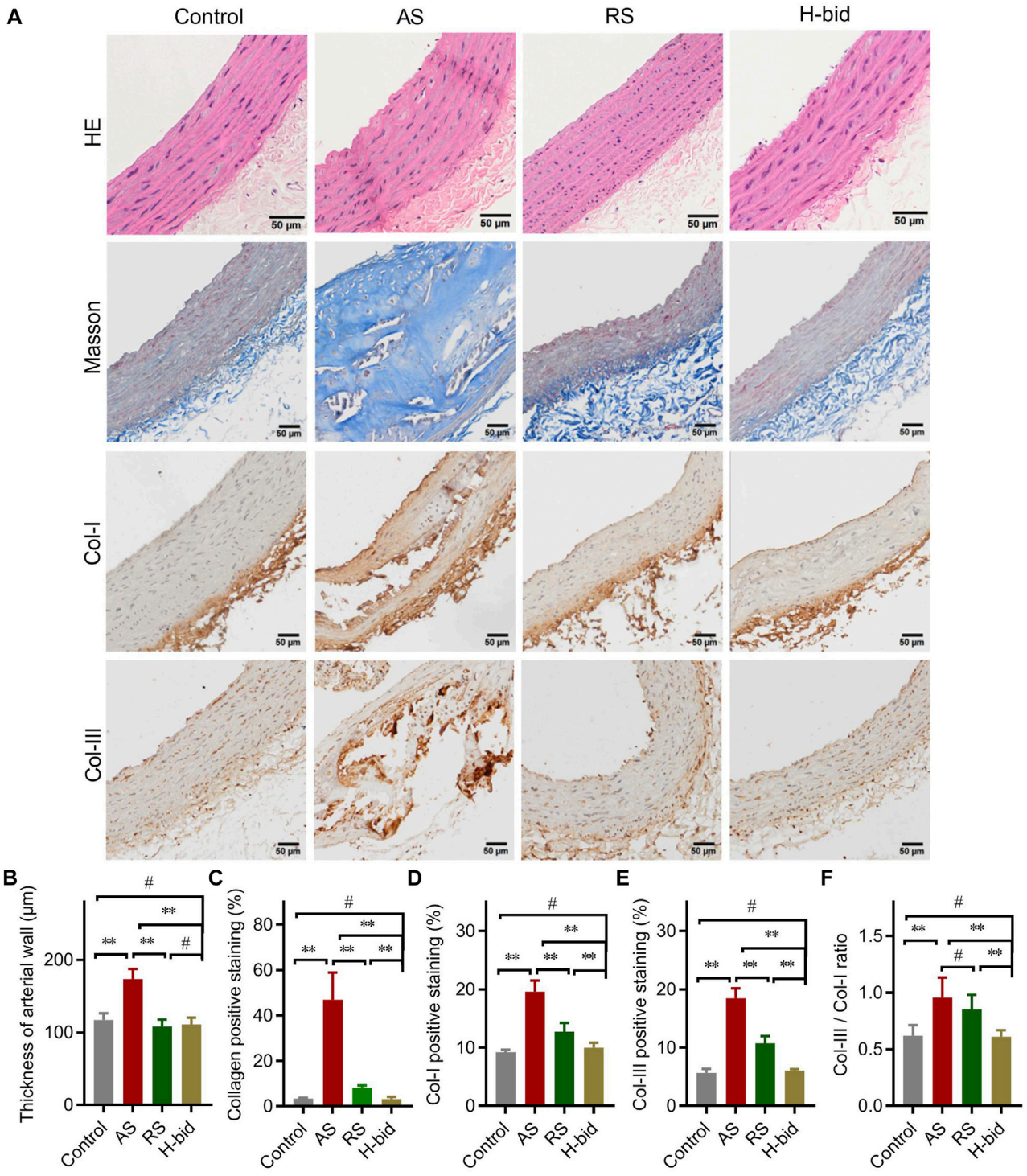


FIGURE 2
Art attenuates arterial remodeling in AS rats. **(A)** HE staining, Masson's staining, and IHC staining of arterial sections. Paraffin sections were stained using HE, Masson's trichrome, or were probed by antibodies against Col-I and Col-III using the IHC method and observed under a light microscope (Bar = 50 μm). **(B)** Thickness of the arterial wall. **(C)** Collagen-positive staining (%). **(D)** Col-I-positive staining (%). **(E)** Col-III-positive staining (%). **(F)** Col-III/Col-I ratio. $n = 5$; $^{**}p < 0.01$; $^{\#}p > 0.05$.

Art attenuates arterial remodeling in AS rats

Arterial remodeling, characterized predominantly by arterial wall thickening and vascular collagen deposition, is also a crucial alteration in AS (Evans et al., 2022). HE staining was performed to detect morphological changes in arteries. Compared to the control, arterial wall thickness of the AS group significantly increased, while that with RS or Art treatment was markedly declined (Figures 2A,B). The results indicate that Art shows inhibition on the arterial wall thickening similar to RS.

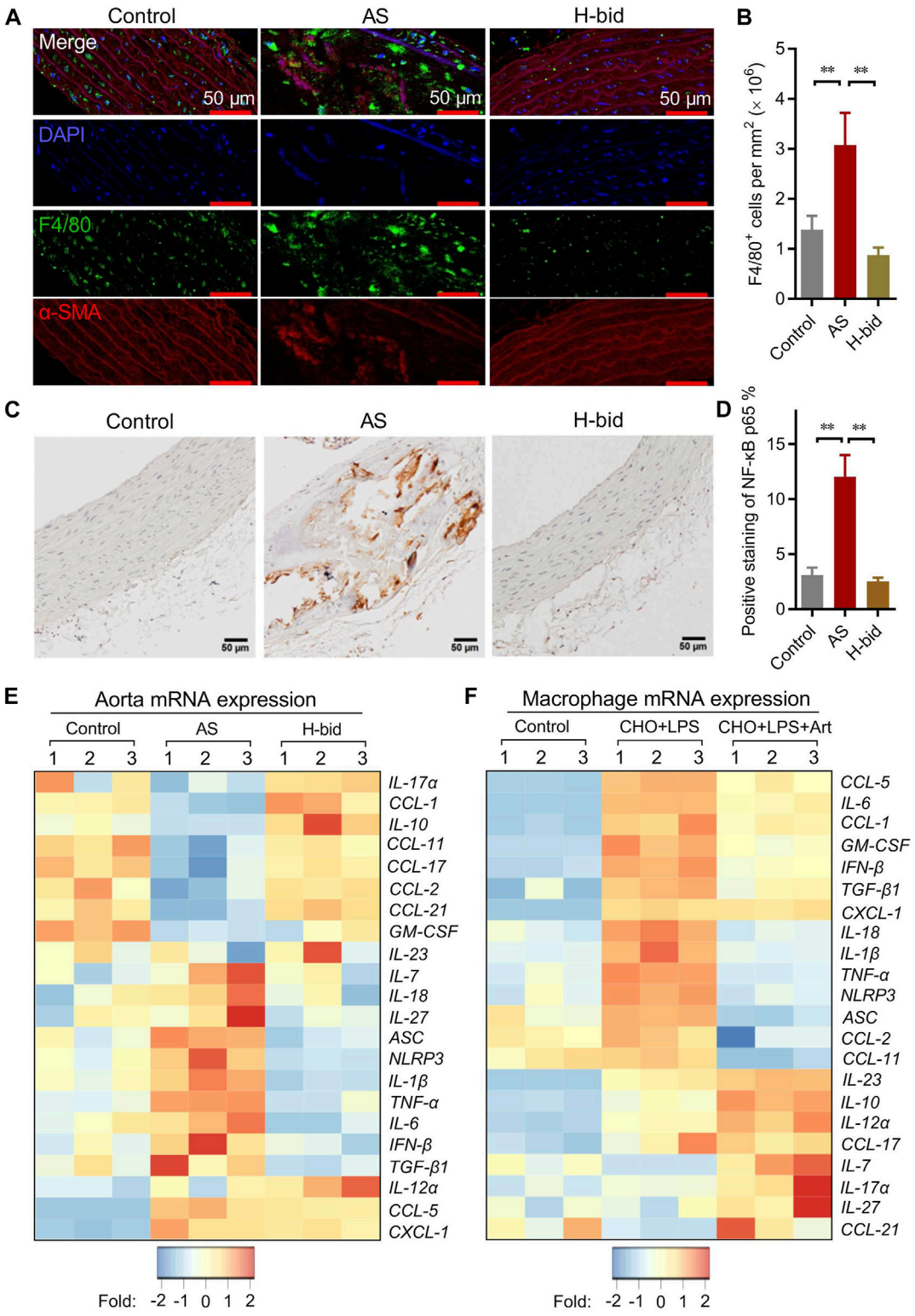


FIGURE 3
Art attenuates arterial inflammation in AS rats. **(A–B)** Immunofluorescence staining of arterial sections. Paraffin sections were probed with antibodies against F4/80 (green) or α-SMA (red), followed by DAPI (blue) staining. Confocal imaging was performed using a confocal microscope, and the $F4/80^+$ cells (macrophages) were measured using ImageJ (Bar = 50 μm; $n = 5$). **(C–D)** IHC staining of arterial sections. Paraffin sections were probed by antibodies against the NF-κB p65 subunit using the IHC method and observed under a light microscope, and the percentage of NF-κB p65-positive staining was calculated using ImageJ (Bar = 50 μm; $n = 5$). **(E)** mRNA levels of a class of inflammatory cytokines and chemokines of aortas from atherosclerotic rats detected by PCR array ($n = 3$). **(F)** PCR array analysis of RPMs treated by water-soluble cholesterol (CHO; 100 μg/mL) plus LPS (10 ng/mL) with or without the presence of artesunate (Art; 20 μg/mL) for 1 h ($n = 3$). $^{**}p < 0.01$.

Collagen is a major component of the vascular intima and wall; hence, collagen deposition is considered to be along with the volume growth of these intimal lesions (Libby et al., 2019). Masson's trichrome staining was applied to detect morphological changes in collagen deposition (Figure 2A). The AS group manifested massive collagen accumulation within the vascular smooth muscle layer, whereas treatment by RS or Art could alleviate collagen deposition induced by AS (Figures 2A,C). Consistently, IHC staining also indicated that the expression of Col-I and Col-III, the main members of interstitial collagen, was remarkably increased in the AS group but lessened significantly by RS or Art treatment (Figures 2A,D,E). Moreover, the Col-III/Col-I ratio, which indicates arterial stiffness and plaque stability, was also calculated herein. The Col-III/Col-I ratio was significantly increased in the AS group, whereas it was decreased by the treatment of only Art, not RS (Figure 2F). Critically, Art could restore collagen deposition and Col-III/Col-I ratio because there was no difference between the control group and the H-bid group ($p > 0.05$). In addition, Art showed better activity on decreasing AS-induced collagen deposition than the RS group ($p < 0.01$, as shown in Figures 2C–F). Taken together, the aforementioned findings suggest that Art can inhibit AS-induced arterial remodeling.

Art attenuates arterial inflammation in AS rats

Arterial macrophages, differentiated from circulating monocytes, are the main contributors responsible for lipid clearance, inflammatory responses, and arterial remodeling of AS (Libby et al., 2019). IF staining was applied to observe the influence of Art treatment on the recruitment of macrophages ($F4/80^+$; green) by the arterial wall (α-SMA^+; red) in AS rats. Our results showed that the macrophage number within plaques increased in the AS group while remarkably decreased by Art (Figures 3A,B). These findings indicate that Art inhibits, rather than increases, the recruitment of macrophages by arterial plaques in AS rats, which provides further evidence that Art inhibits arterial inflammation. Therefore, we detected whether Art affected the level of the NF-κB p65 subunit, which strictly regulated the inflammatory responses (Li et al., 2017), in aortas of AS rats using IHC staining. Not surprisingly, p65 was markedly upregulated in the AS group, especially within the plaques, but downregulated by Art (Figures 3C,D), indicating that Art shows an anti-inflammatory effect on the AS artery which is consistent with its inhibition of arterial macrophage recruitment.

Macrophage-derived pro-inflammatory cytokines and pro-inflammatory chemokines, operated by NF-κB, are responsible for arterial inflammation and plaque progression (Li et al., 2017). Therefore, the arterial mRNA levels of a class of cytokines and chemokines were detected *via* PCR array. As shown in the heatmap (Figure 3E), numerous genes were downregulated or were upregulated in the AS group, while the genes were restored in the H-bid group. Notably, we found several NF-κB-primed pro-inflammatory cytokines, including *IL-1β*, *IL-6*, *IL-18*, and *TNF-α* as well as *NLRP3* and *ASC*, which encoded the NLRP3 inflammasome-related proteins, were upregulated in the AS group, while those were downregulated by Art. To further confirm the findings from AS rats, we also performed similar PCR array experiments using RPMs. Similar to *in vivo* findings, we found that mRNA levels of *IL-1β*, *IL-6*, *IL-18*, *TNF-α*, *NLRP3*, and *ASC* were significantly upregulated by LPS plus CHO, whereas those were declined by Art (Figure 3F). Taken together, our data suggest that the protective effect of Art on AS rats is related to the decrease in inflammatory responses.

Art inhibits arterial NLRP3 inflammasome activation in AS rats

The NLRP3 inflammasome serves as a platform to activate caspase-1, thereby activating IL-1β and IL-18, which ultimately results in extensive collagen production and arterial remodeling (Grebe et al., 2018). Similar to mRNA expression of *IL-1β* and *IL-18*, that of *NLRP3* and *ASC* is also operated by NF-κB (Hoseini et al., 2018). Therefore, we further determined whether Art regulated mRNA expression of *NLRP3* and *ASC* in AS rats. It was shown that *NLRP3* and *ASC* mRNA expression was upregulated in the AS group but were downregulated by Art (Figure 4A). Moreover, the serum level of caspase-1 p10, which is an indicator of the activation of the NLRP3 inflammasome, was also determined. The results indicated that the serum level of caspase-1 p10 significantly increased in the AS group while decreased by Art (Figure 4B). Consistently, we found that the serum IL-1β level also remarkably increased in the AS group, whereas it decreased in the H-bid group (Figure 4B). Combining these findings, we can conclude that Art inhibits arterial NLRP3 inflammasome activation in AS rats.

However, whether Art inhibited NLRP3 inflammasome activation of macrophages or arterial walls was still unclear. Here, we observed NLRP3 expression with the indication of macrophages by F4/80 using immunofluorescence staining and found that NLRP3 was significantly upregulated in the arterial wall of the AS group, whereas it was downregulated by Art (Figures 4C,D). Critically, NLRP3 was significantly upregulated in arterial macrophages of the AS group, but it was downregulated by Art (Figures 4C,E). Collectively, aforementioned data suggest that Art inhibits NLRP3 inflammasome activation of both arterial wall and arterial macrophages in AS rats.

Art inhibits the arterial NLRP3 inflammasome–TGF-β1 pathway in AS rats

We further determined the main members and effectors of the NLRP3 inflammasome pathway in the arterial plaques of AS rats by IHC staining again. The results showed that the protein levels of two closely related inflammasome members, namely, NLRP3 and ASC, as well as two cytokines indicating inflammasome activation, namely, IL-1β and IL-18, were remarkably increased in the AS group, especially within arterial plaques; however, those were significantly decreased by Art (Figures 5A,B). Moreover, the expression of TGF-β1, a downstream AS effector of the NLRP3 inflammasome related to collagen production, was also upregulated in the AS group while downregulated by Art (Figures 5A,B). Thus, the protective effect of Art on AS rats is also related to its inhibition of the arterial NLRP3 inflammasome–TGF-β1 pathway.

Discussion

In this study, we reported that Art showed comparable protective activity on AS rats induced by the atherogenic diet plus inflammatory stimulation compared to RS and further showed a better inhibition on arterial lipid deposition and arterial remodeling than RS. The protective effect of Art was to inhibit arterial macrophage recruitment and NF-κB-

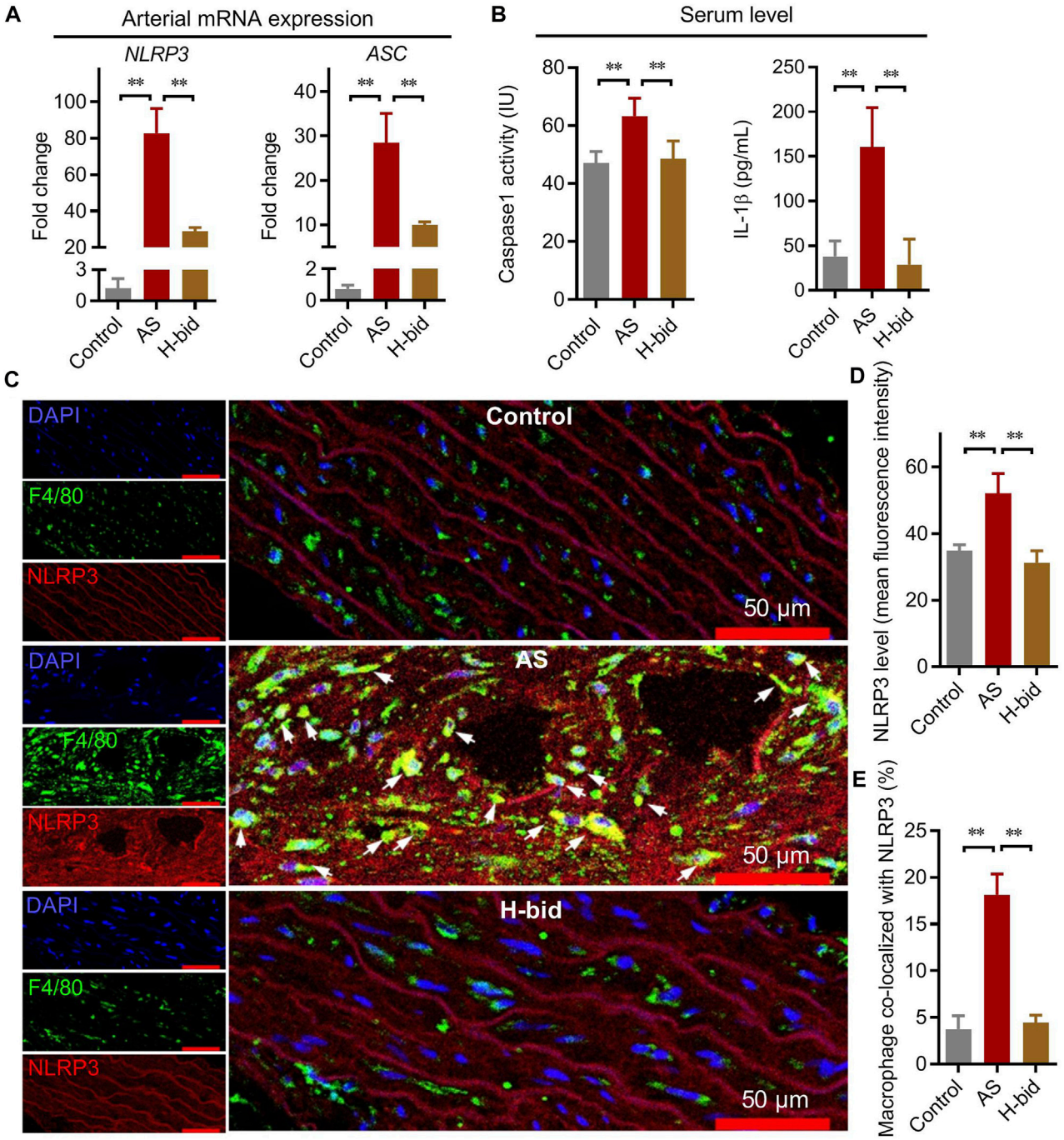


FIGURE 4
Art inhibits arterial NLRP3 inflammasome activation in AS rats. **(A)** mRNA expression of *NLRP3* and *ASC* in aortas from AS rats detected by real-time PCR (n = 4). **(B)** Serum levels of caspase-1 and IL-1β in AS rats detected by ELISA (n = 5). **(C)** Immunofluorescence staining of arterial sections. Experiments were performed as described in Figure 3A, using antibodies against F4/80 (green) or NLRP3 (red), followed by DAPI (blue) staining. The white arrows indicate the macrophages co-located with NLRP3. **(D–E)** Confocal images were analyzed by ImageJ; the arterial NLRP3 expression and colocalization of macrophages with NLRP3 were calculated, respectively (n = 5). *p < 0.05; **p < 0.01.

related excessive arterial inflammation, especially the NLRP3 inflammasome activation that leads to less production of caspase-1, IL-1β, IL-18, and subsequent TGF-β1. In conclusion, Art protects AS rats by modulating the arterial inflammatory responses *via* inhibition of the NF-κB–NLRP3 inflammasome pathway.

Since R. Ross raised the theory that "AS is an inflammatory disease" in 1999, a consensus of the pathogenesis of AS has been reached that inflammation plays a critical role and participates in the effects of many other risk factors for the development and complications of AS (Ross, 1999; Libby et al., 2011). It is well studied that some pathogens like *Chlamydia pneumoniae*, *Helicobacter pylori*, and cytomegalovirus are associated with AS, and the acute rise of C-reactive protein (CRP), which indicates infection, shows a strong positive correlation with AS

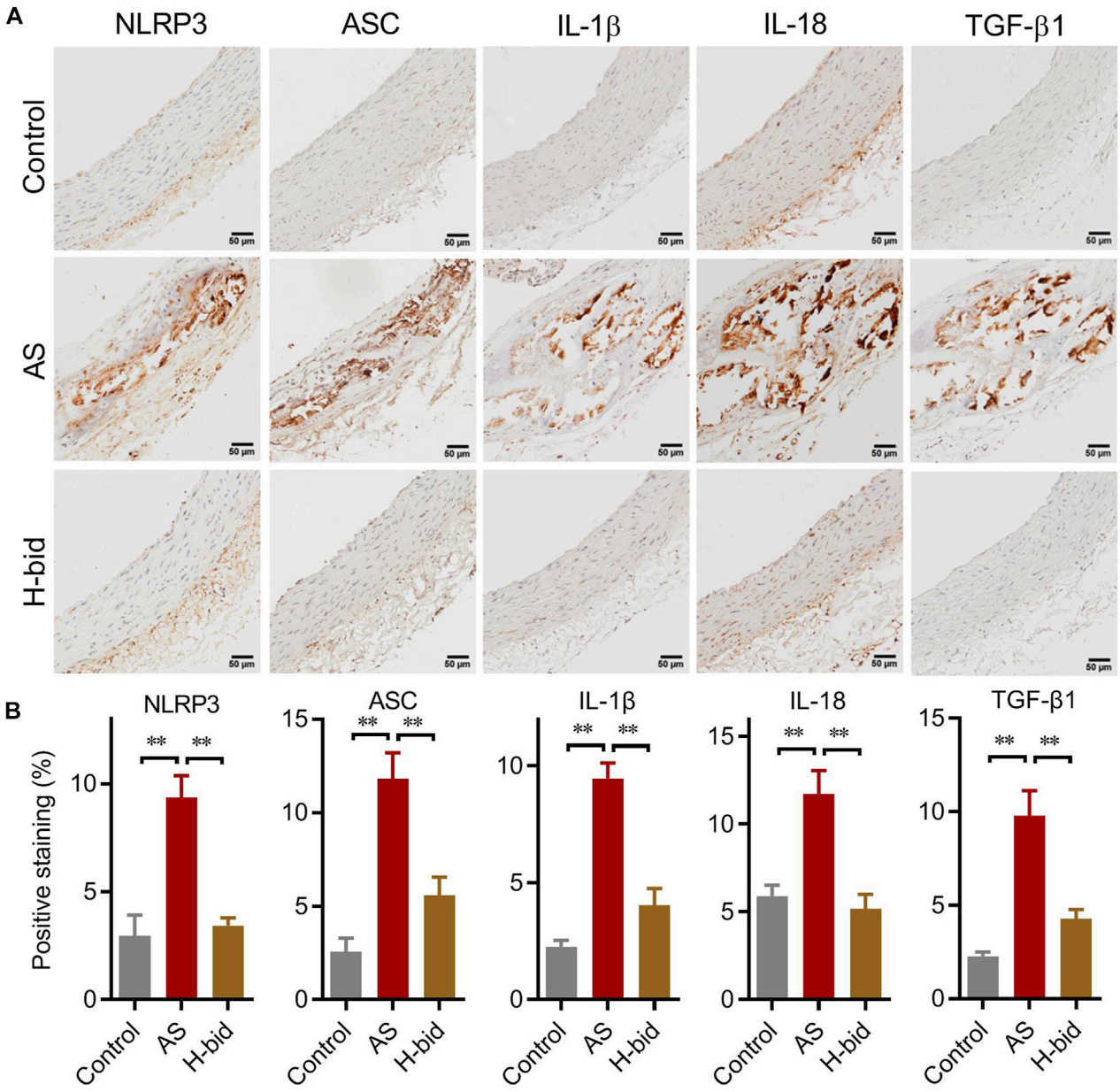


FIGURE 5
Art inhibits the arterial NLRP3 inflammasome–TGF-β1 pathway in AS rats. **(A)** IHC staining of arterial sections. IHC experiments were performed by using antibodies against NLRP3, ASC, IL-1β, IL-18, and TGF-β1 (Bar = 50 μm). **(B)** Percentages of positive staining (n = 5). $^{**}p < 0.01$.

(Stone and Kazil, 2014). Except these infectious factors, common CVDs, such as hypertension, hyperlipidemia, and diabetes, can stimulate the production of various inflammatory factors and thus affect the development of AS (Libby et al., 2011). Nowadays, the principles of immune response in experimental murine AS have been well addressed, and the efficacy of anti-inflammatory therapy in human AS has been confirmed in the clinical trials (Wolf and Ley, 2019). Collectively, inflammation has been validated as a critical mechanism in the pathogenesis of AS, and anti-inflammatory therapy has shown excellent clinical application value.

However, inflammation is indispensable in the pathological process of AS, responsible for waste clearance, vascular metabolic homeostasis, immune response, and repair of impaired vessels (Rosenfeld, 2013; Taleb, 2016). Therefore, researchers have to confront a contradictory challenge of finding a drug with a moderate anti-inflammatory effect while preserving the immune function of immune cells. During the complicated immunity and inflammation of AS, macrophage plays a central role by contributing to the maintenance of local inflammatory response, propagation of plaque development, recruitment of immune cells, and promotion of thrombosis (Barrett, 2020). In detail, macrophages are vital for the clearance of lipids and apoptotic cells; cytokines and chemokines released from macrophages are associated with subsequent recruitment of immune cells, maintenance of the vascular microenvironment, and matrix degradation which may lead to plaque instability (Taleb, 2016). Taking these into consideration,

antimalarial Art, which possesses a moderate anti-inflammatory effect and immunomodulatory effect reported by our laboratory previously (Jiang et al., 2016; Kuang et al., 2018), comes into our sight.

Previously, we have showed that Art conferred a protective effect on the *ApoE*$^{-/-}$ mice AS model by declining release of IL-8 and CCL2 (Jiang et al., 2016). Reports from other groups have reported similar protective activity of Art in the *ApoE*$^{-/-}$ mice model in recent years (He et al., 2020; Wang et al., 2022). However, a typical *ApoE*$^{-/-}$ model confers a significant increase in the TC level and spontaneous AS lesions, due to the increased serum level of very low-density lipoprotein (VLDL) caused by ApoE-deficiency (Veseli et al., 2017). To our knowledge, ApoE function is normal in most human patients with AS. Meanwhile, the ApoE-deficiency model cannot fully account for other pathogenic factors, especially inflammation that lies in the center of AS. Therefore, we employed an AS model using normal rats fed with the atherogenic diet plus LPS, which comprised both the high-fat pathogenic factor and pro-inflammatory pathogenic factor. In this study, Art was administered at an antimalarial dose and showed a good protective effect on AS rats because the occurrence of AS lesions and the serum lipid level were almost completely restored by Art (as shown in Figure 1). Dramatically, Art not only showed comparable activity against AS similar to a potent statin drug, RS, but also showed a stronger inhibition on arterial lipid deposition and vascular remodeling than RS. Moreover, our previous report also indicated that Art showed a synergistic effect with RS against the *ApoE*$^{-/-}$ mice model (Jiang et al., 2016). These findings suggest that Art monotherapy and Art–RS combination therapy have a certain potential for future clinical application.

Considering that Art was more effective than RS in inhibiting lipid deposition and vascular remodeling and had a synergistic effect with RS, we proposed that Art might function through a different mechanism compared to RS. In combination with our previous reports, indicating that Art was an inflammatory modulator in infectious diseases, and the consensus that inflammation played a central role in AS, we began to explore the mechanism from the perspective of inflammation. Art showed a moderate inhibition of arterial recruitment of macrophages. As the dominant producer of cytokines and chemokines, excessively infiltrated macrophages within arteries would probably lead to hyperinflammatory and hyperimmune states, which might result in an increased plaque number in the initiation stage and a higher risk of plaque rupture in the pathological process of AS (Colin et al., 2014). Therefore, this feature of Art might be the basis for its anti-AS activity. In contrast, it is not surprising that hydroxychloroquine, which possesses a strong anti-inflammatory activity, has failed in the treatment of AS, unless AS is accelerated in systemic lupus erythematosus and chronic kidney disease, because patients with autoimmune disorders are in the hyperinflammatory stage which requires treatment with a potent anti-inflammatory agent (Shukla et al., 2015; Floris et al., 2018).

NF-κB is considered the most critical transcription factor, and it controls the transcription of cytokines, chemokines, matrix metalloproteinases, and adhesion factors, which contribute to the pathogenesis of AS (Evans et al., 2022; Li et al., 2022). NF-κB-targeted therapy using inhibitory NF-κB decoy oligodeoxynucleotide in a LPS/high-fat diet-induced AS mice model, which was very similar to our model, has exhibited protective activity on AS mice (Kim et al., 2010). In this study, we showed very consistent findings in a similar rat model using Art, confirming that NF-κB-targeted therapy is a promising strategy for AS treatment. Moreover, NF-κB-targeted therapy reduced pro-inflammatory cytokines, TNF-α and IL-1β, and inflammatory markers, vascular adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1, in AS mice (Lee et al., 2013). In more detail, we showed the alterations of the inflammatory gene profile regulated by NF-κB in AS arteries and further exhibited changes of those in macrophages treated with CHO plus LPS. Art treatment attenuated AS-induced inflammation by declining production of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-18.

Particularly, we found that mRNA expression of *IL-1β*, *IL-18*, and *TGF-β1* was downregulated by Art compared to the AS group *in vivo* and *in vitro*. Although the transcription process of these genes was operated by NF-κB, their protein level was tightly regulated by the activation of NLRP3 inflammasome (Baldrighi et al., 2017; Grebe et al., 2018). In brief, NLRP3 and ASC, the two core members of the NLRP3 inflammasome which were also primed by the NF-κB signaling, form an intact inflammasome with pro-caspase-1, and then mature caspase-1 cleaves pro-IL-1β and pro-IL-18 into IL-1β and IL-18, which in turn initiate TGF-β1 release and Smad activation that culminate in the production of collagen (Mangan et al., 2018). As suggested in recent studies, NLRP3 inflammasome is the main PRR for sensing cholesterol crystals in AS lesions and is essential for vascular inflammation and the progression of AS (Karasawa and Takahashi, 2017). Therefore, the NLRP3 inflammasome is widely recognized as the relevant target for AS treatment, and several NLRP3-targeted studies have confirmed the validity of this viewpoint (Kong et al., 2016; Parsamanesh et al., 2019; Zhang et al., 2019). Here, Art treatment indeed inhibits activation of the NLRP3 inflammasome, thereby reducing TGF-β1 production and decreasing arterial collagen deposition, ultimately leading to better protective activity in AS rats than RS.

Conclusively, Art possesses a good protective activity in AS rats and functions through inhibiting the NF-κB–NLRP3 inflammasome pathway. However, this study still has certain limitations. Although the protective effect of Art is well addressed, the relationship between the activity and the NF-κB–NLRP3 inflammasome pathway needs to be further investigated in NF-κB/NLRP3-deficiency animal models. Moreover, the influence of Art on arterial remodeling in AS rats should be observed more elaborately, such as the relationship between the time point of intervention and arterial remodeling. However, in consideration of the safety records of Art in treating malaria in the past 2 decade and its effectiveness in AS rats herein and *ApoE*$^{-/-}$ mice reported previously, we propose Art to be a promising agent for AS treatment.

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

The animal study was reviewed and approved by the Ethics Committee for Animal Experimentation of Army Medical University.

Author contributions

YC and XP conceived the experiment; YX, HT, and RQ collected the data; QY analyzed the data; YC and XP wrote and reviewed the manuscript.

Funding

This study was supported by the Natural Science Foundation of Chongqing, China (No. cstc2020jcyj-msxmX0055), the National Natural Science Foundation of China (No. 81872911), and the Excellent Talents Pool Project of Army Medical University.

Acknowledgments

The authors thank Fangfang Zheng of the Department of Foreign Language of our university for her kind support in English writing.

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

Afrasyab, A., Qu, P., Zhao, Y., Peng, K., Wang, H., Lou, D., et al. (2016). Correlation of NLRP3 with severity and prognosis of coronary atherosclerosis in acute coronary syndrome patients. *Heart. Vessels* 31, 1218–1229. doi:10.1007/s00380-015-0723-8

Baldrighi, M., Mallat, Z., and Li, X. (2017). NLRP3 inflammasome pathways in atherosclerosis. *Atherosclerosis* 267, 127–138. doi:10.1016/j.atherosclerosis.2017.10.027

Barrett, T. J. (2020). Macrophages in atherosclerosis regression. *Arterioscler. Thromb. Vasc. Biol.* 40, 20–33. doi:10.1161/ATVBAHA.119.312802

Chen, D., Zuo, K., Liang, X., Wang, M., Zhang, H. H., Zhou, R., et al. (2020). Functional mechanism of AMPK activation in mitochondrial regeneration of rat peritoneal macrophages mediated by uremic serum. *PloS One* 15, e0235960. doi:10.1371/journal.pone.0235960

Chistiakov, D. A., Bobryshev, Y. V., and Orekhov, A. N. (2016). Macrophage-mediated cholesterol handling in atherosclerosis. *J. Cell Mol. Med.* 20, 17–28. doi:10.1111/jcmm.12689

Chistiakov, D. A., Melnichenko, A. A., Orekhov, A. N., and Bobryshev, Y. V. (2017). How do macrophages sense modified low-density lipoproteins? *Int. J. Cardiol.* 230, 232–240. doi:10.1016/j.ijcard.2016.12.164

Colin, S., Chinetti-Gbaguidi, G., and Staels, B. (2014). Macrophage phenotypes in atherosclerosis. *Immunol. Rev.* 262, 153–166. doi:10.1111/imr.12218

Evans, B. R., Yerly, A., van der Vorst, E. P. C., Baumgartner, I., Bernhard, S. M., Schindewolf, M., et al. (2022). Inflammatory mediators in atherosclerotic vascular remodeling. *Front. Cardiovasc. Med.* 9, 868934. doi:10.3389/Fcvm.2022.868934

Floris, A., Piga, M., Mangoni, A. A., Bortoluzzi, A., Erre, G. L., and Cauli, A. (2018). Protective effects of hydroxychloroquine against accelerated atherosclerosis in systemic lupus erythematosus. *Mediat. Inflamm.* 2018, 3424136. doi:10.1155/2018/3424136

Fu, W. J., Lei, T., Yin, Z., Pan, J. H., Chai, Y. S., Xu, X. Y., et al. (2017). Anti-atherosclerosis and cardio-protective effects of the Angong Niuhuang Pill on a high fat and vitamin D3 induced rodent model of atherosclerosis. *J. Ethnopharmacol.* 195, 118–126. doi:10.1016/j.jep.2016.11.015

Grebe, A., Hoss, F., and Latz, E. (2018). NLRP3 inflammasome and the IL-1 pathway in atherosclerosis. *Circ. Res.* 122, 1722–1740. doi:10.1161/CIRCRESAHA.118.311362

Grebe, A., and Latz, E. (2013). Cholesterol crystals and inflammation. *Curr. Rheumatol. Rep.* 15, 313. doi:10.1007/s11926-012-0313-z

He, L. H., Gao, J. H., Yu, X. H., Wen, F. J., Luo, J. J., Qin, Y. S., et al. (2020). Artesunate inhibits atherosclerosis by upregulating vascular smooth muscle cells-derived LPL expression via the KLF2/NRF2/TCF7L2 pathway. *Eur. J. Pharmacol.* 884, 173408. doi:10.1016/j.ejphar.2020.173408

Hoseini, Z., Sepahvand, F., Rashidi, B., Sahebkar, A., Masoudifar, A., and Mirzaei, H. (2018). NLRP3 inflammasome: Its regulation and involvement in atherosclerosis. *J. Cell. Physiol.* 233, 2116–2132. doi:10.1002/jcp.25930

Hu, D., Wang, Z., Wang, Y., and Liang, C. (2021). Targeting macrophages in atherosclerosis. *Curr. Pharm. Biotechnol.* 22, 2008–2018. doi:10.2174/1389201022666210122142233

Jiang, W. W., Cen, Y. Y., Song, Y., Li, P., Qin, R. X., Liu, C., et al. (2016). Artesunate attenuated progression of atherosclerosis lesion formation alone or combined with rosuvastatin through inhibition of pro-inflammatory cytokines and pro-inflammatory chemokines. *Phytomedicine* 23, 1259–1266. doi:10.1016/j.phymed.2016.06.004

Karasawa, T., and Takahashi, M. (2017). The crystal-induced activation of NLRP3 inflammasomes in atherosclerosis. *Inflamm. Regen.* 37, 18. doi:10.1186/s41232-017-0050-9

Kim, S. J., Park, J. H., Kim, K. H., Lee, W. R., Lee, S., Kwon, O. C., et al. (2010). Effect of NF-κB decoy oligodeoxynucleotide on LPS/high-fat diet-induced atherosclerosis in an animal model. *Basic. Clin. Pharmacol. Toxicol.* 107, 925–930. doi:10.1111/j.1742-7843.2010.00617.x

Kong, F., Ye, B., Cao, J., Cai, X., Lin, L., Huang, S., et al. (2016). Curcumin represses NLRP3 inflammasome activation via TLR4/MyD88/NF-κB and P2X7R signaling in PMA-induced macrophages. *Front. Pharmacol.* 7, 369. doi:10.3389/fphar.2016.00369

Kuang, M., Cen, Y. Y., Qin, R. X., Shang, S. L., Zhai, Z. X., Liu, C., et al. (2018). Artesunate attenuates pro-inflammatory cytokine release from macrophages by inhibiting TLR4-mediated autophagic activation via the TRAF6-beclin1-PI3KC3 pathway. *Cell. Physiol. biochem.* 47, 475–488. doi:10.1159/000489982

Lee, W. R., Kim, K. H., An, H. J., Park, Y. Y., Kim, K. S., Lee, C. K., et al. (2013). Effects of chimeric decoy oligodeoxynucleotide in the regulation of transcription factors NF-κB and Sp1 in an animal model of atherosclerosis. *Basic. Clin. Pharmacol. Toxicol.* 112, 236–243. doi:10.1111/bcpt.12029

Li, B., Li, W. H., Li, X. L., and Zhou, H. (2017). Inflammation: A novel therapeutic target/direction in atherosclerosis. *Curr. Pharm. Des.* 23, 1216–1227. doi:10.2174/1381612822666161230142931

Li, W., Jin, K., Luo, J., Xu, W., Wu, Y., Zhou, J., et al. (2022). NF-κB and its crosstalk with endoplasmic reticulum stress in atherosclerosis. *Front. Cardiovasc. Med.* 9, 988266. doi:10.3389/fcvm.2022.988266

Li, W. L., Hua, L. G., Qu, P., Yan, W. H., Ming, C., Jun, Y. D., et al. (2016). NLRP3 inflammasome: A novel link between lipoproteins and atherosclerosis. *Arch. Med. Sci.* 12, 950–958. doi:10.5114/aoms.2016.61356

Libby, P., Buring, J. E., Badimon, L., Hansson, C. K., Deanfield, J., Bittencourt, M. S., et al. (2019). Atherosclerosis. *Nat. Rev. Dis. Prim.* 5 (1), 56. doi:10.1038/s41572-019-0106-z

Libby, P., Ridker, P. M., and Hansson, G. K. (2011). Progress and challenges in translating the biology of atherosclerosis. *Nature* 473, 317–325. doi:10.1038/nature10146

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25, 402–408. doi:10.1006/meth.2001.1262

Mangan, M. S. J., Olhava, E. J., Roush, W. R., Seidel, H. M., Glick, G. D., and Latz, E. (2018). Targeting the NLRP3 inflammasome in inflammatory diseases. *Nat. Rev. Drug. Discov.* 17, 688–606. doi:10.1038/nrd.2018.149

Paramel Varghese, G., Folkersen, L., Strawbridge, R. J., Halvorsen, B., Yndestad, A., Ranheim, T., et al. (2016). NLRP3 inflammasome expression and activation in human atherosclerosis. *J. Am. Heart. Assoc.* 5, e003031. doi:10.1161/JAHA.115.003031

Parsamanesh, N., Moossavi, M., Bahrami, A., Fereidouni, M., Barreto, G., and Sahebkar, A. (2019). NLRP3 inflammasome as a treatment target in atherosclerosis: A focus on statin therapy. *Int. Immunopharmacol.* 73, 146–155. doi:10.1016/j.intimp.2019.05.006

Rosenfeld, M. E. (2013). Inflammation and atherosclerosis: Direct versus indirect mechanisms. *Curr. Opin. Pharmacol.* 13, 154–160. doi:10.1016/j.coph.2013.01.003

Ross, R. (1999). Atherosclerosis--an inflammatory disease. *N. Engl. J. Med.* 340, 115–126. doi:10.1056/NEJM199901143400207

Schaftenaar, F., Frodermann, V., Kuiper, J., and Lutgens, E. (2016). Atherosclerosis: The interplay between lipids and immune cells. *Curr. Opin. Lipidol.* 27, 209–215. doi:10.1097/Mol.0000000000000302

Schindelin, J., Rueden, C. T., Hiner, M. C., and Eliceiri, K. W. (2015). The ImageJ ecosystem: An open platform for biomedical image analysis. *Mol. Reprod. Dev.* 82, 518–529. doi:10.1002/mrd.22489

Sharma, B. R., and Kanneganti, T. D. (2021). NLRP3 inflammasome in cancer and metabolic diseases. *Nat. Immunol.* 22, 550–559. doi:10.1038/s41590-021-00886-5

Shukla, A. M., Bose, C., Karaduta, O. K., Apostolov, E. O., Kaushal, G. P., Fahmi, T., et al. (2015). Impact of hydroxychloroquine on atherosclerosis and vascular stiffness in the presence of chronic kidney disease. *PloS One* 10, e0139226. doi:10.1371/journal.pone.0139226

Stone, P. A., and Kazil, J. (2014). The relationships between serum C-reactive protein level and risk and progression of coronary and carotid atherosclerosis. *Semin. Vasc. Surg.* 27, 138–142. doi:10.1053/j.semvascsurg.2015.04.002

Takahashi, M. (2022). NLRP3 inflammasome as a key driver of vascular disease. *Cardiovasc. Res.* 118, 372–385. doi:10.1093/cvr/cvab010

Taleb, S. (2016). Inflammation in atherosclerosis. *Arch. Cardiovasc Dis.* 109, 708–715. doi:10.1016/j.acvd.2016.04.002

Veseli, B. E., Perrotta, P., De Meyer, G. R. A., Roth, L., Van der Donckt, C., Martinet, W., et al. (2017). Animal models of atherosclerosis. *Eur. J. Pharmacol.* 816, 3–13. doi:10.1016/j.ejphar.2017.05.010

Wang, X., Du, H., and Li, X. (2022). Artesunate attenuates atherosclerosis by inhibiting macrophage M1-like polarization and improving metabolism. *Int. Immunopharmacol.* 102, 108413. doi:10.1016/j.intimp.2021.108413

Wiesner, P., Choi, S. H., Almazan, F., Benner, C., Huang, W., Diehl, C. J., et al. (2010). Low doses of lipopolysaccharide and minimally oxidized low-density lipoprotein cooperatively activate macrophages via nuclear factor kappa B and activator protein-1: Possible mechanism for acceleration of atherosclerosis by subclinical endotoxemia. *Circ. Res.* 107, 56–65. doi:10.1161/CIRCRESAHA.110.218420

Wolf, D., and Ley, K. (2019). Immunity and inflammation in atherosclerosis. *Circ. Res.* 124, 315–327. doi:10.1161/CIRCRESAHA.118.313591

Xu, H., Jiang, J., Chen, W., Li, W., and Chen, Z. (2019). Vascular macrophages in atherosclerosis. *J. Immunol. Res.* 2019, 4354786. doi:10.1155/2019/4354786

Yin, K., Tang, S. L., Yu, X. H., Tu, G. H., He, R. F., Li, J. F., et al. (2013). Apolipoprotein A-I inhibits LPS-induced atherosclerosis in ApoE(-/-) mice possibly via activated STAT3-mediated upregulation of tristetraprolin. *Acta. Pharm. Sin.* 34, 837–846. doi:10.1038/aps.2013.10

Zhang, L., Lu, L., Zhong, X., Yue, Y., Hong, Y., Li, Y., et al. (2019). Metformin reduced NLRP3 inflammasome activity in Ox-LDL stimulated macrophages through adenosine monophosphate activated protein kinase and protein phosphatase 2A. *Eur. J. Pharmacol.* 852, 99–106. doi:10.1016/j.ejphar.2019.03.006

Zhuang, T., Liu, J., Chen, X., Zhang, L., Pi, J., Sun, H., et al. (2019). Endothelial Foxp1 suppresses atherosclerosis via modulation of Nlrp3 inflammasome activation. *Circ. Res.* 125, 590–605. doi:10.1161/CIRCRESAHA.118.314402

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 24 February 2023
DOI 10.3389/fphar.2023.1141180

OPEN ACCESS

EDITED BY
Xiaofei Zhang,
Shaanxi University of Chinese Medicine, China

REVIEWED BY
Chuan Wang,
Shaanxi University of Chinese Medicine, China
Ramoji Kosuru,
Versiti Blood Research Institute, United States
Chengjuan Gu,
Fourth Medical College of Guangzhou University of Traditional Chinese Medicine, China

*CORRESPONDENCE
Li-Sha He,
helisha@cdutcm.edu.cn
Xue-Ping Li,
lixueping@cdutcm.edu.cn

SPECIALTY SECTION
This article was submitted to Ethnopharmacology, a section of the journal Frontiers in Pharmacology

RECEIVED 10 January 2023
ACCEPTED 14 February 2023
PUBLISHED 24 February 2023

CITATION
Li R-L, Wang L-Y, Duan H-X, Qian D, Zhang Q, He L-S and Li X-P (2023), Natural flavonoids derived from herbal medicines are potential anti-atherogenic agents by inhibiting oxidative stress in endothelial cells.
Front. Pharmacol. 14:1141180.
doi: 10.3389/fphar.2023.1141180

Natural flavonoids derived from herbal medicines are potential anti-atherogenic agents by inhibiting oxidative stress in endothelial cells

Ruo-Lan Li[1], Ling-Yu Wang[1], Hu-Xinyue Duan[1], Die Qian[1], Qing Zhang[1], Li-Sha He[1]* and Xue-Ping Li[2]*

[1]Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China

As the common pathological basis of various cardiovascular diseases, the morbidity and mortality of atherosclerosis (AS) have increased in recent years. Unfortunately, there are still many problems in the treatment of AS, and the prevention and treatment of the disease is not ideal. Up to now, the occurrence and development of AS can roughly include endothelial cell dysfunction, vascular smooth muscle cell proliferation, inflammation, foam cell production, and neoangiogenesis. Among them, endothelial dysfunction, as an early event of AS, plays a particularly important role in promoting the development of AS. In addition, oxidative stress occurs throughout the causes of endothelial dysfunction. Some previous studies have shown that flavonoids derived from herbal medicines are typical secondary metabolites. Due to its structural presence of multiple active hydroxyl groups, it is able to exert antioxidant activity in diseases. Therefore, in this review, we will search PubMed, Web of Science, Elesvier, Wliey, Springer for relevant literature, focusing on flavonoids extracted from herbal medicines, and summarizing how they can prevent endothelial dysfunction by inhibiting oxidative stress. Meanwhile, in our study, we found that flavonoid represented by quercetin and naringenin showed superior protective effects both *in vivo* and *in vitro*, suggesting the potential of flavonoid compounds in the treatment of AS.

KEYWORDS

atherosclerosis, endothelial dysfunction, oxidative stress, herbal medicines, flavonoids

1 Introduction

Cardiovascular disease (CVD) ranks alongside cancer, diabetes, and chronic respiratory diseases as the four diseases with the highest morbidity and mortality worldwide (Zhong et al., 2019). More than 17 million people die from CVD every year, accounting for more than 31% of global deaths (Townsend et al., 2016; Benjamin et al., 2017). Shockingly, with the acceleration of population aging, the incidence and mortality of CVD are still increasing, and there are large problems in the existing treatment methods need to be solved (Yusuf et al., 2004). Among them, atherosclerosis (AS), as the common pathological basis of CVD, has also received extensive attention in the prevention and treatment of CVD. AS is a chronic, progressive multifocal arterial disease, which

mainly causes damage to large and medium-sized arteries. Unfortunately, although much effort has been invested in AS, the prevention and therapy of the disease are not particularly ideal (Falk, 2006; Crea and Libby, 2017). So far, the measures to alleviate AS have mainly been to reduce hyperlipidemia, slow the disease process, and mitigate the consequences of AS (Khan et al., 2021). Smoking, unhealthy diet, obesity, alcohol consumption and other factors may contribute to the development of AS. However, due to the complexity of AS, the AS pathogenesis is not well understood, which greatly reduced the therapeutic effect of AS (Steven et al., 2019). Based on the evidence from recent years, the occurrence and development of AS mainly involves a variety of cellular events such as endothelial cell dysfunction, vascular smooth muscle cell (VSMC) proliferation, inflammation, foam cell production, and neovascularization (Shemiakova et al., 2020). Pleasantly surprise, endothelial dysfunction appears to be reversible with therapeutic interventions aimed at correcting risk factors for endothelial dysfunction. At the same time, most of the initiation of AS development is located in the subendothelial space, and can be controlled by the endothelium and hormones. The treatment and improvement of endothelial dysfunction also play a particularly important role in AS. At present, there are many theories about the causes of endothelial cell dysfunction. Notably, inflammation, oxidative stress, autophagy and other events are inseparable from endothelial cell dysfunction, while oxidative stress is carried throughout (Li et al., 2022a).

For thousands of years, herbal medicines have been widely used in the prevention and treatment of diseases. With the development of medical information technology, flavonoids derived from herbal medicines have received more and more attention due to their significant efficacy and high safety (Li et al., 2022b). Flavonoids are mainly found in vacuoles of plants and are a secondary metabolite with abundant content. The main function of flavonoids is to protect plants against pathogens and UV radiation, and to participate in pollination by being recognized by pollinators (Pandey and Rizvi, 2009). Previous studies have shown that flavonoids have unique antioxidant activity due to their ability to provide hydrogen atoms or electrons, which can directly remove reactive oxygen species, thereby limiting the effects of oxidative stress (Li et al., 2022a). In addition, a large number of literature studies have shown that flavonoids derived from herbal medicines also have a significant effect on AS. Notably, flavonoids derived from herbal medicines also have been shown to regulate endothelial cell dysfunction during AS development (Yamagata, 2019). Therefore, based on the above explanation, we can propose that flavonoids derived from herbal medicines can inhibit oxidative stress, thereby inhibiting the occurrence of endothelial dysfunction.

2 Endothelial dysfunction contributes to the development of AS

Endothelial cells, as a unique type of epithelial cells, are distributed in a monolayer of blood vessels and constitute the vascular endothelium that maintains vascular homeostasis (Krüger-Genge et al., 2019). The vascular endothelium is a semipermeable barrier between plasma and vascular tissue that extends along the entire circulatory system. Due to its unique location, endothelial cells can not only undergo metabolic exchange with plasma and interstitial fluid, but also interact with cells in the blood vessel wall (Yamaoka-Tojo, 2017). In addition, changes in blood composition and blood flow also have a great influence on the function of endothelial cells, among which mechanical transduction due to shear stress is considered to be the most important factor (Mitra et al., 2017; Mensah et al., 2020). In a healthy state, shear stress can directly promote the activation of endothelial NO synthase (eNOS) in endothelial cells, and also can activate eNOS by inducing rapid influx of Ca^{2+} into cells. eNOS promotes nitric oxide (NO) production by converting L-arginine to L-citrulline and NO (Förstermann and Munzel, 2006; Xu et al., 2021). As all we known, NO is an important vasoactive substance (Figure 1). NO can diffuse into vascular smooth muscle cells (VSMC), promote vasodilation by stimulating soluble guanyl cyclase and increasing cyclic guanosine monophosphate (cGMP), and has an antiproliferative effect on VSMC (Jin and Loscalzo, 2010). In the circulatory system, NO can also inhibit the adhesion and aggregation of platelets and exert anti-inflammatory properties. In addition, molecules represented by hydrogen sulfide (H_2S), carbon monoxide, and arachidonic acid metabolites can also mediate vasodilation by inducing endothelium-derived hyperpolarization (Shimokawa, 2014). Under physiological conditions, in addition to vasodilation, endothelial cells can also mediate vasoconstriction by releasing a variety of vasoconstrictor molecules such as thromboxane A2 (TXA2), angiotensin (Ang) II and endothelin (ET) (Ley et al., 2007; Rao et al., 2007). Besides this, endothelial cells also can regulate platelet activity, coagulation cascade and fibrinolysis system. However, these functions of endothelial cells can be disrupted to varying degrees by diseases, including hyperlipidemia, diabetes, and heart failure (Tuñón et al., 2007; Tonelli et al., 2016; Giannitsi et al., 2019). Apparently, aging and genetic changes can also induce endothelial cell dysfunction.

Inflammation, oxidative stress and autophagy are considered as important cellular events that affect endothelial function. Previous studies have shown that lipids in endothelial cells can be transported to autophagic vesicles for lysosome-mediated degradation after ox-LDL stimulation. At the same time, ER stress is triggered in endothelial cells and further induces autophagy (Torisu et al., 2016). In addition, endothelial cells can also regulate autophagic flux through different transcription factors when shear stress is changed (Hua et al., 2022). Therefore, autophagy has also been proposed as an effective tool to alleviate endothelial dysfunction. Since inflammation is an important factor in inducing endothelial dysfunction, its role in AS cannot be ignored. When endothelial cells are activated, interleukin (IL) −8, chemokines, vascular adhesion molecule-1 (VCAM-1), growth factors and other inflammatory factors are secreted, attracting monocytes and neutrophils to adhere to endothelial cells and penetrate the arterial wall to cause inflammation (Chistiakov et al., 2018). There are many ways to induce endothelial inflammation. For example, lipopolysaccharide release from the blood promotes inflammation by increasing the expression of interferon-induced proteins and tetrapeptide repeats in endothelial cells (Wang et al., 2020). Insulin can increase Ang-II expression through the p38 MAPK-cFOS pathway and enhance inflammation in a paracrine manner (Chandel et al., 2020). In addition, excessive ROS can also induce endothelial dysfunction by enhancing inflammatory response (Zeng et al., 2020).

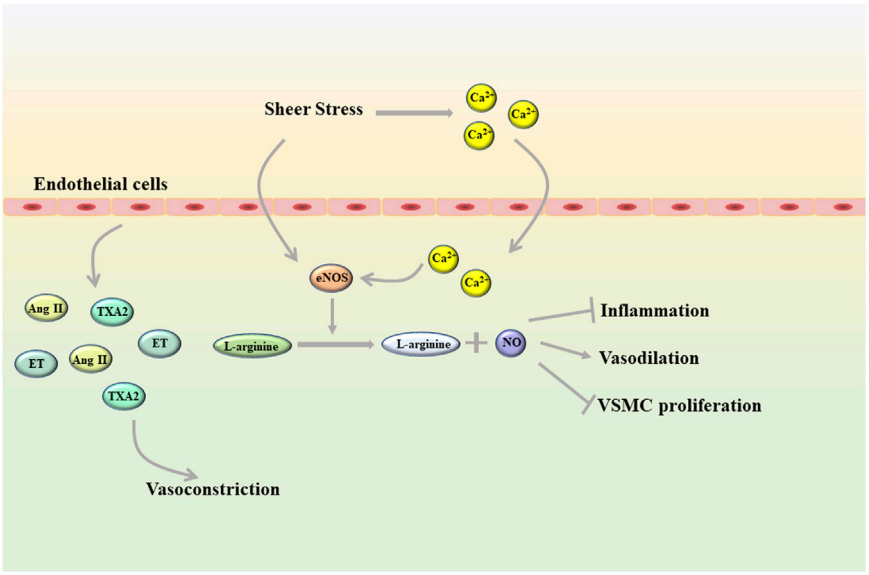


FIGURE 1
Shear stress helps endothelial cells maintain homeostasis in a healthy state (Ang II, angiotensin II; eNOS, endothelial NO synthase; ET, endothelin; TXA2, thromboxane A2; VSMC, vascular smooth muscle cells).

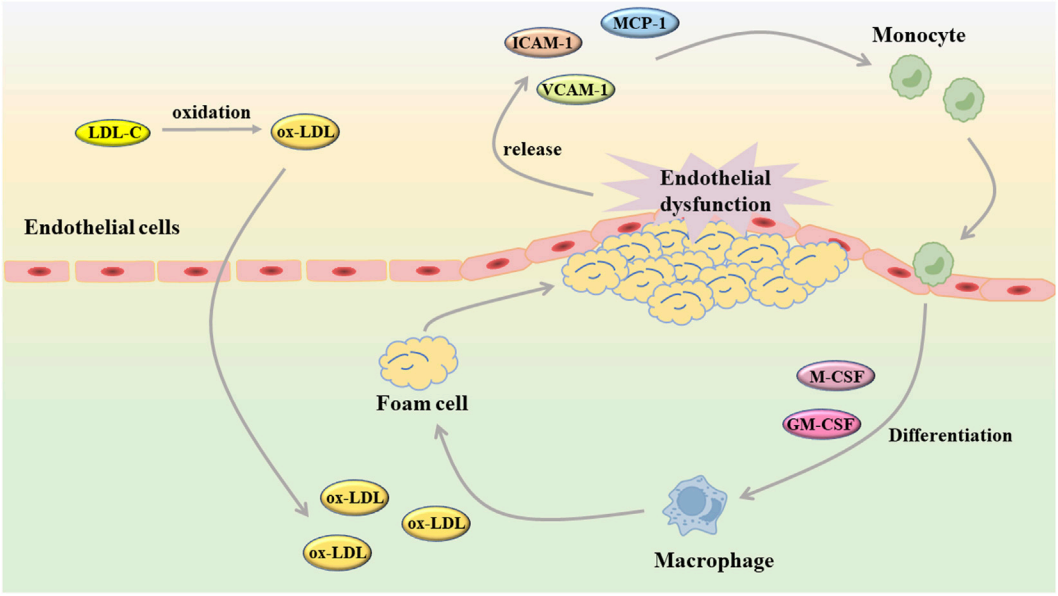


FIGURE 2
Endothelial dysfunction contributes to the development of atherosclerosis (GM-CSF: granulocyte macrophage colony stimulating factor; ICAM-1, intercellular cell adhesion molecule-1; LDL-C, low-density lipoprotein cholesterol; MCP-1, monocyte chemoattractant protein-1; M-CSF, macrophage colony stimulating factor; ox-LDL, oxidized low-density lipoprotein; VCAM-1, vascular cell adhesion molecule-1).

When endothelial injury occurs in blood vessels, white blood cells will combine with fibrin tissue to form fibrin network, which plays a role in the repair of endothelial injury (Ley et al., 2007; Rao et al., 2007). Unfortunately, when the body suffers from a wide range of pathological damage, vascular endothelial cells are continuously damaged and stimulated, and the repair effect is ineffective. Under these conditions, the endothelial cells undergo a phenotypic shift, the balance between vasodilator and vasoconstrictor is disrupted, and the arterial structure is destroyed (Incalza et al., 2018; Kim et al., 2019). As an early event of AS, endothelial cell dysfunction plays a

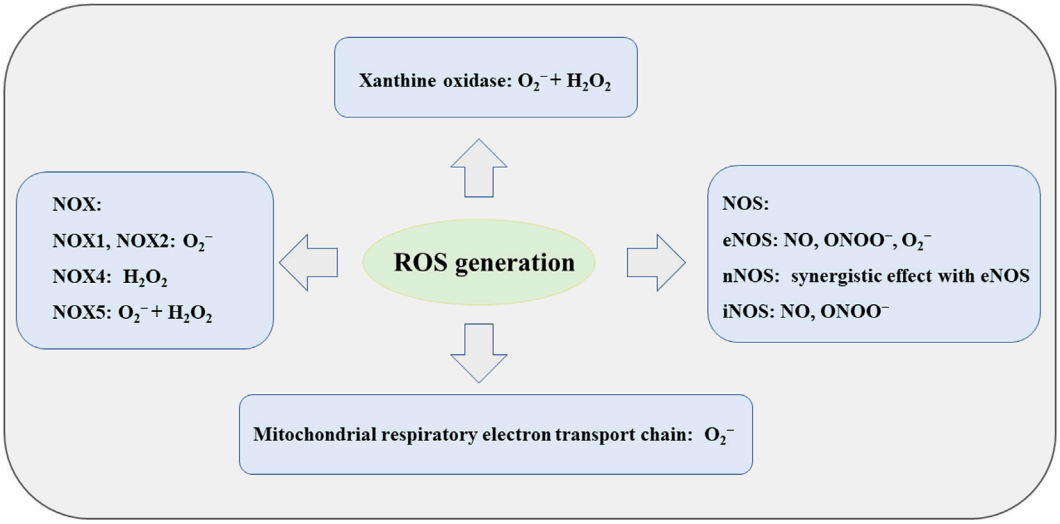


FIGURE 3
The generation of ROS.

role in the development of AS (Figure 2). After the occurrence of endothelial cell dysfunction, the vascular barrier function is weakened, and the low-density lipoprotein cholesterol (LDL-C) in the blood is more likely to accumulate in the intima and undergo oxidation reaction, and then produce oxidized low-density lipoprotein (ox-LDL) (Gao et al., 2017). The injured endothelial cells will release monocyte chemoattractant protein-1 (MCP-1), intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and so on to induce monocyte and macrophages to adhere to the vessel wall (Clapp et al., 2004; Chi and Melendez, 2007). Subsequently, macrophage colony stimulating factor (M-CSF) and granulocyte macrophage colony stimulating factor (GM-CSF) stimulate mononuclear macrophages to differentiate into macrophages, which will take up ox-LDL to generate foam cells and further aggravate AS (Trus et al., 2020; Zhi et al., 2023). As an important component of vascular composition, VSMC will switch from a contractile to a synthetic phenotype after endothelial cell injury. Similarly, VSMC also undergo abnormal proliferation and migration induced by chemokines and matrix metalloproteinases (MMP), which destroys the stability of plaques. In the intima, VSMC not only uptake ox-LDL to generate foam cells, but also secrete extracellular matrix components to form fibrous caps (Liang et al., 2018; Muqri et al., 2020).

3 Oxidative stress and endothelial dysfunction in AS

As mentioned above, factors such as hyperlipidemia, diabetes, heart failure, aging, and genetics may contribute to the development of endothelial dysfunction. Among these factors, we can find the presence of oxidative stress and ox-LDL. At present, many studies believe that excessive reactive oxygen species (ROS) can induce oxidative stress on the one hand, and aggravate the oxidative modification of LDL on the other hand. Subsequently, oxidative stress interacts with ox-LDL to jointly promote the occurrence of endothelial dysfunction.

3.1 Mechanisms of ROS generation

ROS is an endogenous and important mediator involved in various biological processes of the organism and can serve as a second messenger in cell signaling. Because ROS can easily acquire or loss electrons, it is widely involved in redox reactions. However, when the content of ROS exceeds limitation, it will disrupt the redox balance in the body, which in turn leads to the occurrence of oxidative stress, thereby affecting all aspects of physiological functions (Kattoor et al., 2017). Nowadays, the ROS family includes many small molecules and ions, such as superoxide, hydroxyl radicals, hydrogen peroxide and so on. It is well known that almost all cells in blood vessels can produce ROS, and its generation mechanism mainly includes NADPH oxidase (NOX), xanthine oxidase, mitochondrial respiratory chain and NOS (Figure 3) (Goszcz et al., 2015).

As a membrane-binding enzyme complex, NOX is the only family of enzymes whose main function is to produce ROS. NOX is widely present in various vascular cells and is the main source of ROS by transferring electrons from NADPH to O_2 and generate O_2^-. When the body has hypertension, diabetes or high cholesterol, it is easy to increase the expression of NOX and thus increase the content of ROS in the body (Balaban et al., 2005). Existing studies have shown that the congeners of NOX are expressed in various types of vascular cells, but the difference in their content cannot be ignored. For example, NOX2, NOX4, and NOX5 are predominantly expressed in EC, whereas NOX1 and NOX4 are predominantly expressed in VSMC. Different NOXs produce different types of ROS, with NOX1 and NOX2 generating O_2^-, NOX4 generating H_2O_2, and NOX5 generating O_2^- and H_2O_2 (Dikalov et al., 2008;

Lassègue et al., 2012). At the same time, different NOX have different effects on AS. For example, downregulation of NOX1, NOX2, and NOX5 can inhibit AS, while NOX-4 has a cardioprotective effect (possibly due to the fact that NOX4 mainly produces H_2O_2) (Guzik et al., 2008; Takac et al., 2011; Fulton and Barman, 2016). Xanthine oxidase is another important enzymatic source of ROS and is mainly present in EC. Xanthine oxidase can generate O_2^- and H_2O_2 by oxidation of xanthine and hypoxanthine. In addition, xanthine oxidase can also elevate LOX-1 and CD-36 in macrophages and VSMCS, disrupt intracellular lipid metabolism, and increase the risk of AS (Kattoor et al., 2017).

Mitochondria, as an important organelle within the cells, is an important source of energy required for cellular activities. Oxygen, which is required for cell survival, is converted to O_2^- in the mitochondrial respiratory electron transport chain mainly by electron grant in complexes I, II, and III for energy production and oxidative phosphorylation (Peng et al., 2019; Peoples et al., 2019). This process is recognized as the main way to generate ROS. Normally, ROS generated by this process can be removed by various oxidoreductases to maintain homeostasis. Under pathological conditions, the disruption of this balance will lead to excessive accumulation of ROS and further induce ROS leakage (Peoples et al., 2019).

NOS has three distinct isoforms, namely, neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). Among them, eNOS is most closely associated with AS. Notably, although eNOS could produce NO in the presence of tetrahydrobiopterin (BH4) to scour oxygen radicals and thus exert anti-atherosclerosis effect, it has been shown in previous studies that overexpression of eNOS may also promote the development of AS. The possible mechanism lies in the decoupling of eNOS caused by excessive BH4 depletion (Ozaki et al., 2002; Hossain et al., 2012). This hypothesis has been confirmed by a recent study. It was shown that when BH4 was scarce, eNOS uncouples to generate O_2^- and combines with NO to generate peroxynitrite ($ONOO^-$). $ONOO^-$ is a potent oxidant that induces the occurrence of oxidative stress damage (Li et al., 2015). nNOS can exert a synergistic effect with eNOS in anti-atherosclerosis by regulating vascular tone (Capettini et al., 2011). However, iNOS can not only induce excessive production of NO, but also compete with eNOS to bind BH4, promote the generation of $ONOO^-$, and aggravate the occurrence and development of AS (Gunnett et al., 2005).

3.2 ROS promotes ox-LDL production and aggravates endothelial dysfunction

It was shown that excessive ROS-induced oxidative stress can directly affect intracellular biomacromolecules to cause damage. ROS and its oxidation products can act as signal transduction molecules to activate related pathways, damage endothelial cells, and promote the development of AS.

As one of the oxidation products, ox-LDL is thought to play a major role in lipid metabolic disorders. LDL-related modifications include oxidation, deacetylation, glycosylation and aggregation, among which the oxidation of LDL is closely related to AS (Nègre-Salvayre et al., 2017). ROS can oxidise a variety of polyunsaturated lipids in blood vessels, and the by-products formed can react with apolipoprotein B-100 and damage its function. Subsequently, modified ApoB-100 retards LDL removal and prolongs the exposure of lipids and apoB-100 to ROS attack, which further enhances LDL oxidation (Negre-Salvayre et al., 2008; Rabbani et al., 2010; Nègre-Salvayre et al., 2017). When endothelial cells are exposed to oxidative stress for a long time, their structure and function are continuously damaged, which also leads to the continuous oxidation of LDL to form ox-LDL (Stocker and Keaney, 2004). However, after numerous studies on the oxidation mechanism of LDL, it has been found that ox-LDL is heterogeneous, and different concentrations of ox-LDL also have a dual effect on vascular cells. For example, low concentrations of ox-LDL can induce cell migration and proliferation, and create a pro-inflammatory environment for AS, while high concentrations of ox-LDL can promote apoptosis (Dandapat et al., 2007; Cinq-Frais et al., 2013; Camaré et al., 2015). Excessive ROS can cause endothelial cell apoptosis through several major pathways. Firstly, ROS can not only activate nuclear factor kappa-B (NF-κB) through redox factor-1 (Ref-1), but also directly activate NF-κB. Subsequently, activated NF-κB translocates into the nucleus where it binds to the apoptosis-related gene c-Myc, promoting gene transcription and inducing apoptosis. The p38 pathway and c-Jun N-terminal kinase pathways are also strongly associated with ROS-induced apoptosis (Haghi Aminjan et al., 2019; Zhang L. et al., 2022). Notably, excessive ROS causes lipid peroxidation, damages the inner mitochondrial membrane, and ultimately induces both endogenous and exogenous endothelial cell apoptosis (Sinha et al., 2013). In addition, the generated ox-LDL disrupts the structure of actin filaments upon contact with endothelial cells, causing disruption of the cytoskeleton, which in turn alters endothelial cell permeability. The increased permeability of endothelial cells makes it easier for lipids to pass through the cells, further aggravating the development of AS (Chouinard et al., 2008; Zhang et al., 2022).

Ox-LDL can enter endothelial cells through a variety of cell-surface expressed scavenger receptors, the most typical of which are LOX-1 and CD36 (Nègre-Salvayre et al., 2017). LOX-1 is the main receptor for ox-LDL uptake by endothelial cells. The combination of LOX-1 and ox-LDL can enhance the expression of NOX, promote the generation of O_2^-, and aggravate the oxidative stress response in cells (Lu et al., 2011; Yoshimoto et al., 2011). At the same time, the oxLDL/LOX-1/ROS axis is activated, which promotes the production of various inflammatory cytokines, chemokines, adhesion molecules, and ultimately leads to the recruitment and adhesion of monocytes to the activated endothelium (Kamei and Carman, 2010; Lubrano and Balzan, 2016). As a multifunctional receptor, CD36 recognizes oxidized phospholipids and other ligands in addition to ox-LDL. When ox-LDL binds to CD36, MAPK, NF-κB and Toll-like receptors (TLR) are activated, which enhance the local response (Park et al., 2013).

4 Natural flavonoids derived from herbal medicines are potential anti-AS agents by inhibiting oxidative stress in endothelial cells

Flavonoids are a class of secondary metabolites widely found in plants and fungi. Their characteristic structure mainly contains 15 carbon atoms. Flavonoids can be subdivided according to their structure into anthocyanins, flavonoids, flavanones, flavonols, anthoxanthins, and isoflavonoids. Because flavonoids

have hydroxyl groups in their structure, they can play an antioxidant role both *in vivo* and *in vitro*. In this review, we searched the relevant literature on flavonoids inhibiting oxidative stress to treat endothelial dysfunction in AS, and selected some important compounds to elaborate.

Quercetin is a natural polyhydroxy flavonoid found in a variety of plants such as *Bupleurum chinense* DC, *Bupleurum scorzonerifolium* Willd (Apiaceae), mulberry leaves, *Crataegus pinnatifida* Bunge, and *C. pinnatifida* var. *Major* N. E. Br. It is a plant secondary metabolite with antioxidant activity (Zhi et al., 2023). In the past decades, quercetin has been widely used in clinical practice for various diseases due to its superior activity, including cancer, arthritis, neurodegenerative diseases and cardiovascular diseases (Wang et al., 2022). There are numerous studies on quercetin in the treatment of AS. *In vivo* and *in vitro* studies have shown that quercetin exerts multiple effects on various processes of AS development, including foam cell formation, lipid metabolism, monocyte migration, and endothelial cell dysfunction. Firstly, intragastric administration of quercetin ameliorated arterial lipid deposition in high-fat diet fed ApoE mice. In ox-LDL-induced human umbilical vein endothelial cells (HUVECs), quercetin reduced intracellular ROS and increased mitochondrial membrane potential. At the same time, apoptosis and senescence induced by ox-LDL were also alleviated, lipid droplet deposition was reduced, and cell morphology was improved. By exploring the underlying mechanism, p53 and mTOR signaling pathways were found to be involved in the pharmacological mechanism of quercetin (Jiang et al., 2020).

Naringenin, a flavonoid extracted from the pericarp of *Citrus reticulata* Blanco, is a trihydroxy flavanone. It can be found in past reports that naringenin exerts antioxidant activity directly through free radical scavenging activity, and has the ability to induce endogenous antioxidant system (Hernández-Aquino and Muriel, 2018). The comparison of the antioxidant capacity of naringenin with that of quercetin has been controversial in some studies. It was reported that naringenin equivalent antioxidant activity was 1.53 mmol/L, a small value compared to the 4.7 mmol/L of quercetin (Rice-Evans et al., 1996). However, in the study of Cavia-Saiz et al., the antioxidant capacity of naringenin was worse than that of quercetin (Cavia-Saiz et al., 2010). Therefore, further studies are needed to compare the antioxidant capacity of naringenin with other flavonoids. However, it was no doubt about the role of naringenin in protecting endothelial dysfunction in AS. In previous experiments, naringenin was found to inhibit AS by ameliorating dyslipidemia, and subsequently it was found to protect mitochondrial membrane potential to ameliorate ischemic damage (Mulvihill et al., 2010; Testai et al., 2017). Therefore, in the study of Li et al., it was hypothesized that naringenin could ameliorate endothelial injury through a mitochondria-dependent pathway. After homocysteine-induced HUVECs injury, naringenin could inhibit the generation of ROS in mitochondria and cytoplasm, restore mitochondrial membrane potential, but there was no significant difference in Ca^{2+} concentration. RNA-seq transcriptome analysis and experimental validation showed that naringenin significantly restored the expression of Sirt1, AMPKα and eNOS. In addition, knockdown of Sirt1 and AMPKα by siRNA almost abolished this protective effect (Li et al., 2021). *In vivo*, endothelial injury was defined as plasma homocysteine levels higher than 15 μmol/L. Naringenin could significantly inhibit the damage of arterial wall and protect endothelial function after treatment, and its mechanism was consistent with the results *in vitro* (Li et al., 2021). Therefore, we can conclude that naringenin ameliorates homocysteine-induced endothelial injury through the AMPKα/Sirt1 pathway.

Carthamus tinctorius L. has been used as a traditional medicinal plant for thousands years. According to Kaibao Materia Medica, the dried flowers of *C. tinctorius* L. can promote blood circulation and relieve pain. So far, *C. tinctorius* L. has been developed as *Danhong* injection, safflower injection and other preparations for the treatment of coronary heart disease and angina pectoris. Hydroxysafflor yellow A is an important active component of *C. tinctorius* L., and it is also the most abundant component of safflower yellow, an indicator component of *C. tinctorius* L (Xue et al., 2021). In recent years, hydroxysafflor yellow A has been shown to protect endothelial cells by inhibiting inflammation and apoptosis. First, Ji et al. found that hydroxysafflor yellow A could increase the ratio of Bcl-2/Bax at the mRNA and protein levels and reduce mitochondrial-dependent apoptosis in hypoxia-induced HUVECs (Ji et al., 2009). This phenomenon was further illustrated in the experiments of Xie et al., which showed that hydroxysafflor yellow A could regulate cell survival and proliferation by promoting AKT and inhibiting PTEN expression. Meanwhile, hydroxysafflor yellow A reduced ROS generation and restored intracellular redox balance by increasing intracellular superoxide dismutase (SOD) in H_2O_2-induced HUVECs (Xie et al., 2020). In addition, in ox-LDL-induced HUVECs, hydroxysafflor yellow A could upregulate VDAC2 or inhibit apoptosis through AMPK signaling, in which VDAC2 could exert an anti-apoptotic effect by interfering with Bak-mediated apoptosis (Ye et al., 2017; Zhang H. et al., 2022).

Genistein is a natural isoflavone first obtained from *Genista tinctoria* L. It is mainly derived from *Euchresta japonica* Hook. f. ex Regel, *Sophora japonica* L. and so on. Currently, methanol, ethanol, acetonitrile and other organic solvents are used to extract genistein. Meanwhile, the chemical synthesis of genistein is simple and feasible (Spagnuolo et al., 2015). The structure of genistein is similar to that of endogenous estrogen, so it can bind to estrogen receptors and exert estrogen-like effects after being absorbed by the body. In addition, as a typical flavonoid, it is connected with multiple hydroxyl groups on the phenyl ring, which makes it have excellent antioxidant effects and can be applied to the treatment of cardiovascular diseases, diabetes, depression and other diseases (Borrás et al., 2006; Jafari et al., 2022). In endothelial dysfunction, genistein can effectively inhibit ROS and malondialdehyde (MDA) in cells, and restore the four oxidoreductases activities including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GPx). In this way, the redox balance of endothelial cells is maintained (Zhang et al., 2017). Further exploration revealed that the antioxidant activity of genistein was closely related to MR-34a/sirtuin-1/foxo3a. Genistein can downregulate the expression of MiR-34a in ox-LDL-induced HUVES, thereby promoting the expression of sirtuin-1. In addition, sirtuin-1 is known to exert antioxidant activity by activating fxo3a in previous studies. However, after genistein treatment, the expression of fxo3a was significantly increased (Zhang et al., 2017).

Baicalein, also known as 5, 6, 7-trihydroxyflavone, is a well-recognized natural flavonoid with antioxidant and anti-inflammatory activities. Baicalein is the most abundant component in the root of Scutellaria baicalensis (*S. baicalensis*) Georgi, a traditional Chinese medicine (also known as *Huangqin* in Chinese) (Huang et al., 2005). In a previous study, it was shown that baicalein inhibited IL-1β-induced ICAM-1 expression in HUVECs, suggesting that baicalein could protect endothelial cell function (Kimura et al., 1997). In a recent study, ox-LDL was used to induce apoptosis in HUVECs and baicalein was preincubated before induction. It was showed that baicalein effectively inhibited the generation of intracellular ROS and the release of cytochrome C from mitochondria, and increased mitochondrial membrane potential. The expression of pro-apoptotic protein BAX was downregulated, while the expression of anti-apoptotic protein Bcl-2 was upregulated. In addition, the bioavailability of NO was also improved (Chan et al., 2016). Subsequently, it was also shown that baicalein pretreatment could inhibit the binding ability of ox-LDL by reducing the expression of LOX-1, thereby inhibiting the generation of ROS. In addition, baicalein inhibited the protein expression of NADPH oxidase and increased the phosphorylation level of AMPK, thereby inhibiting the activation of protein kinase C (PKC)-α and PKC-β (Tsai et al., 2016).

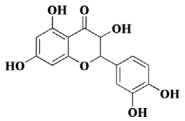
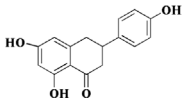
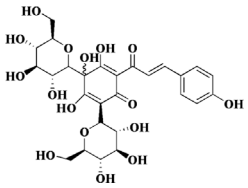
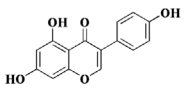
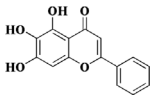
Luteolin is a common flavonoid, which is usually found in the form of glycosylated in celery, green pepper, *Perilla frutescens* (L.) Britt., and *Matricaria recutita* L. Luteolin possesses the antioxidant properties, as well as anti-inflammatory ability. Therefore, it also has a good advantage in the treatment of AS (Prasher et al., 2022). Up to now, the antioxidant activity of luteolin has been fully confirmed. It can exert efficacy in all stages of AS, such as VSMC migration and proliferation, cell adhesion molecule secretion and endothelial cell dysfunction (Luo et al., 2017). When endothelial cells are dysfunctional, luteolin can inhibit the generation of intracellular ROS, while the phosphorylation of p38MAPK and nuclear translocation of NF-kB induced by ox-LDL are reversed. At the same time, the mRNA levels of ICAM-1, VCAM-1, selectin, MMP-1, MMP-2, and MMP-9 are also downregulated by luteolin (Yi et al., 2012). In another study, this conclusion was further developed. In other words, luteolin inhibited TNF-α-induced transcriptional activities of NF-κB and p38 as well as ERK1/2 phosphorylation, while it also exerted its inhibitory effect on Nox4 expression. Ultimately, luteolin restored the redox balance in endothelial cells, that is, the contents of GSH and SOD were restored and LDH was decreased (Xia et al., 2014).

Erigeron breviscapus (Vant.) Hand.-Mazz is a traditional natural medicine used to treat heart and brain ischemic diseases. The modern pharmacological studies have shown that the main active substance is scutellarin. Scutellarin, also known as 4′, 5, 6-trihydroxyflavone-7-glucuronde, is a member of the natural flavonoid family. Previous studies have found that scutellarin not only prevents cerebral ischemia by inhibiting inflammatory response, but also improves liver damage by inhibiting oxidative stress (Yuan et al., 2016). In addition, scutellarin also plays a role in endothelial dysfunction through its antioxidant effect in AS. Scutellarin scavenged excess ROS and increased the bioavailability of NO in HAECs induced by either angiotensin II or H_2O_2. The contents of oxidoreductases, including SOD, GPx, CAT and Nox, could be restored to varying degrees after treatment with scutellarin. Subsequently, the mechanism of scutellarin against endothelial cell injury and apoptosis was further studied, and the results showed that the protective effect of scutellarin was closely related to Hippo-FOXO3A and PI3K/AKT signaling pathways. After treating with scutellarin, the mRNA levels of mammalian sterile-20-like kinases 1 (Mst1), Yes-associated protein (YAP) and FOXO3A were significantly downregulated, as well as the protein levels of p-Mst1, p-YAP and nuclear translocation of FOXO3A. At the same time, PI3K/AKT signaling pathway was activated, and its downstream apoptosis-related Bax and Bcl-2 proteins were also changed (Mo et al., 2018; Fu et al., 2019). We can draw the same conclusion *in vivo* that scutellarin can alleviate lipid metabolism disorder and maintain redox balance in AS rats through Hippo-FoxO3A and PI3K/AKT signaling pathways (Fu et al., 2019). The specific indicators are referred to Table 1.

Acacetin, also known as 5, 7-dihydroxy-4′-methoxy flavone, is a monomethoxy flavonoid widely found in medicinal plants such as *Robinia pseudoacacia* L., *Dendranthema morifolium* (Ramat.)Tzvel., and *Saussurea involucrata* (Kar. et Kir.) Sch.-Bip. In nature, acacetin mostly exists in the form of free or glycosides, and has pharmacological activities on cancer, obesity, diabetes, etc (Wu et al., 2022). In recent years, acacetin has been found to have a protective effect on endothelial dysfunction in AS, which has attracted extensive attention from the scientific community. *In vivo* study believed that acacetin significantly accelerated lipid metabolism in AS mice and reduced the levels of inflammatory factors in plasma (Han et al., 2020). *In vitro* experiment confirmed that acacetin could protect mitochondrial function, reverse mitochondrial depolarization, and inhibit the excessive production of ROS and MDA in HUVECs induced by high glucose. On the other hand, the mitoBcl-2/mitoBax ratio in mitochondria was increased after acacetin administration. This protective effect was closely related to the SIRT1-mediated activation of Sirt3/AMPK signaling, and the protein expression of SOD, Bcl-2 and PGC-1α was increased during this process (Han et al., 2020). In addition, the study has shown that acacetin may restore the antioxidant function of endothelial cells by promoting the phosphorylation of Nrf2, the degradation of Keap1 and the expression of methionine sulfite reductase (Wu et al., 2021).

Eupatilin is a flavonoid mainly found in *Artemisia princeps* Pampanini, and also known as 2- (3, 4-dimethoxyphenyl) −5, 7-dihydroxy-6-methoxy-ychromen-4-one. *Artemisia princeps* Pampanini has been widely used as a medicinal plant in Asia over the last thousands of years. In modern times, due to the rapid development of modern pharmacology, eupatilin has been found to have a wider range of pharmacological activities (Lim et al., 2021). For example, eupatilin has therapeutic potential in diseases such as oncology, allergy, and inflammation (Park, 2014; Jeong et al., 2015). In AS, eupatilin has been shown to inhibit the proliferation and migration of human aortic smooth muscle cells. The oxidative stress as well as inflammatory responses occurring in endothelial cells could also be inhibited by eupatilin. In addition, Yu et al. has been confirmed that eupatilin could effectively reduce the ROS content in TNF-α-induced HUVECs, inhibit the expression of VCAM-1 and ICAM-1, and thus reduce the adhesion ability of U937 cells to endothelial cells. The mechanism by which eupatilin exerted its therapeutic effect was closely related to MAPK-NF-κB. The phosphorylation of NF-kB p65 and MAPK was significantly

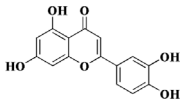
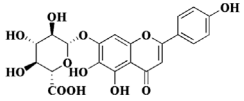
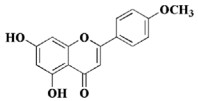
TABLE 1 Natural flavonoids derived from herbal medicines are potential anti-AS agents by inhibiting oxidative stress in endothelial cells.

Components	Plant source	Structure	Experimental model	Effective dose	Effect and mechanism	Ref
Quercetin	*Bupleurum chinense* DC, *Bupleurum scorzonerifolium* Willd (Apiaceae), mulberry leaves, *Crataegus pinnatifida Bunge, Crataegus pinnatifida* var. *Major* N. E. Br		Ox-LDL-induced HUVECs	0.3, 1, 3 μM	MMP↑ ROS↓, lipid droplet deposition↓, p53↓, mTOR↓	Jiang et al. (2020)
			High-fat diet fed ApoE mice	20 mg/kg/d	Arterial lipid deposition↓	
Naringenin	*Citrus reticulata* Blanco		Homocysteine-induced HUVECs	200 μM	MMP↑, the mRNA of AMPKα and Sirt1↑, the protein of AMPKα, Sirt1 and eNOS↑ ROS↓, cytoplasmic cytochrome c↓	Li et al. (2021)
			High-methionine induced SD rat	100 mg/kg/d	SOD↑, NO↑, AMPKα↑, Sirt1↑, eNOS↑ Homocysteine↓, MDA↓	
Hydroxysafflor yellow A	*Carthamus tinctorius* L		Hypoxia-induced HUVECs	1, 10, 100 μM	Bcl-2/Bax↑, eNOS↑ p53↓	Ji et al. (2009)
			Ox-LDL-induced HUVECs	50 μM	SOD↑, NO↑, NOX4↑, AMPKα↑, p-AMPKα↑ ROS↓	Zhang et al. (2022b)
			Ox-LDL-induced HUVECs	1, 5, 25 μM	NO↑, VDAC2↑ SOD↓	Ye et al. (2017)
			H_2O_2-induced HUVECs	4 and 8 μg/mL	GSH/GSSG↑, SOD↑, Bcl-2↑, AKT↑ ROS↓, Bax↓, PTEN↓	Xie et al. (2020)
Genistein	*Euchresta japonica* Hook. f. ex Regel, *Sophora japonica* L. and so on		Ox-LDL-induced HUVECs	10, 100, 1,000 nM	SOD↑, CAT↑, GSH↑, GPx↑, sirtuin-1↑, foxo3a↑ MiR-34a↓	Zhang et al. (2017)
Baicalein	Scutellaria baicalensis (*S. baicalensis*) Georgi		Ox-LDL-induced HUVECs	2.5–20 μM	NO↑, cytochrome C in mitochondria↑, mitochondrial membrane potential↑, Bcl-2↑ ROS↓, BAX↓	Chan et al. (2016)

(Continued on following page)

10.3389/fphar.2023.1141180

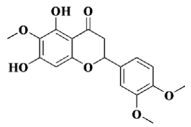
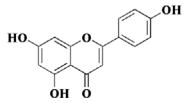
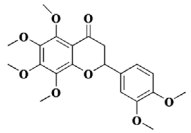
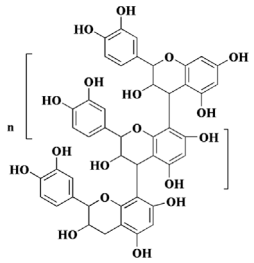
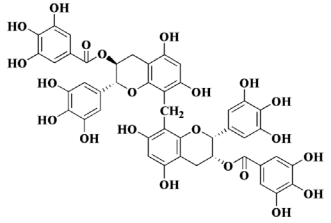
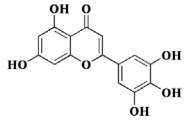
TABLE 1 (*Continued*) Natural flavonoids derived from herbal medicines are potential anti-AS agents by inhibiting oxidative stress in endothelial cells.

Components	Plant source	Structure	Experimental model	Effective dose	Effect and mechanism	Ref
			Ox-LDL-induced HUVECs	2.5 μM	p-MAPK↑	Tsai et al. (2016)
					LOX-1↓, NADPH↓, p-PKC-α↓, p-PKC-β↓, p47phox↓, Rac-1↓	
Luteolin	Celery, green pepper, *Perilla frutescens* (L.) Britt., and *Matricaria recutita* L		Ox-LDL-induced EA.hy926	40 μM	p-p38MAPK↑, nuclear translocation of NF-kB↑	Yi et al. (2012)
					ROS↓, ICAM-1↓, VCAM-1↓, selectin↓, MMP-1↓, MMP-2↓, MMP-9↓	
			TNF-α-induced HUVECs	6.25, 12.5, 25 μM	GSH↑, SOD↑, Bcl-2↑	Xia et al. (2014)
					ROS↓, LDH↓, NF-κB↓, p38↓, p-ERK1/2↓, ICAM-1↓, VCAM-1↓, Nox4↓	
Scutellarin	*Erigeron breviscapus* (Vant.) Hand.-Mazz		H_2O_2-induced HUVECs	12.5, 50, 200 μM	SOD1↑, NO↑, SOD↑, GPx↑, CAT↑	Mo et al. (2018)
					MDA↓, Ca^{2+}↓, Nox4↓	
			Angiotensin II -induced HUVECs	50, 100, 200 μM	SOD↑, CAT↑, Bcl-2↑, PI3K↑, p-AKT↑	Fu et al. (2019)
					MDA↓, ROS↓, Caspase-3↓, FAS↓, BAX↓, Bim↓, p-Mst1↓, p-YAP↓, p-FOXO3A↓	
			HFD-induced rat	6.25 and 25 mg/kg/d	HDL↑, IL-1α↑, SOD↑, CAT↑, Bcl-2↑, PI3K↑, p-AKT↑	
					TG↓, TC↓, LDL↓, VCAM-1↓, ICAM-1↓, IL-6↓, TNF-α↓, MDA↓, Caspase-3↓, Fas↓, Bim↓, Bax↓, p-Mst1↓, p-YAP↓, p-FOXO3A↓, FOXO3A↓	
Acacetin	*Robinia pseudoacacia L., Dendranthema morifolium* (Ramat.)Tzvel., and *Saussurea involucrata* (Kar. et Kir.) Sch.-Bip		High glucose-induced HUVECs	0.3, 1, 3 μM	MMP↑, SOD↑, mitoBcl-2/mitoBax↑, Sirt3↑, pAMPK↑, PGC-1α↑	Han et al. (2020)
					ROS↓, MDA↓	
			Streptozotocin-induced diabetic $ApoE^{-/-}$ mice	20 mg/kg/d	SOD1↑, SOD2↑, Sirt1↑, PGC-1α↑, Sirt3↑, pAMPK↑, Bcl2↑	
					TG↓, TC↓, LDL↓, lipoprotein A↓, lipoprotein B↓, Bax↓	
			Ox-LDL-induced EA.hy926	3 μM	Bcl-2↑, MsrA↑, Nrf2↑, HO-1↑, CAT↑	Wu et al. (2021)
					ROS↓, Bax↓, caspase-3↓, Keap1↓	

(Continued on following page)

10.3389/fphar.2023.1141180

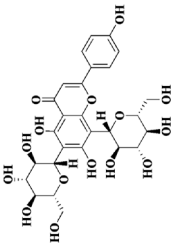
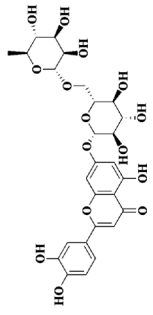
TABLE 1 (*Continued*) Natural flavonoids derived from herbal medicines are potential anti-AS agents by inhibiting oxidative stress in endothelial cells.

Components	Plant source	Structure	Experimental model	Effective dose	Effect and mechanism	Ref
Eupatilin	*Artemisia princeps* Pampanini		TNF-α-induced HUVECs	6.25, 12.5, 25 μM	ROS↓, VCAM-1↓, ICAM-1↓, NF-kB p65↓, p-MAPK↓	Yu et al. (2015)
Apigenin	*Clinopodium chinense* (Benth.) O. Kuntze		High glucose-induced HUVECs	3, 30 μM	NO↑, p-Akt↑, Bcl-2↑ ROS↓, caspase-3↓, Bax↓, p-PKCβII↓, p-p65↓	Qin et al. (2016)
Nobiletin	*Citrus depressa* (shiikuwasa), *Citrus sinensis* (oranges), and *Citrus limon* (lemons)		Ox-LDL-induced HUVECs	10–50 μM	ROS↓, MDA↓, TF↓, NF-κB↓	Cirillo et al. (2017)
Oligomeric proanthocyanidins	*Crataegus oxyacantha* berries		Ox-LDL and C-reactive protein-induced HUVECs	100 μg/mL	NO↑, MMP↑ ROS↓, IL-6↓, MCP-1↓, IL-1β↓, LOX-1↓, eNOS↓	Jamuna et al. (2022)
Oolonghomobisflavan A	Leaves of *Camellia sinensis*		LDL	0.5, 1, 2 μM	Cholesterol ester hydroperoxides↓, thiobarbituric acid reactive substances↓	Sukhbold et al. (2017)
Tricetin	Cereal crops and the pollen of members of the Myrtaceae family		Ox-LDL-induced HUVECs	5, 10 μM	ROS↓, MCP-1↓, IL-1β↓, ICAM-1↓, VCAM-1↓, LOX-1↓, Egr-1↓, ERK1/2↓	Cai et al. (2020)

(Continued on following page)

10.3389/fphar.2023.1141180

TABLE 1 (*Continued*) Natural flavonoids derived from herbal medicines are potential anti-AS agents by inhibiting oxidative stress in endothelial cells.

Components	Plant source	Structure	Experimental model	Effective dose	Effect and mechanism		Ref
Vicenin-2	*Cyclopia subternata*		High-glucose-induced HUVECs	20 μM	SOD↑, CAT↑		Ku and Bae (2016)
					ROS↓, MCP-1↓, IL-18↓, ICAM-1↓, VCAM-1↓, NF-κB p65↓		
Scolymoside	*Cyclopia subternata*		High-glucose-induced HUVECs	20 μM	SOD↑, CAT↑		Ku and Bae (2016)
					ROS↓, MCP-1↓, IL-18↓, ICAM-1↓, VCAM-1↓, NF-κB p65↓		

Egr-1, early growth response 1; eNOS, endothelial NO, synthase; ERK1/2, extracellular signal-regulated protein kinase 1 and 2; GPx, glutathione peroxidase; GSH, glutathione; HFD, high-cholesterol diet; HUVECs, Human umbilical vein endothelial cells; ICAM, intercellular adhesion molecule-1; LOX-1, lectin-like ox-LDL, receptor-1; MCP-1, monocyte chemotactic protein-1; MDA, malondialdehyde; MMP, mitochondrial membrane potential; Msr A, methionine sulphoxide reductase; ox-LDL, oxidized low-density lipoprotein; ROS, reactive oxygen species; SD, sprague dawley; SOD, superoxide dismutase; TC, total cholesterol; TF, tissue factor; TG, triglyceride; VCAM-1, vascular cell adhesion molecule-1.

inhibited by eupatilin. Taken together, it was suggested that eupatilin could protect endothelial cell function through ROS/MAPK-NF-κB (Yu et al., 2015).

From the foregoing, it is known that the preceding flavonoid compounds can protect the cells from oxidative stress damage by restoring the antioxidant capacity of endothelial cells. However, glabridin extracted from the root of *Glycyrrhiza glabra* (licorice) could attenuate the oxidative stress injury to endothelial cells by inhibiting the oxidative sensitivity of LDL. Incubation of LDL with $CuSO_4$ or 2,2' -azobis (2-amidino-propane) dihydrochloride resulted in varying degrees of oxidation of LDL. However, the degree of LDL oxidation was significantly reduced after glabridin treatment, and glabridin inhibited the formation of lipid peroxides and cholesterol linoleic acid hydroperoxides (CLOOH) (Belinky et al., 1998). This protective effect of glabridin provides a novel form of protection for flavonoids. The protective effects of other flavonoid compounds on endothelial cells are shown in Table 1.

5 Conclusion and problems

In this review, we summarized the pathogenesis of endothelial dysfunction in AS, and then selected representative flavonoids with anti-oxidative stress effects for relevant elaboration. After summarizing, we have found that flavonoids from natural herbal medicines not only inhibit oxidative stress, but also have anti-inflammatory and anti-adhesion effects in the treatment of endothelial dysfunction. This result is consistent with the multi-level and multi-target advantages of traditional Chinese medicine. In modern clinical practice, it has been demonstrated that flavonoids can be used to reduce the incidence of AS. First of all, epidemiological investigations have shown that increasing the intake of flavonoids in daily diet can effectively reduce the risk of AS (Lagiou et al., 2006; Mursu et al., 2007). Subsequently, more and more evidence has shown that the intake anthocyanins, tea (the main components are flavan-3-ols), *etc.*, can directly reduce the occurrence of AS (Jennings et al., 2012; Ivey et al., 2013). However, after in-depth understanding, flavonoids from natural herbal medicines also have certain limitations and problems that need to be solved urgently. Firstly, most of the models used in the existing studies are *in vitro* models. Flavonoids have been shown to exert protective effects on endothelial cells in experiments, but it is not clear whether this protective effect will change with the transformation of drug structure due to complex changes after drug entry into the body. Secondly, although some researchers have confirmed the protective effect of flavonoids on AS from *in vivo* and *in vitro* experiments, there is no relevant clinical data to support. At the same time, the toxicity and safety of drugs are also essential before the development of drugs. In the case of quercetin, after long-term addition of quercetin to the diet of F344/N rats, there was no obvious toxic damage in the rats at the beginning, but their weight gain was slow and they showed kidney carcinogenic activity in males after 2 years (Dunnick and Hailey, 1992). The oncogenic activity of quercetin remains controversial. However, it is generally believed that quercetin is safe when used under the intended conditions, and caution should be taken when taking quercetin in high doses or for a long time. Therefore, the safety and toxicity of flavonoids should be considered before they are used in clinical

practice, and more work needs to be done. Finally, because the flavonoid compounds have more phenolic hydroxyl groups in their structure, it makes their structure unstable. Therefore, it is necessary to consider how to solve the problem of drug stability before developing flavonoid compounds into drugs. Looking at the existing flavonoid drug development, it can be found that the research on the treatment of endothelial dysfunction in AS is still relatively basic, and has not yet considered what kind of preparation the flavonoid is made into, or how it is administered. The development of flavonoids into modern formulations such as nanoparticles may change the instability of the compounds, which can also become the future development direction of flavonoids for the treatment of endothelial dysfunction. In summary, flavonoid compounds hold great promise in the treatment of endothelial dysfunction in AS, but further exploration is needed.

Author contributions

All authors made a significant contribution to the work reported, whether that is in the conception, execution, acquisition of data, analysis and interpretation. R-LL and L-YW took part in drafting, revising and critically reviewing the article; H-XD, DQ, and QZ gave final approval of the version to be published; L-SH and X-PL have agreed on the journal to which the article has been submitted and agree to be accountable for all aspects of the work.

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

Balaban, R. S., Nemoto, S., and Finkel, T. (2005). Mitochondria, oxidants, and aging. *Cell.* 120 (4), 483–495. doi:10.1016/j.cell.2005.02.001

Belinky, P. A., Aviram, M., Fuhrman, B., Rosenblat, M., and Vaya, J. (1998). The antioxidative effects of the isoflavan glabridin on endogenous constituents of LDL during its oxidation. *Atherosclerosis* 137 (1), 49–61. doi:10.1016/s0021-9150(97)00251-7

Benjamin, E. J., Blaha, M. J., Chiuve, S. E., Cushman, M., Das, S. R., Deo, R., et al. (2017). Heart disease and stroke statistics—2017 update: A report from the American heart association. *Circulation* 135 (10), e146–e603. doi:10.1161/CIR.0000000000000485

Borrás, C., Gambini, J., Gómez-Cabrera, M. C., Sastre, J., Pallardó, F. V., Mann, G. E., et al. (2006). Genistein, a soy isoflavone, up-regulates expression of antioxidant genes: Involvement of estrogen receptors, ERK1/2, and NFkappaB. *FASEB J. official Publ. Fed. Am. Soc. Exp. Biol.* 20 (12), 2136–2138. doi:10.1096/fj.05-5522fje

Cai, L., Zhang, X., Hou, M., and Gao, F. (2020). Natural flavone tricetin suppresses oxidized LDL-induced endothelial inflammation mediated by Egr-1. *Int. Immunopharmacol.* 80, 106224. doi:10.1016/j.intimp.2020.106224

Camaré, C., Trayssac, M., Garmy-Susini, B., Mucher, E., Sabbadini, R., Salvayre, R., et al. (2015). Oxidized LDL-induced angiogenesis involves sphingosine 1-phosphate: Prevention by anti-S1P antibody. *Br. J. Pharmacol.* 172 (1), 106–118. doi:10.1111/bph.12897

Capettini, L. S., Cortes, S. F., Silva, J. F., Alvarez-Leite, J. I., and Lemos, V. S. (2011). Decreased production of neuronal NOS-derived hydrogen peroxide contributes to endothelial dysfunction in atherosclerosis. *Br. J. Pharmacol.* 164 (6), 1738–1748. doi:10.1111/j.1476-5381.2011.01500.x

Cavia-Saiz, M., Busto, M. D., Pilar-Izquierdo, M. C., Ortega, N., Perez-Mateos, M., and Muñiz, P. (2010). Antioxidant properties, radical scavenging activity and biomolecule protection capacity of flavonoid naringenin and its glycoside naringin: A comparative study. *J. Sci. food Agric.* 90 (7), 1238–1244. doi:10.1002/jsfa.3959

Chan, S. H., Hung, C. H., Shih, J. Y., Chu, P. M., Cheng, Y. H., Tsai, Y. J., et al. (2016). Baicalein is an available anti-atherosclerotic compound through modulation of nitric oxide-related mechanism under oxLDL exposure. *Oncotarget* 7 (28), 42881–42891. doi:10.18632/oncotarget.10263

Chandel, S., Sathis, A., Dhar, M., Giri, H., Nathan, A. A., Samawar, S. K. R., et al. (2020). Hyperinsulinemia promotes endothelial inflammation via increased expression and release of Angiopoietin-2. *Atherosclerosis* 307, 1–10. doi:10.1016/j.atherosclerosis.2020.06.016

Chi, Z., and Melendez, A. J. (2007). Role of cell adhesion molecules and immune-cell migration in the initiation, onset and development of atherosclerosis. *Cell. adhesion Migr.* 1 (4), 171–175. doi:10.4161/cam.1.4.5321

Chistiakov, D. A., Melnichenko, A. A., Grechko, A. V., Myasoedova, V. A., and Orekhov, A. N. (2018). Potential of anti-inflammatory agents for treatment of atherosclerosis. *Exp. Mol. pathology* 104 (2), 114–124. doi:10.1016/j.yexmp.2018.01.008

Chouinard, J. A., Grenier, G., Khalil, A., and Vermette, P. (2008). Oxidized-LDL induce morphological changes and increase stiffness of endothelial cells. *Exp. Cell. Res.* 314 (16), 3007–3016. doi:10.1016/j.yexcr.2008.07.020

Cinq-Frais, C., Coatrieux, C., Grazide, M. H., Hannun, Y. A., Nègre-Salvayre, A., Salvayre, R., et al. (2013). A signaling cascade mediated by ceramide, src and PDGFRβ coordinates the activation of the redox-sensitive neutral sphingomyelinase-2 and sphingosine kinase-1. *Biochimica biophysica acta* 1831 (8), 1344–1356. doi:10.1016/j.bbalip.2013.04.014

Cirillo, P., Conte, S., Cimmino, G., Pellegrino, G., Ziviello, F., Barra, G., et al. (2017). Nobiletin inhibits oxidized-LDL mediated expression of Tissue Factor in human endothelial cells through inhibition of NF-κB. *Biochem. Pharmacol.* 128, 26–33. doi:10.1016/j.bcp.2016.12.016

Clapp, B. R., Hingorani, A. D., Kharbanda, R. K., Mohamed-Ali, V., Stephens, J. W., Vallance, P., et al. (2004). Inflammation-induced endothelial dysfunction involves reduced nitric oxide bioavailability and increased oxidant stress. *Cardiovasc. Res.* 64 (1), 172–178. doi:10.1016/j.cardiores.2004.06.020

Crea, F., and Libby, P. (2017). Acute coronary syndromes: The way forward from mechanisms to precision treatment. *Circulation* 136 (12), 1155–1166. doi:10.1161/CIRCULATIONAHA.117.029870

Dandapat, A., Hu, C., Sun, L., and Mehta, J. L. (2007). Small concentrations of oxLDL induce capillary tube formation from endothelial cells via LOX-1-dependent redox-sensitive pathway. *Arteriosclerosis, thrombosis, Vasc. Biol.* 27 (11), 2435–2442. doi:10.1161/ATVBAHA.107.152272

Dikalov, S. I., Dikalova, A. E., Bikineyeva, A. T., Schmidt, H. H., Harrison, D. G., and Griendling, K. K. (2008). Distinct roles of Nox1 and Nox4 in basal and angiotensin II-stimulated superoxide and hydrogen peroxide production. *Free Radic. Biol. Med.* 45 (9), 1340–1351. doi:10.1016/j.freeradbiomed.2008.08.013

Dunnick, J. K., and Hailey, J. R. (1992). Toxicity and carcinogenicity studies of quercetin, a natural component of foods. *Fundam. Appl. Toxicol. official J. Soc. Toxicol.* 19 (3), 423–431. doi:10.1016/0272-0590(92)90181-g

Falk, E. (2006). Pathogenesis of atherosclerosis. *J. Am. Coll. Cardiol.* 47 (8), C7–C12. doi:10.1016/j.jacc.2005.09.068

Förstermann, U., and Münzel, T. (2006). Endothelial nitric oxide synthase in vascular disease: From marvel to menace. *Circulation* 113 (13), 1708–1714. doi:10.1161/CIRCULATIONAHA.105.602532

Fu, Y., Sun, S., Sun, H., Peng, J., Ma, X., Bao, L., et al. (2019). Scutellarin exerts protective effects against atherosclerosis in rats by regulating the Hippo-FOXO3A and PI3K/AKT signaling pathways. *J. Cell. physiology* 234 (10), 18131–18145. doi:10.1002/jcp.28446

Fulton, D. J., and Barman, S. A. (2016). Clarity on the isoform-specific roles of NADPH oxidases and NADPH oxidase-4 in atherosclerosis. *Arteriosclerosis, thrombosis, Vasc. Biol.* 36 (4), 579–581. doi:10.1161/ATVBAHA.116.307096

Gao, S., Zhao, D., Wang, M., Zhao, F., Han, X., Qi, Y., et al. (2017). Association between circulating oxidized LDL and atherosclerotic cardiovascular disease: A meta-analysis of observational studies. *Can. J. Cardiol.* 33 (12), 1624–1632. doi:10.1016/j.cjca.2017.07.015

Giannitsi, S., Bougiakli, M., Bechlioulis, A., and Naka, K. (2019). Endothelial dysfunction and heart failure: A review of the existing bibliography with emphasis on flow mediated dilation. *JRSM Cardiovasc. Dis.* 8, 2048004019843047. doi:10.1177/2048004019843047

Goszcz, K., Deakin, S. J., Duthie, G. G., Stewart, D., Leslie, S. J., and Megson, I. L. (2015). Antioxidants in cardiovascular therapy: Panacea or false hope? *Front. Cardiovasc. Med.* 2, 29. doi:10.3389/fcvm.2015.00029

Gunnett, C. A., Lund, D. D., McDowell, A. K., Faraci, F. M., and Heistad, D. D. (2005). Mechanisms of inducible nitric oxide synthase-mediated vascular dysfunction. *Arterioscler. Thromb. Vasc. Biol.* 25 (8), 1617–1622. doi:10.1161/01.ATV.0000172626.00296.ba

Guzik, T. J., Chen, W., Gongora, M. C., Guzik, B., Lob, H. E., Mangalat, D., et al. (2008). Calcium-dependent NOX5 nicotinamide adenine dinucleotide phosphate oxidase contributes to vascular oxidative stress in human coronary artery disease. *J. Am. Coll. Cardiol.* 52 (22), 1803–1809. doi:10.1016/j.jacc.2008.07.063

Haghi Aminjan, H., Abtahi, S. R., Hazrati, E., Chamanara, M., Jalili, M., and Paknejad, B. (2019). Targeting of oxidative stress and inflammation through ROS/NF-kappaB pathway in phosphine-induced hepatotoxicity mitigation. *Life Sci.* 232, 116607. doi:10.1016/j.lfs.2019.116607

Han, W. M., Chen, X. C., Li, G. R., and Wang, Y. (2020). Acacetin protects against high glucose-induced endothelial cells injury by preserving mitochondrial function via activating sirt1/sirt3/AMPK signals. *Front. Pharmacol.* 11, 607796. doi:10.3389/fphar.2020.607796

Hernández-Aquino, E., and Muriel, P. (2018). Beneficial effects of naringenin in liver diseases: Molecular mechanisms. *World J. gastroenterology* 24 (16), 1679–1707. doi:10.3748/wjg.v24.i16.1679

Hossain, M., Qadri, S. M., and Liu, L. (2012). Inhibition of nitric oxide synthesis enhances leukocyte rolling and adhesion in human microvasculature. *J. Inflamm. Lond. Engl.* 9 (1), 28. doi:10.1186/1476-9255-9-28

Hua, Y., Zhang, J., Liu, Q., Su, J., Zhao, Y., Zheng, G., et al. (2022). The induction of endothelial autophagy and its role in the development of atherosclerosis. *Front. Cardiovasc. Med.* 9, 831847. doi:10.3389/fcvm.2022.831847

Huang, Y., Tsang, S. Y., Yao, X., and Chen, Z. Y. (2005). Biological properties of baicalein in cardiovascular system. *Cardiovasc. Haematol. Disord.* 5 (2), 177–184. doi:10.2174/1568006043586206

Incalza, M. A., D'Oria, R., Natalicchio, A., Perrini, S., Laviola, L., and Giorgino, F. (2018). Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Vasc. Pharmacol.* 100, 1–19. doi:10.1016/j.vph.2017.05.005

Ivey, K. L., Lewis, J. R., Prince, R. L., and Hodgson, J. M. (2013). Tea and non-tea flavonol intakes in relation to atherosclerotic vascular disease mortality in older women. *Br. J. Nutr.* 110 (9), 1648–1655. doi:10.1017/S0007114513000780

Jafari, A., Esmaeilzadeh, Z., Khezri, M. R., Ghasemnejad-Berenji, H., Pashapour, S., Sadeghpour, S., et al. (2022). An overview of possible pivotal mechanisms of genistein as a potential phytochemical against SARS-CoV-2 infection: A hypothesis. *J. food Biochem.* 46 (10), e14345. doi:10.1111/jfbc.14345

Jamuna, S., Ashokkumar, R., and Devaraj, S. N. (2022). Amelioration of C-reactive protein and lectin like oxidized low density lipoprotein receptor complex induced endothelial dysfunction by oligomeric proanthocyanidins. *Appl. Biochem. Biotechnol.* doi:10.1007/s12010-021-03792-610.1007/s12010-021-03792-6

Jennings, A., Welch, A. A., Fairweather-Tait, S. J., Kay, C., Minihane, A. M., Chowienczyk, P., et al. (2012). Higher anthocyanin intake is associated with lower arterial stiffness and central blood pressure in women. *Am. J. Clin. Nutr.* 96 (4), 781–788. doi:10.3945/ajcn.112.042036

Jeong, J. H., Moon, S. J., Jhun, J. Y., Yang, E. J., Cho, M. L., and Min, J. K. (2015). Eupatilin exerts antinociceptive and chondroprotective properties in a rat model of osteoarthritis by downregulating oxidative damage and catabolic activity in chondrocytes. *PloS one* 10 (6), e0130882. doi:10.1371/journal.pone.0130882

Ji, D. B., Zhang, L. Y., Li, C. L., Ye, J., and Zhu, H. B. (2009). Effect of Hydroxysafflor yellow A on human umbilical vein endothelial cells under hypoxia. *Vasc. Pharmacol.* 50 (3-4), 137–145. doi:10.1016/j.vph.2008.11.009

Jiang, Y. H., Jiang, L. Y., Wang, Y. C., Ma, D. F., and Li, X. (2020). Quercetin attenuates atherosclerosis via modulating oxidized LDL-induced endothelial cellular senescence. *Front. Pharmacol.* 11, 512. doi:10.3389/fphar.2020.00512

Jin, R. C., and Loscalzo, J. (2010). Vascular nitric oxide: Formation and function. *J. blood Med.* 2010 (1), 147–162. doi:10.2147/JBM.S7000

Kamei, M., and Carman, C. V. (2010). New observations on the trafficking and diapedesis of monocytes. *Curr. Opin. Hematol.* 17 (1), 43–52. doi:10.1097/MOH.0b013e3283333949

Kattoor, A. J., Pothineni, N. V. K., Palagiri, D., and Mehta, J. L. (2017). Oxidative stress in atherosclerosis. *Curr. Atheroscler. Rep.* 19 (11), 42. doi:10.1007/s11883-017-0678-6

Khan, J., Deb, P. K., Priya, S., Medina, K. D., Devi, R., Walode, S. G., et al. (2021). Dietary flavonoids: Cardioprotective potential with antioxidant effects and their pharmacokinetic, toxicological and therapeutic concerns. *Mol. (Basel, Switz.* 26 (13), 4021. doi:10.3390/molecules26134021

Kim, S. M., Huh, J. W., Kim, E. Y., Shin, M. K., Park, J. E., Kim, S. W., et al. (2019). Endothelial dysfunction induces atherosclerosis: Increased aggrecan expression promotes apoptosis in vascular smooth muscle cells. *BMB Rep.* 52 (2), 145–150. doi:10.5483/BMBRep.2019.52.2.282

Kimura, Y., Matsushita, N., and Okuda, H. (1997). Effects of baicalein isolated from Scutellaria baicalensis on interleukin 1 beta- and tumor necrosis factor alpha-induced adhesion molecule expression in cultured human umbilical vein endothelial cells. *J. Ethnopharmacol.* 57 (1), 63–67. doi:10.1016/s0378-8741(97)00045-7

Krüger-Genge, A., Blocki, A., Franke, R. P., and Jung, F. (2019). Vascular endothelial cell biology: An update. *Int. J. Mol. Sci.* 20 (18), 4411. doi:10.3390/ijms20184411

Ku, S. K., and Bae, J. S. (2016). Vicenin-2 and scolymoside inhibit high-glucose-induced vascular inflammation *in vitro* and *in vivo*. *Can. J. physiology Pharmacol.* 94 (3), 287–295. doi:10.1139/cjpp-2015-0215

Lagiou, P., Samoli, E., Lagiou, A., Skalkidis, Y., Katsouyanni, K., Petridou, E., et al. (2006). Flavonoid classes and risk of peripheral arterial occlusive disease: A case-control study in Greece. *Eur. J. Clin. Nutr.* 60 (2), 214–219. doi:10.1038/sj.ejcn.1602291

Lassègue, B., San Martín, A., and Griendling, K. K. (2012). Biochemistry, physiology, and pathophysiology of NADPH oxidases in the cardiovascular system. *Circulation Res.* 110 (10), 1364–1390. doi:10.1161/CIRCRESAHA.111.243972

Ley, K., Laudanna, C., Cybulsky, M. I., and Nourshargh, S. (2007). Getting to the site of inflammation: The leukocyte adhesion cascade updated. *Nat. Rev. Immunol.* 7 (9), 678–689. doi:10.1038/nri2156

Li, H., Liu, L., Cao, Z., Li, W., Liu, R., Chen, Y., et al. (2021). Naringenin ameliorates homocysteine induced endothelial damage via the AMPKα/Sirt1 pathway. *J. Adv. Res.* 34, 137–147. doi:10.1016/j.jare.2021.01.009

Li, Q., Youn, J. Y., and Cai, H. (2015). Mechanisms and consequences of endothelial nitric oxide synthase dysfunction in hypertension. *J. Hypertens.* 33 (6), 1128–1136. doi:10.1097/HJH.0000000000000587

Li, R. L., Wang, L. Y., Duan, H. X., Zhang, Q., Guo, X., Wu, C., et al. (2022b). Regulation of mitochondrial dysfunction induced cell apoptosis is a potential therapeutic strategy for herbal medicine to treat neurodegenerative diseases. *Front. Pharmacol.* 13, 937289. doi:10.3389/fphar.2022.937289

Li, R. L., Wang, L. Y., Liu, S., Duan, H. X., Zhang, Q., Zhang, T., et al. (2022a). Natural flavonoids derived from fruits are potential agents against atherosclerosis. *Front. Nutr.* 9, 862277. doi:10.3389/fnut.2022.862277

Liang, X., Hou, X., Yang, Y., Liu, H., Guo, R., Yang, Z., et al. (2018). The feedback loop of "EMMPRIN/NF-κB" worsens atherosclerotic plaque via suppressing autophagy in macrophage. *J. Mol. Cell. Cardiol.* 114, 129–140. doi:10.1016/j.yjmcc.2017.11.008

Lim, W. X. J., Gammon, C. S., von Hurst, P., Chepulis, L., and Page, R. A. (2021). A narrative review of human clinical trials on the impact of phenolic-rich plant extracts on prediabetes and its subgroups. *Nutrients* 13 (11), 3733. doi:10.3390/nu13113733

Lu, J., Mitra, S., Wang, X., Khaidakov, M., and Mehta, J. L. (2011). Oxidative stress and lectin-like ox-LDL-receptor LOX-1 in atherogenesis and tumorigenesis. *Antioxidants redox Signal.* 15 (8), 2301–2333. doi:10.1089/ars.2010.3792

Lubrano, V., and Balzan, S. (2016). Roles of LOX-1 in microvascular dysfunction. *Microvasc. Res.* 105, 132–140. doi:10.1016/j.mvr.2016.02.006

Luo, Y., Shang, P., and Li, D. (2017). Luteolin: A flavonoid that has multiple cardio-protective effects and its molecular mechanisms. *Front. Pharmacol.* 8, 692. doi:10.3389/fphar.2017.00692

Mensah, S. A., Nersesyan, A. A., Harding, I. C., Lee, C. I., Tan, X., Banerjee, S., et al. (2020). Flow-regulated endothelial glycocalyx determines metastatic cancer cell activity. *FASEB* 34 (5), 6166–6184. doi:10.1096/fj.201901920R

Mitra, R., O'Neil, G. L., Harding, I. C., Cheng, M. J., Mensah, S. A., and Ebong, E. E. (2017). Glycocalyx in atherosclerosis-relevant endothelium function and as a therapeutic target. *Curr. Atheroscler. Rep.* 19, 63. doi:10.1007/s11883-017-0691-9

Mo, J., Yang, R., Li, F., Zhang, X., He, B., Zhang, Y., et al. (2018). Scutellarin protects against vascular endothelial dysfunction and prevents atherosclerosis via antioxidation. *Phytomedicine Int. J. phytotherapy Phytopharm.* 42, 66–74. doi:10.1016/j.phymed.2018.03.021

Mulvihill, E. E., Assini, J. M., Sutherland, B. G., DiMattia, A. S., Khami, M., Koppes, J. B., et al. (2010). Naringenin decreases progression of atherosclerosis by improving dyslipidemia in high-fat-fed low-density lipoprotein receptor-null mice. *Arterioscler. Thromb. Vasc. Biol.* 30 (4), 742–748. doi:10.1161/ATVBAHA.109.201095

Muqri, F., Helkin, A., Maier, K. G., and Gahtan, V. (2020). Thrombospondin-5 and fluvastatin promote angiogenesis and are protective against endothelial cell apoptosis. *J. Cell. Biochem.* 121 (10), 4154–4165. doi:10.1002/jcb.29686

Mursu, J., Nurmi, T., Tuomainen, T. P., Ruusunen, A., Salonen, J. T., and Voutilainen, S. (2007). The intake of flavonoids and carotid atherosclerosis: The kuopio ischaemic heart disease risk factor study. *Br. J. Nutr.* 98 (4), 814–818. doi:10.1017/S0007114507744410

Nègre-Salvayre, A., Augé, N., Camaré, C., Bacchetti, T., Ferretti, G., and Salvayre, R. (2017). Dual signaling evoked by oxidized LDLs in vascular cells. *Free Radic. Biol. Med.* 106, 118–133. doi:10.1016/j.freeradbiomed.2017.02.006

Negre-Salvayre, A., Coatrieux, C., Ingueneau, C., and Salvayre, R. (2008). Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. *Br. J. Pharmacol.* 153 (1), 6–20. doi:10.1038/sj.bjp.0707395

Ozaki, M., Kawashima, S., Yamashita, T., Hirase, T., Namiki, M., Inoue, N., et al. (2002). Overexpression of endothelial nitric oxide synthase accelerates atherosclerotic lesion formation in apoE-deficient mice. *J. Clin. investigation* 110 (3), 331–340. doi:10.1172/JCI15215

Pandey, K. B., and Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Med. Cell. Longev.* 2 (5), 270–278. doi:10.4161/oxim.2.5.9498

Park, B. B., Yoon, J. s., Kim, E. s., Choi, J., Won, Y. w., Choi, J. h., et al. (2013). Inhibitory effects of eupatilin on tumor invasion of human gastric cancer MKN-1 cells. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* 34 (2), 875–885. doi:10.1007/s13277-012-0621-y

Park, Y. M. (2014). CD36, a scavenger receptor implicated in atherosclerosis. *Exp. Mol. Med.* 46 (6), e99. doi:10.1038/emm.2014.38

Peng, W., Cai, G., Xia, Y., Chen, J., Wu, P., Wang, Z., et al. (2019). Mitochondrial dysfunction in atherosclerosis. *DNA Cell. Biol.* 38 (7), 597–606. doi:10.1089/dna.2018.4552

Peoples, J. N., Saraf, A., Ghazal, N., Pham, T. T., and Kwong, J. Q. (2019). Mitochondrial dysfunction and oxidative stress in heart disease. *Exp. Mol. Med.* 51 (12), 1–13. doi:10.1038/s12276-019-0355-7

Prasher, P., Sharma, M., Singh, S. K., Gulati, M., Chellappan, D. K., Zacconi, F., et al. (2022). Luteolin: A flavonoid with a multifaceted anticancer potential. *Cancer Cell. Int.* 22 (1), 386. doi:10.1186/s12935-022-02808-3

Qin, W., Ren, B., Wang, S., Liang, S., He, B., Shi, X., et al. (2016). Apigenin and naringenin ameliorate PKCβII-associated endothelial dysfunction via regulating ROS/caspase-3 and NO pathway in endothelial cells exposed to high glucose. *Vasc. Pharmacol.* 85, 39–49. doi:10.1016/j.vph.2016.07.006

Rabbani, N., Chittari, M. V., Bodmer, C. W., Zehnder, D., Ceriello, A., and Thornalley, P. J. (2010). Increased glycation and oxidative damage to apolipoprotein B100 of LDL cholesterol in patients with type 2 diabetes and effect of metformin. *Diabetes* 59 (4), 1038–1045. doi:10.2337/db09-1455

Rao, R. M., Yang, L., Garcia-Cardena, G., and Luscinskas, F. W. (2007). Endothelial-dependent mechanisms of leukocyte recruitment to the vascular wall. *Circulation Res.* 101 (3), 234–247. doi:10.1161/CIRCRESAHA.107.151860b

Rice-Evans, C. A., Miller, N. J., and Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.* 20 (7), 933–956. doi:10.1016/0891-5849(95)02227-9

Shemiakova, T., Ivanova, E., Grechko, A. V., Gerasimova, E. V., Sobenin, I. A., and Orekhov, A. N. (2020). Mitochondrial dysfunction and DNA damage in the context of pathogenesis of atherosclerosis. *Biomedicines* 8 (6), 166. doi:10.3390/biomedicines8060166

Shimokawa, H. (2014). 2014 williams harvey lecture: Importance of coronary vasomotion abnormalities-from bench to bedside. *Eur. heart J.* 35 (45), 3180–3193. doi:10.1093/eurheartj/ehu427

Sinha, K., Das, J., Pal, P. B., and Sil, P. C. (2013). Oxidative stress: The mitochondria-dependent and mitochondria-independent pathways of apoptosis. *Archives Toxicol.* 87 (7), 1157–1180. doi:10.1007/s00204-013-1034-4

Spagnuolo, C., Russo, G. L., Orhan, I. E., Habtemariam, S., Daglia, M., Sureda, A., et al. (2015). Genistein and cancer: Current status, challenges, and future directions. *Adv. Nutr. (Bethesda, Md* 6 (4), 408–419. doi:10.3945/an.114.008052

Steven, S., Frenis, K., Oelze, M., Kalinovic, S., Kuntic, M., Bayo Jimenez, M. T., et al. (2019). Vascular inflammation and oxidative stress: Major triggers for cardiovascular disease. *Oxidative Med. Cell. Longev.* 2019, 7092151. doi:10.1155/2019/7092151

Stocker, R., and Keaney, J. F., Jr (2004). Role of oxidative modifications in atherosclerosis. *Physiol. Rev.* 84 (4), 1381–1478. doi:10.1152/physrev.00047.2003

Sukhbold, E., Sekimoto, S., Watanabe, E., Yamazaki, A., Yang, L., Takasugi, M., et al. (2017). Effects of oolonghomobisflavan A on oxidation of low-density lipoprotein. *Biosci. Biotechnol. Biochem.* 81 (8), 1569–1575. doi:10.1080/09168451.2017.1314758

Takac, I., Schröder, K., Zhang, L., Lardy, B., Anilkumar, N., Lambeth, J. D., et al. (2011). The E-loop is involved in hydrogen peroxide formation by the NADPH oxidase Nox4. *J. Biol. Chem.* 286 (15), 13304–13313. doi:10.1074/jbc.M110.192138

Testai, L., Da Pozzo, E., Piano, I., Pistelli, L., Gargini, C., Breschi, M. C., et al. (2017). The Citrus flavanone naringenin produces cardioprotective effects in hearts from 1 Year old rat, through activation of mitoBK channels. *Front. Pharmacol.* 8, 71. doi:10.3389/fphar.2017.00071

Tonelli, M., Karumanchi, S. A., and Thadhani, R. (2016). Epidemiology and mechanisms of uremia-related cardiovascular disease. *Circulation* 133 (5), 518–536. doi:10.1161/CIRCULATIONAHA.115.018713

Torisu, K., Singh, K. K., Torisu, T., Lovren, F., Liu, J., Pan, Y., et al. (2016). Intact endothelial autophagy is required to maintain vascular lipid homeostasis. *Aging Cell.* 15 (1), 187–191. doi:10.1111/acel.12423

Townsend, N., Wilson, L., Bhatnagar, P., Wickramasinghe, K., Rayner, M., and Nichols, M. (2016). Cardiovascular disease in europe: Epidemiological update 2016. *Eur. heart J.* 37 (42), 3232–3245. doi:10.1093/eurheartj/ehw334

Trus, E., Basta, S., and Gee, K. (2020). Who's in charge here? Macrophage colony stimulating factor and granulocyte macrophage colony stimulating factor: Competing factors in macrophage polarization. *Cytokine* 127, 154939. doi:10.1016/j.cyto.2019.154939

Tsai, K. L., Hung, C. H., Chan, S. H., Shih, J. Y., Cheng, Y. H., Tsai, Y. J., et al. (2016). Baicalein protects against oxLDL-caused oxidative stress and inflammation by modulation of AMPK-alpha. *Oncotarget* 7 (45), 72458–72468. doi:10.18632/oncotarget.12788

Tuñón, J., Martín-Ventura, J. L., Blanco-Colio, L. M., Tarín, N., and Egido, J. (2007). Common pathways of hypercholesterolemia and hypertension leading to atherothrombosis: The need for a global approach in the management of cardiovascular risk factors. *Vasc. health risk Manag.* 3 (4), 521–526.

Wang, G., Wang, Y., Yao, L., Gu, W., Zhao, S., Shen, Z., et al. (2022). Pharmacological activity of quercetin: An updated review. *eCAM* 2022, 3997190. doi:10.1155/2022/3997190

Wang, J. L., Cai, F., Liu, X. H., Li, L. M., He, X., Hu, X. M., et al. (2020). Lipopolysaccharide promotes inflammatory response via enhancing IFIT1 expression in human umbilical vein endothelial cells. *DNA Cell. Biol.* 39 (7), 1274–1281. doi:10.1089/dna.2020.5454

Wu, C., Yan, J., and Li, W. (2022). Acacetin as a potential protective compound against cardiovascular diseases. *Evidence-based complementary Altern. Med. eCAM* 2022, 6265198. doi:10.1155/2022/6265198

Wu, Y., Song, F., Li, Y., Li, J., Cui, Y., Hong, Y., et al. (2021). Acacetin exerts antioxidant potential against atherosclerosis through Nrf2 pathway in apoE-/- Mice. *J. Cell. Mol. Med.* 25 (1), 521–534. doi:10.1111/jcmm.16106

Xia, F., Wang, C., Jin, Y., Liu, Q., Meng, Q., Liu, K., et al. (2014). Luteolin protects HUVECs from TNF-α-induced oxidative stress and inflammation via its effects on the Nox4/ROS-NF-κB and MAPK pathways. *J. Atheroscler. thrombosis* 21 (8), 768–783. doi:10.5551/jat.23697

Xie, Y., Guo, Y., Cao, S., Xue, M., Fan, Z., Gao, C., et al. (2020). Hydroxysafflor yellow A attenuates hydrogen peroxide-induced oxidative damage on human umbilical vein endothelial cells. *Evidence-based complementary Altern. Med. eCAM* 2020, 8214128. doi:10.1155/2020/8214128

Xu, S., Ilyas, I., Little, P. J., Li, H., Kamato, D., Zheng, X., et al. (2021). Endothelial dysfunction in atherosclerotic cardiovascular diseases and beyond: From mechanism to pharmacotherapies. *Pharmacol. Rev.* 73 (3), 924–967. doi:10.1124/pharmrev.120.000096

Xue, X., Deng, Y., Wang, J., Zhou, M., Liao, L., Wang, C., et al. (2021). Hydroxysafflor yellow A, a natural compound from Carthamus tinctorius L with good effect of alleviating atherosclerosis. *Phytomedicine Int. J. phytotherapy Phytopharm.* 91, 153694. doi:10.1016/j.phymed.2021.153694

Yamagata, K. (2019). Polyphenols regulate endothelial functions and reduce the risk of cardiovascular disease. *Curr. Pharm. Des.* 25 (22), 2443–2458. doi:10.2174/1381612825666190722100504

Yamaoka-Tojo, M. (2017). Endothelial function for cardiovascular disease prevention and management. *Int. J. Clin. Cardiol.* 4 (3), 1–11. doi:10.23937/2378-2951/1410103

Ye, F., Wang, J., Meng, W., Qian, J., and Jin, M. (2017). Proteomic investigation of effects of hydroxysafflor yellow A in oxidized low-density lipoprotein-induced endothelial injury. *Sci. Rep.* 7 (1), 17981. doi:10.1038/s41598-017-18069-4

Yi, L., Chen, C. Y., Jin, X., Zhang, T., Zhou, Y., Zhang, Q. Y., et al. (2012). Differential suppression of intracellular reactive oxygen species-mediated signaling pathway in vascular endothelial cells by several subclasses of flavonoids. *Biochimie* 94 (9), 2035–2044. doi:10.1016/j.biochi.2012.05.027

Yoshimoto, R., Fujita, Y., Kakino, A., Iwamoto, S., Takaya, T., and Sawamura, T. (2011). The discovery of LOX-1, its ligands and clinical significance. *Cardiovasc. drugs Ther.* 25 (5), 379–391. doi:10.1007/s10557-011-6324-6

Yu, K., Li, X. M., Xu, X. L., Zhang, R. Y., and Cong, H. L. (2015). Eupatilin protects against tumor necrosis factor-α-mediated inflammation inhuman umbilical vein endothelial cells. *Int. J. Clin. Exp. Med.* 8 (12), 22191–22197.

Yuan, Y., Fang, M., Wu, C. Y., and Ling, E. A. (2016). Scutellarin as a potential therapeutic agent for microglia-mediated neuroinflammation in cerebral ischemia. *Neuromolecular Med.* 18 (3), 264–273. doi:10.1007/s12017-016-8394-x

Yusuf, S., Hawken, S., Ounpuu, S., Dans, T., Avezum, A., Lanas, F., et al.INTERHEART Study Investigators (2004). Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART

study): Case-control study. *Lancet (London, Engl.* 364 (9438), 937–952. doi:10.1016/S0140-6736(04)17018-9

Zeng, Y., Xu, J., Hua, Y. Q., Peng, Y., and Xu, X. L. (2020). MDM2 contributes to oxidized low-density lipoprotein-induced inflammation through modulation of mitochondrial damage in endothelial cells. *Atherosclerosis* 305, 1–9. doi:10.1016/j.atherosclerosis.2020.05.020

Zhang, H., Fan, L. J., Liu, J., Zhu, J. Q., Tan, T. T., Li, M., et al. (2022a). Safflor yellow A protects vascular endothelial cells from ox-LDL-mediated damage. *J. Recept. signal Transduct. Res.* 42 (1), 52–59. doi:10.1080/10799893.2020.1843492

Zhang, H., Zhao, Z., Pang, X., Yang, J., Yu, H., Zhang, Y., et al. (2017). MiR-34a/sirtuin-1/foxo3a is involved in genistein protecting against ox-LDL-induced oxidative damage in HUVECs. *Toxicol. Lett.* 277, 115–122. doi:10.1016/j.toxlet.2017.07.216

Zhang, L., Huang, J., Zhang, D., Lei, X., Ma, Y., Cao, Y., et al. (2022b). Targeting reactive oxygen species in atherosclerosis via Chinese herbal medicines. *Oxidative Med. Cell. Longev.* 2022, 1852330. doi:10.1155/2022/1852330

Zhi, W., Liu, Y., Wang, X., and Zhang, H. (2023). Recent advances of traditional Chinese medicine for the prevention and treatment of atherosclerosis. *J. Ethnopharmacol.* 301, 115749. doi:10.1016/j.jep.2022.115749

Zhong, S., Li, L., Shen, X., Li, Q., Xu, W., Wang, X., et al. (2019). An update on lipid oxidation and inflammation in cardiovascular diseases. *Free Radic. Biol. Med.* 144, 266–278. doi:10.1016/j.freeradbiomed.2019.03.036

frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 21 March 2023
DOI 10.3389/fphar.2023.1064567

OPEN ACCESS

EDITED BY
Cailu Lin,
Monell Chemical Senses Center,
United States

REVIEWED BY
Jorge Machado-Alba,
Technological University of Pereira,
Colombia
Tangjie Zhang,
Yangzhou University, China

*CORRESPONDENCE
Qiang Wang,
wangqiang130@scu.edu.cn

SPECIALTY SECTION
This article was submitted to Ethnopharmacology, a section of the journal Frontiers in Pharmacology

RECEIVED 08 October 2022
ACCEPTED 28 February 2023
PUBLISHED 21 March 2023

CITATION
Dai M, Furuya-Kanamori L, Syed A, Lin L and Wang Q (2023), An empirical comparison of the harmful effects for randomized controlled trials and non-randomized studies of interventions. *Front. Pharmacol.* 14:1064567. doi: 10.3389/fphar.2023.1064567

An empirical comparison of the harmful effects for randomized controlled trials and non-randomized studies of interventions

Minhan Dai[1], Luis Furuya-Kanamori[2], Asma Syed[3], Lifeng Lin[4] and Qiang Wang[1]*

[1]Mental Health Center, West China Hospital of Sichuan University, Chengdu, China, [2]School of Public Health, Faculty of Medicine, The University of Queensland, Herston, QL, Australia, [3]Department of Population Medicine, College of Medicine, Qatar University, Doha, Qatar, [4]Department of Epidemiology and Biostatistics, University of Arizona, Tucson, AZ, United States

Introduction: Randomized controlled trials (RCTs) are the gold standard to evaluate the efficacy of interventions (e.g., drugs and vaccines), yet the sample size of RCTs is often limited for safety assessment. Non-randomized studies of interventions (NRSIs) had been proposed as an important alternative source for safety assessment. In this study, we aimed to investigate whether there is any difference between RCTs and NRSIs in the evaluation of adverse events.

Methods: We used the dataset of systematic reviews with at least one meta-analysis including both RCTs and NRSIs and collected the 2 × 2 table information (i.e., numbers of cases and sample sizes in intervention and control groups) of each study in the meta-analysis. We matched RCTs and NRSIs by their sample sizes (ratio: 0.85/1 to 1/0.85) within a meta-analysis. We estimated the ratio of the odds ratios (RORs) of an NRSI against an RCT in each pair and used the inverse variance as the weight to combine the natural logarithm of ROR (lnROR).

Results: We included systematic reviews with 178 meta analyses, from which we confirmed 119 pairs of RCTs and NRSIs. The pooled ROR of NRSIs compared to that of RCTs was estimated to be 0.96 (95% confidence interval: 0.87 and 1.07). Similar results were obtained with different sample size subgroups and treatment subgroups. With the increase in sample size, the difference in ROR between RCTs and NRSIs decreased, although not significantly.

Discussion: There was no substantial difference in the effects between RCTs and NRSIs in safety assessment when they have similar sample sizes. Evidence from NRSIs might be considered a supplement to RCTs for safety assessment.

KEYWORDS

randomized controlled trial, non-randomized studies of intervention, adverse events, harmful effect, empirical comparison

1 Introduction

Randomized controlled trials (RCTs) are considered the most unbiased study design and represent the current gold standard for assessment of efficacy of interventions (Guyatt et al., 2008). Through the randomization process, RCTs would mostly avoid the bias of confounding factors by indicating the intervention effect (Shrier et al., 2007). However, RCTs are expensive, and thus most RCTs only cover a small number of patients with a short follow-up period (Van Spall et al., 2007; Kennedy-Martin et al., 2015). In addition, sample size estimates for RCTs are usually based on the main outcome, that is, efficacy, rather than adverse events. This makes it challenging to assess safety outcomes since many outcomes occur at a low frequency—the observed events would be rare and even zero for certain outcomes. Therefore, statistical inference faces significant uncertainty caused by random errors (Bhaumik et al., 2012; Efthimiou, 2018). In addition, recruiting subjects usually involves strict inclusion criteria, and researchers tend to exclude high-risk patients, such as children, elderly people, pregnant women, patients with multiple complications, and those with potential drug interactions. These restrictions limit the representativeness of the findings of RCTs (Chou and Helfand, 2005; Golder et al., 2011).

Non-randomized studies of interventions (NRSIs) are an alternative to overcome the aforementioned issues for assessing safety. It is widely known that a case-control study is designed for when the cases of events are rare (Vandenbroucke and Pearce, 2012). There are two sources of error that could impact the estimates of NRSIs, namely, systematic error (bias) and random error. For effectiveness of intervention, the bias of NRSIs is deemed to be the main effect modifier on the results, and the random error may have limited impacts due to the large sample size and sufficient outcomes (Higgins et al., 2011). Methods such as stratification, matching, and regression analysis have been proposed to address the confounding bias for NRSIs (McNamee, 2005; Austin, 2011). Simulation studies have verified that these methods work well to control the impact of confounders on the effects (Jreich and Sebastien, 2021). However, for rare adverse events, such methods may not be feasible due to the limited number of cases. For example, when the event risk is 1/1000, even for an NRSI with a sample size of 2000, the expected number of cases would only be two, which is insufficient for the aforementioned methods. In such a case, in safety assessment, the random error may have a larger impact than the systematic error (bias), which dominates the results.

One increasingly popular method was to pool all available RCTs of the same topic together, i.e., *via* a meta-analysis, to increase the statistical power, and it has the ability to increase the power in testing whether the true effect actually exists. Nevertheless, the statistical power of these meta-analyses was still seriously insufficient (Jia et al., 2021). Researchers then proposed to include NRSIs in the meta-analysis because, for safety outcomes, the primary aim is to capture any signal of harm (Reeves et al., 2013; Valentine and Thompson, 2013). This is somewhat reasonable as we mentioned previously that for safety outcomes of rare events, systematic error may have a limited impact on the results. Even so, this has raised wide controversy as the concerns about the confounding bias still exist for NRSIs and will be synthesized into the pooled effect (Benson and Hartz, 2000; Concato et al., 2000; Ioannidis et al., 2001; Abraham et al., 2010; Hemkens et al., 2016; Soni et al., 2019).

To address this concern, we designed an empirical study based on a database of systematic reviews of safety that compared the effects of RCTs and NRSIs to see whether there was any difference in the evaluation of adverse events between them.

2 Materials and methods

The current study findings are reported according to the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) checklist for case-control studies (von Elm et al., 2008). A brief description of the study is as follows. First, we searched for the published systematic reviews of safety and screened for those with safety as exclusive outcomes. Then, we checked the eligible systematic review for those including both RCTs and NRSIs in the meta-analyses. The RCTs and NRSIs were further matched by sample size (1:1) within each meta-analysis. Finally, the effects of each pair of RCT and NRSI were compared.

2.1 Sample size estimation

To ensure a sufficient sample size (pairs) for the statistical test, we used the following formula to estimate the minimum sample size for the current study: $n = (z_{\alpha/2} \times d/E)^2$ (Donner, 1984). Here, E indicates the margin of error and d represents the expected standard deviation of the difference of the effects (i.e., ln odds ratio, lnOR) across the pairs. For the margin of error, it is a concept similar to the bias in a simulation study, namely, how close the estimated effect is to the true effect (Donner, 1984). For the standard deviation, it is a concept similar to the between-study heterogeneity in a meta-analysis (Pateras et al., 2018). Therefore, we took 25% as the tolerable margin of error and 1 as standard deviation, indicating that there would be substantial-to-large heterogeneity across pairs (Ju et al., 2020; Xu et al., 2021a). Based on these parameters, the estimated sample size of the current study is 96.04; that is, we need at least 97 pairs of RCTs and NRSIs to ensure the statistical power to test whether the difference of the effects across the pairs was significant.

2.2 Data source

We used a dataset collected in 2020, which was primarily established to improve the evidence-based practice for safety assessment and has been documented elsewhere (Xu et al., 2021b). The dataset consists of 640 systematic reviews of healthcare interventions published in two time periods (2008–2011 and 2015–2020), with adverse events as exclusive outcomes and at least one meta-analysis. The two different periods were primarily designed for comparing how double-zero studies were dealt with by systematic review authors over time (Xu et al., 2021b). For each time period, a comprehensive literature search was performed to ensure the representativeness of the sample (systematic reviews of safety). A detailed description of the dataset can be found in our previous works (Zorzela et al., 2014; Xu et al., 2021b).

2.3 Eligibility criteria

We screened 640 systematic reviews for those with at least one outcome (each outcome referred to a separate meta-analysis) that included both RCTs and NRSIs in order to compare the effects of NRSI *vs.* RCT. In addition, considering that data extraction error is commonly seen in published meta-analyses, we only considered those providing summarized 2 x 2 table data for each study in the meta-analysis; a further double-checking process for such data through original studies is possible. Based on the same consideration, those reviews directly reporting the effect size (e.g., OR) and standard error for the meta-analysis were not considered; for such systematic reviews, it is impossible to check whether the effect sizes they used were correctly estimated or extracted, especially for NRSIs. We collected RCTs and NRSIs in systematic reviews under the condition that each pair of the RCT and NRSI has the same topic. Thus, the potential impact of different topics on the results was eliminated. In addition, only pairwise meta-analyses were considered to ensure the interventions were homogeneous.

2.4 Data collection

The meta-analytic data of each outcome from each eligible systematic review were extracted by two review authors independently. Any disagreements were solved by discussing with the lead author. These include the 2 x 2 table information (i.e., numbers of cases and sample sizes in intervention and control groups) of each study in the meta-analysis, type of design of each study (i.e., RCT or NRSI), first author of the systematic reviews, and first author and year of publication of included studies. During data extraction, any disagreements were solved by discussion. The primary data were collected from the systematic reviews, and to ensure the quality of the data, we further double-checked the data of matched pairs from the original studies included in the corresponding systematic reviews.

2.5 Data analysis

Previous studies pooled the effects of NRSIs and RCTs by treating them as subgroups in a meta-analysis and compared the pooled effects across each meta-analysis (Mathes et al., 2021). However, this method has a big disadvantage in that it requires a sufficient number of studies (i.e., 10) in each subgroup to ensure the robustness of the pooled effects. Under such a limitation, there would be very few meta-analyses that would meet the requirement and may further impact the generalizability of the findings.

In the current study, in order to compare the potential difference of the effects, we matched RCTs and NRSIs within the same meta-analysis by their sample sizes to control the impact of random error on the effects. In brief, we first calculated the sample size of each study in each meta-analysis and ranked the sample sizes within the meta-analysis. Then, those RCTs and NRSIs with similar sample sizes were matched as a pair, using the "nearest neighbor matching" method (Austin, 2011). To ensure the matched RCT and NRIS have almost the same sample size, we calculated the ratio of their sample size; only those with a ratio from 0.85/1 to 1/0.85 were considered to avoid the potential influence of sample size on the results (Xu et al., 2021c).

In each pair, the OR and its standard error of the RCTs and NRSIs were estimated as it has been considered one of the optimal effect estimators (Doi et al., 2020; Doi et al., 2021). For those studies with zero events in single or double groups, the continuity correction was applied by adding 0.5 to each cell to produce an approximate evaluation of the OR and its standard error (Xu et al., 2021d). Furthermore, the ratio of the ORs (ROR) of NRSI against RCT was calculated to reflect the deviation of the effects; the ROR is the primary outcome of the current study (Dechartres et al., 2018). This statistics allows us to further test whether there is a difference in the effect of RCTs and NRSIs. When the weighted mean value of the ROR across the pairs is 1, there would be no difference between the effect of RCTs and NRSIs. In order to obtain the weighted mean value of the ROR, we calculated the natural logarithm of ROR (lnROR) and its standard error and then used the inverse variance heterogeneous model to combine these lnRORs (Doi et al., 2015; Doi and Furuya-Kanamori, 2020). The standard error of the lnROR of each pair can be estimated using the SEs for the RCT and NRSI estimates (Golder et al., 2011).

$$SE(\ln ROR) = \sqrt{(SE \ln OR_{rct})^2 + (SE \ln OR_{nrsi})^2}.$$

The pooled effect is the weighted mean value. A statistical null hypothesis would be then the pooled lnROR = 0. We used the two-sided t -test with the significant level of alpha = 0.05. Sensitivity analysis was employed by cluster robust error meta-regression to consider the potential correlation of lnRORs for the pairs within each systematic review (Xu and Doi, 2018). Further subgroup analysis by the maximum sample size of each pair was employed to see if the potential difference of the effects varies by sample size. The following five groups were prespecified: 1–50, 51–100, 101–200, 201–500, and >501. Statistical analyses were conducted in MetaXL 5.3 software (EpiGear International, Australia) and Stata 14/SE (Stata, College Station, TX).

3 Results

3.1 Basic characteristics

Of the 640 systematic reviews of adverse events, 87 included both RCTs and NRSIs. We further excluded 12 with the NRSIs only used for incidence of adverse events or did not include both RCTs and NRSIs within a meta-analysis. Of the remaining 75 systematic reviews, 31 were eligible, which had at least one outcome, contained both RCTs and NRSIs, and provided summarized 2 x 2 table data for each study in the meta-analysis (Grootscholten et al., 2008; Sun et al., 2008; Torloni et al., 2009; Touzé et al., 2009; Slobogean et al., 2010; Yaghoobi et al., 2010; Aires et al., 2015; Geng et al., 2015; Ghayoumi et al., 2015; Inokuchi et al., 2015; Wang et al., 2015; Yoon et al., 2015; Zhang and Ma, 2015; Keir et al., 2016; Peng et al., 2016; Vavken et al., 2016; Balasubramanian et al., 2017; Geminiani et al., 2017; Pecorelli et al., 2017; Cheng et al., 2018; Shah et al., 2018; Zhao et al., 2018; Ceresoli et al., 2019; Craveiro et al., 2019; Jiang et al., 2019; Menne et al., 2019; Nagy et al., 2019; Shah et al., 2019; Vaos et al., 2019; Winberg et al., 2019; Yang et al., 2019). The selection process is reported in Supplementary Figure S1, and the characteristics of the included systematic reviews are shown in Supplementary Table S1.

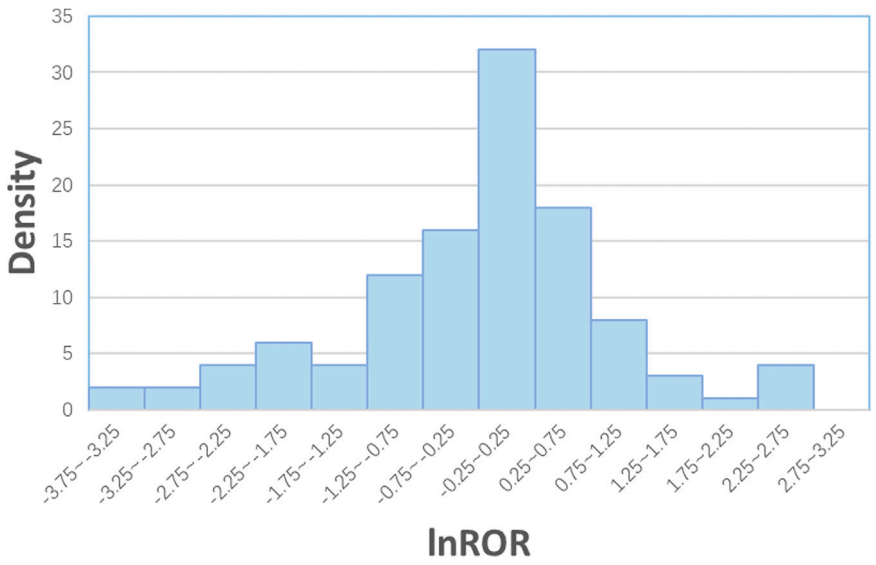


FIGURE 1
Distribution of lnRORs.

From the 31 systematic reviews, 178 meta-analyses contained both RCTs and NRSIs with a total of 1,404 studies. 119 pairs of RCTs and NRSIs were successfully matched for the analysis (Supplementary Figure S1). In a further analysis of the 238 studies from 119 pairs, we recorded two (0.84%) had data extraction errors, which were further addressed by correcting these errors. The sample size of the current study is bigger than the minimum requirement (*Sample size estimation*). Among these 119 pairs, there were 19 (15.97%) with the sample size ranging from 1 to 50, 41 (34.45%) pairs ranging from 51 to 100, 19 (15.97%) ranging from 101–200, 17 (14.29%) ranging from 201–500, and 23 (19.33%) with the sample size >500.

3.2 RCTs *vs.* NRSIs on the effects

Figure 1 shows the distribution of the lnRORs, which has an approximately normal distribution (p = 0.446 for skewness and p = 0.13 for kurtosis). The unweighted mean value of the lnROR was −0.14 with a standard deviation of 1.23, and the single-sample t -test showed no substantial difference of lnROR over zero (t = − 1.25, p = 0.21).

Supplementary Figure S2 shows the forest plot of the weighted average lnRORs. Again, no difference was observed between the effects of NRSIs against RCTs. The pooled ROR across the 119 pairs was 0.96 (95% confidence interval [CI]: 0.87, 1.07; p = 0.49), with no obvious between-study heterogeneity (I^2 = 0%). A robust meta-regression model that considers the correlation between the pairs within a systematic review showed a similar result, with the pooled ROR as 0.96 (95% CI: 0.90, 1.03; p = 0.27).

3.3 Subgroup analysis

Similar conclusions were obtained from the analysis of different sample size subgroups. There was no significant difference between the weighted mean value of lnROR and 0 in each subgroup, that is, there was no significant difference in the effects between RCTs and NRSIs, regardless of sample size. The forest plots of the subgroup analyses are shown in Figure 2. However, there was a slight difference in the absolute value of the weighted mean of lnROR for each sample size subgroup, which decreased lnROR with increasing sample size (Figure 3). With the increase in sample size, the difference between RCTs and NRSIs diminished.

In addition, the treatment used in the original study had no significant effect on the results. We compared the weighted mean of lnROR in the treatment subgroup, and the results of either surgical treatment or drug therapy were close to 0, and there was no significant difference (Supplementary Figure S3).

4 Discussion

In this study, we compared the effects of RCTs and NRSIs on safety assessment based on empirical evidence. Our results showed that there was no significant difference between RCTs and NRSIs in the evaluation of adverse events of the same topic, and there was no significant difference in sample size or treatment subgroups.

In our research, although different sample size subgroups yielded similar results, there was still a slight difference in the weighted average RORs of different sample size subgroups. As shown in Figure 3, with the increase in the sample size, the value of lnROR decreases gradually; that is, the difference between RCTs and NRSIs gradually decreases. This is likely because the random error decreased as the sample size increased, and the estimated effect is therefore closer to the true effect (i.e., lnROR = 0) (Moher et al., 1994; Wang and Ji, 2020). This also indicates that small studies may lead to biased estimation of the effects and should be addressed and interpreted appropriately in further original studies as well as meta-analyses.

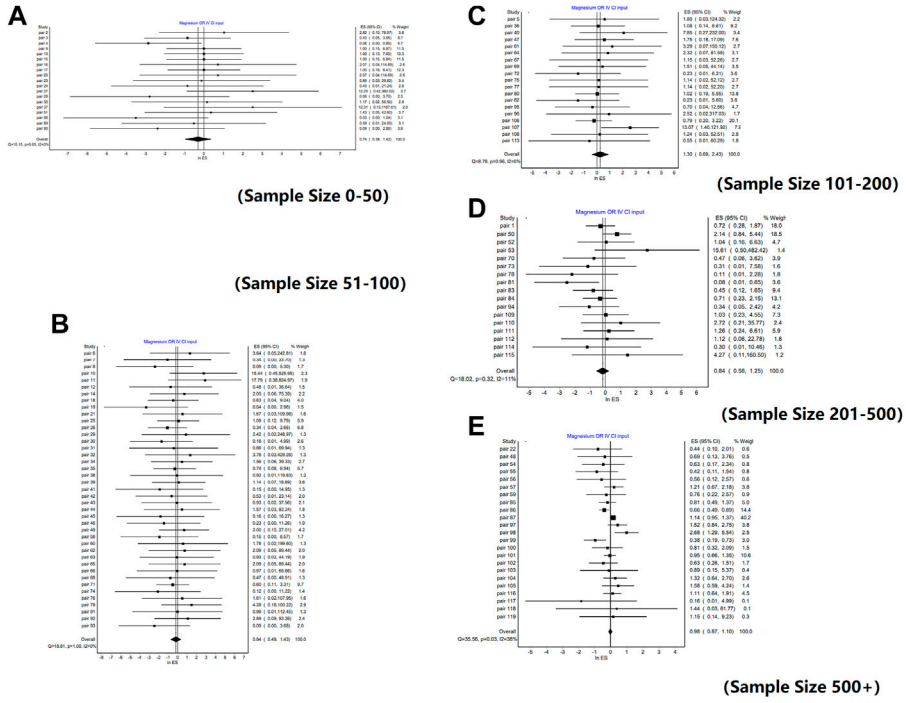


FIGURE 2
Forest plots of lnROR by sample size. [**(A)** Forest plot of lnROR for pairs with sample sizes between 0 and 50; **(B)** Forest plot of lnROR for pairs with sample sizes between 51 and 100; **(C)** Forest plot of lnROR for pairs with sample sizes between 101 and 200; **(D)** Forest plot of lnROR for pairs with sample sizes between 201 and 500; **(E)** Forest plot of lnROR for pairs with sample sizes, ore than 500].

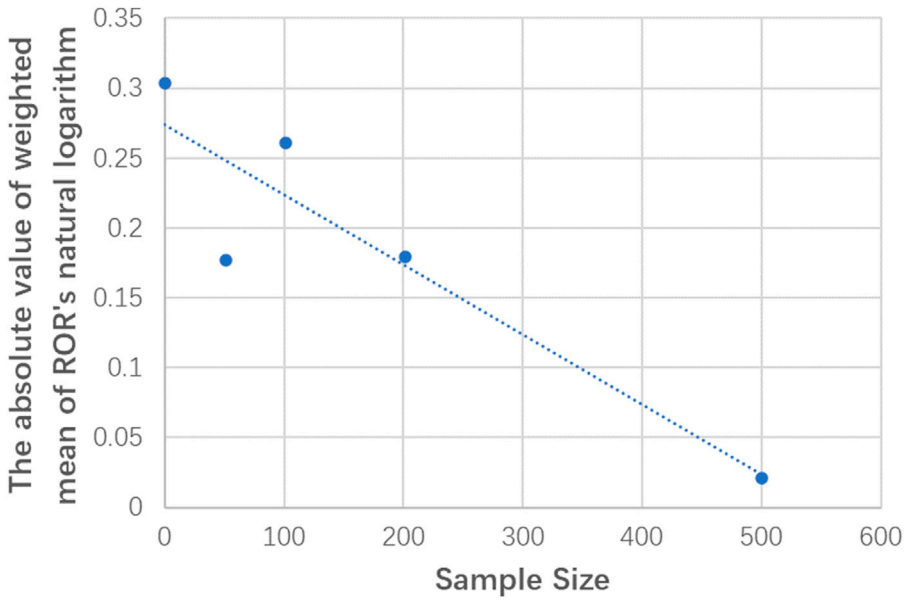


FIGURE 3
Scatter plot between the sample size and the absolute value of the weighted mean lnRORs.

Several previous studies have systematically evaluated the differences in the effects of adverse events between RCTs and NRSIs. One study included 19 systematic reviews, and the pooled ROR of RCTs compared to observational studies was estimated to be 1.03 (95% confidence interval 0.93–1.15) (Golder et al., 2011). The other two studies showed similar results (Grodstein et al., 2003; Edwards et al., 2012). These results are similar to our results and further confirm that there is no difference in the average risk

estimates of intervention adverse events between RCTs and NRSIs. One possible explanation for the findings is that for safety outcomes, the events are rare, and the sample sizes are also limited, which makes the random error the predominant error impact the effect over the systematic errors (e.g., error from confounding), and therefore under the same sample size with almost the same amount of random error, the effects are similar for RCTs and NRSIs.

However, some minor differences in the effects were observed. A study of postmenopausal hormone therapy on breast cancer survivors found that the results of observational studies were inconsistent with those of randomized trials (Col et al., 2005). This may be due to inconsistencies among the study population that they excluded people with a high incidence of adverse events. In Papanikolaou et al. (2006) study, the authors compared risks of 13 major harms of medical interventions using data from both RCTs and observational studies, and the non-randomized studies were often more conservative in their estimates of risk than the randomized trials. The study attributed these differences to the higher rate of adverse reactions reported by the RCTs because adverse events are recorded more thoroughly in RCTs, owing to regulatory requirements. It may also be caused by the different study populations. Further research on measuring the amount of random error and systematic error on NRSIs for rare events could be useful for the community to better understand the mechanism and deserves more attention.

4.1 Strengths and limitations

To the best of our knowledge, our study is currently the largest empirical study that compared the difference of the effects between RCTs and NRSIs for safety outcomes. The sample is representative, and the findings could provide indications for further evidence-based practice for assessing adverse events. In addition, we attempted to source the primary studies contained in each meta-analysis. This can avoid the errors that may exist in the extraction of data by the authors of meta-analyses. Moreover, we matched RCTs or NRSIs with the same outcome in the same systematic review according to their sample sizes, which can avoid the influence of different sample sizes on the results.

The current study has several limitations. First, we did not analyze and evaluate the bias of the included systematic review and possible confounding factors in the original study, such as drug dose, treatment duration, or study population. These confounding factors may affect the outcome of adverse events. In addition, even for the same adverse event, there are differences in how these events were defined or recorded, especially in composite outcomes. The absence of such methodological information increases the potential heterogeneity of the results and even biases the conclusion. Therefore, in the original study, detailed information on outcome collection should be sufficiently provided. Second, selection bias may occur in the current study. It has been estimated that only about 43% of the published studies reported adverse events, while the proportion is 88% in unpublished studies (Golder et al., 2016). This means in the current study, the studies included were those with better reporting on safety outcomes; thus, our results may not be representative of those with poor reporting. Third, we used the matching method for comparison; during the matching process, only 17% were matched among 1,405 studies from the 178 meta-analyses. This means the majority of RCTs and NRSIs have different sample sizes, and therefore whether the effects of them were similar or not is unclear. This is hard to be estimated as the sample size itself is a source of bias. In addition, systematic reviews of adverse events potentially have serious issues in data extraction, and these errors can mislead the conclusions (Xu et al., 2022). Even if data extraction is checked and corrected in this study, there may still be some errors. Further studies are warranted to address these issues.

5 Conclusion

In conclusion, the current study identified that there was no significant difference between RCTs and NRSIs in the evaluation of the effect of adverse events for the same topic when they have similar sample sizes. It is of great significance to the systematic reviews of adverse events that well-conducted NRSIs may provide valid results, which is similar to RCTs. Evidence from NRSIs might be considered a supplement to RCTs to improve the generalizability and comprehensiveness of the review.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

MD conceived and designed the study; MD analyzed the data and drafted the manuscript; AS collected the data, assessed the methodological quality, and edited the manuscript; LF-K, QW, and LL screened the literature; LL and LF-K provided methodological comments and revised the manuscript. All authors approved the final version for publication.

Funding

This study was supported by the Chinese National Programs for Brain Science and Brain-like Intelligence Technology, China Depression Cohort Study (2021ZD0200700) and grants for Key Project 82171499 from the National Natural Science Foundation of China. LF-K is funded by an Australian National Health and Medical Research Council Fellowship (APP1158469). The funding body had no role in any process of the study (i.e., study design, statistical analysis, and result reporting).

Acknowledgments

The authors would like to thank Dr. Chang Xu for his effort in the conception, design, and sharing of data for this study. Dr. Chang Xu provided helpful and constructive comments that improved the manuscript substantially.

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.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1064567/full#supplementary-material

References

Abraham, N., Byrne, C., Young, J., and Solomon, M. (2010). Meta-analysis of well-designed nonrandomized comparative studies of surgical procedures is as good as randomized controlled trials. *J. Clin. Epidemiol.* 63 (3), 238–245. doi:10.1016/j.jclinepi.2009.04.005

Aires, F. T., Dedivitis, R. A., Petrarolha, S. M., Bernardo, W. M., Cernea, C. R., and Brandão, L. G. (2015). Early oral feeding after total laryngectomy: A systematic review. *Head neck* 37 (10), 1532–1535. doi:10.1002/hed.23755

Austin, P. (2011). An introduction to propensity score methods for reducing the effects of confounding in observational studies. *Multivar. Behav. Res.* 46 (3), 399–424. doi:10.1080/00273171.2011.568786

Balasubramanian, I., Fleming, C., Mohan, H. M., Schmidt, K., Haglind, E., and Winter, D. C. (2017). Out-patient management of mild or uncomplicated diverticulitis: A systematic review. *Dig. Surg.* 34 (2), 151–160. doi:10.1159/000450865

Benson, K., and Hartz, A. (2000). A comparison of observational studies and randomized, controlled trials. *N. Engl. J. Med.* 342 (25), 1878–1886. doi:10.1056/NEJM200006223422506

Bhaumik, D., Amatya, A., Normand, S., Greenhouse, J., Kaizar, E., Neelon, B., et al. (2012). Meta-analysis of rare binary adverse event data. *J. Am. Stat. Assoc.* 107 (498), 555–567. doi:10.1080/01621459.2012.664484

Ceresoli, M., Tamini, N., Gianotti, L., Braga, M., and Nespoli, L. (2019). Are endoscopic loop ties safe even in complicated acute appendicitis? A systematic review and meta-analysis. *Int. J. Surg. Lond. Engl.* 68, 40–47. doi:10.1016/j.ijsu.2019.06.011

Cheng, D., Gao, H., and Li, W. (2018). Long-term risk of rosiglitazone on cardiovascular events - a systematic review and meta-analysis. *Endokrynol. Pol.* 69 (4), 381–394. doi:10.5603/EP.a2018.0036

Chou, R., and Helfand, M. (2005). Challenges in systematic reviews that assess treatment harms. *Ann. Intern. Med.* 142, 1090–1099. doi:10.7326/0003-4819-142-12_part_2-200506211-00009

Col, N., Kim, J., and Chlebowski, R. (2005). Menopausal hormone therapy after breast cancer: A meta-analysis and critical appraisal of the evidence. *Breast cancer Res. BCR* 7 (4), R535–R540. doi:10.1186/bcr1035

Concato, J., Shah, N., and Horwitz, R. (2000). Randomized, controlled trials, observational studies, and the hierarchy of research designs. *N. Engl. J. Med.* 342 (25), 1887–1892. doi:10.1056/NEJM200006223422507

Craveiro, N. S., Silva Lopes, B., Tomás, L., Fraga Almeida, S., Palma, H., Afreixo, V., et al. (2019). L-TRUST: Long-term risk of cancer in patients under statins therapy. A systematic review and meta-analysis. *Pharmacoepidemiol. drug Saf.* 28 (11), 1431–1439. doi:10.1002/pds.4895

Dechartres, A., Atal, I., Riveros, C., Meerpohl, J., and Ravaud, P. (2018). Association between publication characteristics and treatment effect estimates: A meta-epidemiologic study. *Ann. Intern. Med.* 169 (6), 385–393. doi:10.7326/M18-1517

Doi, S., Barendregt, J., Khan, S., Thalib, L., and Williams, G. (2015). Advances in the meta-analysis of heterogeneous clinical trials I: The inverse variance heterogeneity model. *Contemp. Clin. trials* 45, 130–138. doi:10.1016/j.cct.2015.05.009

Doi, S., and Furuya-Kanamori, L. (2020). Selecting the best meta-analytic estimator for evidence-based practice: A simulation study. *Int. J. evidence-based Healthc.* 18 (1), 86–94. doi:10.1097/XEB.0000000000000207

Doi, S., Furuya-Kanamori, L., Xu, C., Chivese, T., Lin, L., Musa, O., et al. (2021). The OR is "portable" but not the RR: Time to do away with the log link in binomial regression. *J. Clin. Epidemiol.* 142, 288–293. doi:10.1016/j.jclinepi.2021.08.003

Doi, S., Furuya-Kanamori, L., Xu, C., Lin, L., Chivese, T., and Thalib, L. (2020). Questionable utility of the relative risk in clinical research: A call for change to practice. *J. Clin. Epidemiol.* 142, 271–279. doi:10.1016/j.jclinepi.2020.08.019

Donner, A. (1984). Approaches to sample size estimation in the design of clinical trials--a review. *Statistics Med.* 3 (3), 199–214. doi:10.1002/sim.4780030302

Edwards, J., Kelly, E., Lin, Y., Lenders, T., Ghali, W., and Graham, A. (2012). Meta-analytic comparison of randomized and nonrandomized studies of breast cancer surgery. *Can. J. Surg. J. Can. de Chir.* 55 (3), 155–162. doi:10.1503/cjs.023410

Efthimiou, O. (2018). Practical guide to the meta-analysis of rare events. *Evidence-based Ment. health* 21 (2), 72–76. doi:10.1136/eb-2018-102911

Geminiani, A., Tsigarida, A., Chochlidakis, K., Papaspyridakos, P. V., Feng, C., and Ercoli, C. A meta-analysis of complications during sinus augmentation procedure. *Quintessence Int.* 2017;48(3):231–2. doi:10.3290/j.qi.a37644

Geng, H. Z., Nasier, D., Liu, B., Gao, H., and Xu, Y. K. (2015). Meta-analysis of elective surgical complications related to defunctioning loop ileostomy compared with loop colostomy after low anterior resection for rectal carcinoma. *Ann. R. Coll. Surg. Engl.* 97 (7), 494–501. doi:10.1308/003588415X14181254789240

Ghayoumi, P., Kandemir, U., and Morshed, S. (2015). Evidence based update: Open versus closed reduction. *Injury* 46 (3), 467–473. doi:10.1016/j.injury.2014.10.011

Golder, S., Loke, Y., and Bland, M. (2011). Meta-analyses of adverse effects data derived from randomised controlled trials as compared to observational studies: Methodological overview. *PLoS Med.* 8 (5), e1001026. doi:10.1371/journal.pmed.1001026

Golder, S., Loke, Y. K., Wright, K., and Norman, G. (2016). Reporting of adverse events in published and unpublished studies of Health care interventions: A systematic review. *PLoS Med.* 13 (9), e1002127. doi:10.1371/journal.pmed.1002127

Grodstein, F., Clarkson, T., and Manson, J. (2003). Understanding the divergent data on postmenopausal hormone therapy. *N. Engl. J. Med.* 348 (7), 645–650. doi:10.1056/NEJMsb022365

Grootscholten, K., Kok, M., Oei, S. G., Mol, B. W., and van der Post, J. A. (2008). External cephalic version-related risks: A meta-analysis. *Obstetrics Gynecol.* 112 (5), 1143–1151. doi:10.1097/AOG.0b013e31818b4ade

Guyatt, G., Oxman, A., Vist, G., Kunz, R., Falck-Ytter, Y., Alonso-Coello, P., et al. (2008). Grade: An emerging consensus on rating quality of evidence and strength of recommendations. *BMJ Clin. Res. ed)* 336 (7650), 924–926. doi:10.1136/bmj.39489.470347.AD

Hemkens, L., Contopoulos-Ioannidis, D., and Ioannidis, J. (2016). Agreement of treatment effects for mortality from routinely collected data and subsequent randomized trials: meta-epidemiological survey. *BMJ Clin. Res. ed)* 352, i493. doi:10.1136/bmj.i493

Higgins, J., Altman, D., Gøtzsche, P., Jüni, P., Moher, D., Oxman, A., et al. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ Clin. Res. ed)* 343, d5928. doi:10.1136/bmj.d5928

Inokuchi, M., Sugita, H., Otsuki, S., Sato, Y., Nakagawa, M., and Kojima, K. (2015). Laparoscopic distal gastrectomy reduced surgical site infection as compared with open distal gastrectomy for gastric cancer in a meta-analysis of both randomized controlled and case-controlled studies. *Int. J. Surg. Lond. Engl.* 15, 61–67. doi:10.1016/j.ijsu.2015.01.030

Ioannidis, J., Haidich, A., Pappa, M., Pantazis, N., Kokori, S., Tektonidou, M., et al. (2001). Comparison of evidence of treatment effects in randomized and nonrandomized studies. *JAMA* 286 (7), 821–830. doi:10.1001/jama.286.7.821

Jia, P., Lin, L., Kwong, J., and Xu, C. (2021). Many meta-analyses of rare events in the Cochrane Database of Systematic Reviews were underpowered. *J. Clin. Epidemiol.* 131, 113–122. doi:10.1016/j.jclinepi.2020.11.017

Jiang, Z., Xiao, H., Zhang, H., Liu, S., and Meng, J. (2019). Comparison of adverse events between cluster and conventional immunotherapy for allergic rhinitis patients with or without asthma: A systematic review and meta-analysis. *Am. J. otolaryngology* 40 (6), 102269. doi:10.1016/j.amjoto.2019.07.013

Jreich, R., and Sebastien, B. (2021). Comparison of statistical methodologies used to estimate the treatment effect on time-to-event outcomes in observational studies. *J. Biopharm. statistics* 31, 469–489. doi:10.1080/10543406.2021.1918140

Ju, K., Lin, L., Chu, H., Cheng, L., and Xu, C. (2020). Laplace approximation, penalized quasi-likelihood, and adaptive gauss-hermite quadrature for generalized linear mixed models: Towards meta-analysis of binary outcome with sparse data. *BMC Med. Res. Methodol.* 20 (1), 152. doi:10.1186/s12874-020-01035-6

Keir, A., Pal, S., Trivella, M., Lieberman, L., Callum, J., Shehata, N., et al. (2016). Adverse effects of red blood cell transfusions in neonates: A systematic review and meta-analysis. *Transfusion* 56 (11), 2773–2780. doi:10.1111/trf.13785

Kennedy-Martin, T., Curtis, S., Faries, D., Robinson, S., and Johnston, J. (2015). A literature review on the representativeness of randomized controlled trial samples and implications for the external validity of trial results. *Trials* 16, 495. doi:10.1186/s13063-015-1023-4

Mathes, T., Rombey, T., Kuss, O., and Pieper, D. (2021). No inexplicable disagreements between real-world data-based nonrandomized controlled studies and randomized controlled trials were found. *J. Clin. Epidemiol.* 133, 1–13. doi:10.1016/j.jclinepi.2020.12.019

McNamee, R. Regression modelling and other methods to control confounding. *Occup. Environ. Med.* 2005;62(7):500–50. doi:10.1136/oem.2002.001115

Menne, J., Dumann, E., Haller, H., and Schmidt, B. M. W. (2019). Acute kidney injury and adverse renal events in patients receiving SGLT2-inhibitors: A systematic review and meta-analysis. *PLoS Med.* 16 (12), e1002983. doi:10.1371/journal.pmed.1002983

Moher, D., Dulberg, C., and Wells, G. (1994). Statistical power, sample size, and their reporting in randomized controlled trials. *JAMA* 272 (2), 122–124. doi:10.1001/jama.1994.03520020048013

Nagy, A., Mátrai, P., Hegyi, P., Alizadeh, H., Bajor, J., Czopf, L., et al. (2019). The effects of TNF-alpha inhibitor therapy on the incidence of infection in JIA children: A meta-analysis. *Pediatr. rheumatology online J.* 17 (1), 4. doi:10.1186/s12969-019-0305-x

Papanikolaou, P., Christidi, G., and Ioannidis, J. (2006). Comparison of evidence on harms of medical interventions in randomized and nonrandomized studies. *CMAJ Can. Med. Assoc. J. = J. de l'Association medicale Can.* 174 (5), 635–641. doi:10.1503/cmaj.050873

Pateras, K., Nikolakopoulos, S., and Roes, K. (2018). Data-generating models of dichotomous outcomes: Heterogeneity in simulation studies for a random-effects meta-analysis. *Statistics Med.* 37 (7), 1115–1124. doi:10.1002/sim.7569

Pecorelli, N., Greco, M., Amodeo, S., and Braga, M. (2017). Small bowel obstruction and incisional hernia after laparoscopic and open colorectal surgery: A meta-analysis of comparative trials. *Surg. Endosc.* 31 (1), 85–99. doi:10.1007/s00464-016-4995-6

Peng, C., Ling, Y., Ma, C., Ma, X., Fan, W., Niu, W., et al. (2016). Safety outcomes of notes cholecystectomy versus laparoscopic cholecystectomy: A systematic review and meta-analysis. *Surg. Laparosc. Endosc. percutaneous Tech.* 26 (5), 347–353. doi:10.1097/SLE.0000000000000284

Reeves, B., Higgins, J., Ramsay, C., Shea, B., Tugwell, P., and Wells, G. (2013). An introduction to methodological issues when including non-randomised studies in systematic reviews on the effects of interventions. *Res. synthesis methods* 4 (1), 1–11. doi:10.1002/jrsm.1068

Shah, K., Chaker, Z., Busu, T., Badhwar, V., Alqahtani, F., Alvi, M., et al. (2018). Meta-analysis comparing the frequency of stroke after transcatheter versus surgical aortic valve replacement. *Am. J. Cardiol.* 122 (7), 1215–1221. doi:10.1016/j.amjcard.2018.06.032

Shah, K., Chaker, Z., Busu, T., Shah, R., Osman, M., Alqahtani, F., et al. (2019). Meta-analysis comparing renal outcomes after transcatheter versus surgical aortic valve replacement. *J. interventional Cardiol.* 2019, 3537256. doi:10.1155/2019/3537256

Shrier, I., Boivin, J. F., Steele, R. J., Platt, R. W., Furlan, A., Kakuma, R., et al. (2007). Should meta-analyses of interventions include observational studies in addition to randomized controlled trials? A critical examination of underlying principles. *Am. J. Epidemiol.* 166 (10), 1203–1209. doi:10.1093/aje/kwm189

Slobogean, B. L., Jackman, H., Tennant, S., Slobogean, G. P., and Mulpuri, K. (2010). Iatrogenic ulnar nerve injury after the surgical treatment of displaced supracondylar fractures of the humerus: Number needed to harm, a systematic review. *J. Pediatr. Orthop.* 30 (5), 430–436. doi:10.1097/BPO.0b013e3181e00c0d

Soni, P., Hartman, H., Dess, R., Abugharib, A., Allen, S., Feng, F., et al. (2019). Comparison of population-based observational studies with randomized trials in oncology. *J. Clin. Oncol. official J. Am. Soc. Clin. Oncol.* 37 (14), 1209–1216. doi:10.1200/JCO.18.01074

Sun, J. C., Whitlock, R., Cheng, J., Eikelboom, J. W., Thabane, L., Crowther, M. A., et al. (2008). The effect of pre-operative aspirin on bleeding, transfusion, myocardial infarction, and mortality in coronary artery bypass surgery: A systematic review of randomized and observational studies. *Eur. heart J.* 29 (8), 1057–1071. doi:10.1093/eurheartj/ehn104

Torloni, M. R., Vedmedovska, N., Merialdi, M., Betrán, A. P., Allen, T., González, R., et al. (2009). Safety of ultrasonography in pregnancy: WHO systematic review of the literature and meta-analysis. *Ultrasound obstetrics Gynecol. official J. Int. Soc. Ultrasound Obstetrics Gynecol.* 33 (5), 599–608. doi:10.1002/uog.6328

Touzé, E., Trinquart, L., Chatellier, G., and Mas, J. L. (2009). Systematic review of the perioperative risks of stroke or death after carotid angioplasty and stenting. *Stroke* 40 (12), e683–e693. doi:10.1161/STROKEAHA.109.562041

Valentine, J., and Thompson, S. (2013). Issues relating to confounding and meta-analysis when including non-randomized studies in systematic reviews on the effects of interventions. *Res. synthesis methods* 4 (1), 26–35. doi:10.1002/jrsm.1064

Van Spall, H., Toren, A., Kiss, A., and Fowler, R. (2007). Eligibility criteria of randomized controlled trials published in high-impact general medical journals: A systematic sampling review. *JAMA* 297 (11), 1233–1240. doi:10.1001/jama.297.11.1233

Vandenbroucke, J., and Pearce, N. (2012). Case-control studies: Basic concepts. *Int. J. Epidemiol.* 41 (5), 1480–1489. doi:10.1093/ije/dys147

Vaos, G., Dimopoulou, A., Gkioka, E., and Zavras, N. (2019). Immediate surgery or conservative treatment for complicated acute appendicitis in children? A meta-analysis. *J. Pediatr. Surg.* 54 (7), 1365–1371. doi:10.1016/j.jpedsurg.2018.07.017

Vavken, J., Mameghani, A., Vavken, P., and Schaeren, S. (2016). Complications and cancer rates in spine fusion with recombinant human bone morphogenetic protein-2 (rhBMP-2). *Eur. spine J.* 25 (12), 3979–3989. doi:10.1007/s00586-015-3870-9

von Elm, E., Altman, D. G., Egger, M., Pocock, S. J., Gøtzsche, P. C., Vandenbroucke, J. P., et al. (2008). The strengthening the reporting of observational studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. *J. Clin. Epidemiol.* 61 (4), 344–349. doi:10.1016/j.jclinepi.2007.11.008

Wang, F. B., Pu, Y. W., Zhong, F. Y., Lv, X. D., Yang, Z. X., and Xing, C. G. (2015). Laparoscopic permanent sigmoid stoma creation through the extraperitoneal route versus transperitoneal route. A meta-analysis of stoma-related complications. *Saudi Med. J.* 36 (2), 159–163. doi:10.15537/smj.2015.2.10203

Wang, X., and Ji, X. (2020). Sample size estimation in clinical research: From randomized controlled trials to observational studies. *Chest* 158, S12–S20. doi:10.1016/j.chest.2020.03.010

Winberg, H., Arnbjörnsson, E., Anderberg, M., and Stenström, P. (2019). Postoperative outcomes in distal hypospadias: A meta-analysis of the mathieu and tubularized incised plate repair methods for development of urethrocutaneous fistula and urethral stricture. *Pediatr. Surg. Int.* 35 (11), 1301–1308. doi:10.1007/s00383-019-04523-z

Xu, C., and Doi, S. (2018). The robust error meta-regression method for dose-response meta-analysis. *Int. J. evidence-based Healthc.* 16 (3), 138–144. doi:10.1097/XEB.0000000000000132

Xu, C., Furuya-Kanamori, L., and Lin, L. (2021). Synthesis of evidence from zero-events studies: A comparison of one-stage framework methods. *Res. synthesis methods* 13, 176–189. doi:10.1002/jrsm.1521

Xu, C., Furuya-Kanamori, L., Zorzela, L., Lin, L., and Vohra, S. (2021). A proposed framework to guide evidence synthesis practice for meta-analysis with zero-events studies. *J. Clin. Epidemiol.* 135, 70–78. doi:10.1016/j.jclinepi.2021.02.012

Xu, C., Ju, K., Lin, L., Jia, P., Kwong, J., Syed, A., et al. (2021). Rapid evidence synthesis approach for limits on the search date: How rapid could it be? *Res. synthesis methods* 13, 68–76. doi:10.1002/jrsm.1525

Xu, C., Yu, T., Furuya-Kanamori, L., Lin, L., Zorzela, L., Zhou, X., et al. (2022). Validity of data extraction in evidence synthesis practice of adverse events: Reproducibility study. *Bmj* 377, e069155. doi:10.1136/bmj-2021-069155

Xu, C., Zhou, X., Zorzela, L., Ju, K., Furuya-Kanamori, L., Lin, L., et al. (2021). Utilization of the evidence from studies with no events in meta-analyses of adverse events: An empirical investigation. *BMC Med.* 19 (1), 141. doi:10.1186/s12916-021-02008-2

Yaghoobi, M., Farrokhyar, F., Yuan, Y., and Hunt, R. H. (2010). Is there an increased risk of GERD after *Helicobacter pylori* eradication?: A meta-analysis. *Am. J. gastroenterology* 105 (5), 1007–1013. quiz 6, 14. doi:10.1038/ajg.2009.734

Yang, C., Yi, Q., Zhang, L., Cui, H., and Mao, J. (2019). Safety of aripiprazole for tics in children and adolescents: A systematic review and meta-analysis. *Medicine* 98 (22), e15816. doi:10.1097/MD.0000000000015816

Yoon, B. H., Ha, Y. C., Lee, Y. K., and Koo, K. H. (2015). Postoperative deep infection after cemented versus cementless total hip arthroplasty: A meta-analysis. *J. arthroplasty* 30 (10), 1823–1827. doi:10.1016/j.arth.2015.04.041

Zhang, Y., and Ma, L. (2015). Effect of preoperative angiotensin-converting enzyme inhibitor on the outcome of coronary artery bypass graft surgery. *Eur. J. cardio-thoracic Surg. official J. Eur. Assoc. Cardio-thoracic Surg.* 47 (5), 788–795. doi:10.1093/ejcts/ezu298

Zhao, Y., Peng, H., Li, X., Qin, Y., Cao, F., Peng, D., et al. (2018). Dual antiplatelet therapy after coronary artery bypass surgery: Is there an increase in bleeding risk? A meta-analysis. *Interact. Cardiovasc. Thorac. Surg.* 26 (4), 573–582. doi:10.1093/icvts/ivx374

Zorzela, L., Golder, S., Liu, Y., Pilkington, K., Hartling, L., Joffe, A., et al. (2014). Quality of reporting in systematic reviews of adverse events: Systematic review. *Bmj* 348, f7668. doi:10.1136/bmj.f7668

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 24 March 2023
DOI 10.3389/fphar.2023.1144878

OPEN ACCESS

EDITED BY
Wei Peng,
Chengdu University of Traditional Chinese Medicine, China

REVIEWED BY
Songqi Tang,
Hainan Medical University, China
Tian E. Zhang,
Chengdu University of Traditional Chinese Medicine, China

*CORRESPONDENCE
Fengjie Zheng,
zhengfengjie@bucm.edu.cn
Yuhang Li,
liyuhang@bucm.edu.cn
Yan Sun,
suny@bucm.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Ethnopharmacology, a section of the journal Frontiers in Pharmacology

RECEIVED 15 January 2023
ACCEPTED 15 March 2023
PUBLISHED 24 March 2023

CITATION
Sheng Y, Sun Y, Tang Y, Yu Y, Wang J, Zheng F, Li Y and Sun Y (2023), Catechins: Protective mechanism of antioxidant stress in atherosclerosis.
Front. Pharmacol. 14:1144878.
doi: 10.3389/fphar.2023.1144878

Catechins: Protective mechanism of antioxidant stress in atherosclerosis

Yuhan Sheng†, Yizhuo Sun†, Yang Tang†, Yanru Yu, Jiarou Wang, Fengjie Zheng*, Yuhang Li* and Yan Sun*

School of Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing, China

Tea has long been valued for its health benefits, especially its potential to prevent and treat atherosclerosis (AS). Abnormal lipid metabolism and oxidative stress are major factors that contribute to the development of AS. Tea, which originated in China, is believed to help prevent AS. Research has shown that tea is rich in catechins, which is considered a potential source of natural antioxidants. Catechins are the most abundant antioxidants in green tea, and are considered to be the main compound responsible for tea's antioxidant activity. The antioxidant properties of catechins are largely dependent on the structure of molecules, and the number and location of hydroxyl groups or their substituents. As an exogenous antioxidant, catechins can effectively eliminate lipid peroxidation products. They can also play an antioxidant role indirectly by activating the endogenous antioxidant system by regulating enzyme activity and signaling pathways. In this review, we summarized the preventive effect of catechin in AS, and emphasized that improving the antioxidant effect and lipid metabolism disorders of catechins is the key to managing AS.

KEYWORDS

catechins, oxidative stress, atherosclerosis, lipid metabolism disorders, tea

1 Introduction

Atherosclerosis (AS) is characterized by lesions of the affected artery starting from the intima with accumulation of lipid and/or fibrous material. It is the underlying cause of many cardiovascular diseases, including myocardial infarction, ischaemic strokes and peripheral arterial diseases that can endanger limb viability (Libby et al., 2019).

Dyslipidemia, which is defined as derangement of the lipid profile, is one of the important factors that promote the development of atherosclerosis. It is characterized by elevated low density lipoprotein cholesterol (LDL-C) and/or decreased high density lipoprotein cholesterol (HDL-C). Examples include hypercholesterolemia and hypertriglyceridemia (Gupta et al., 2020). Oxidative stress promotes modification in lipid metabolism (Hu et al., 2019; Wójcik et al., 2019). Excessive reactive oxygen species (ROS) can destroy cellular proteins, lipids, and DNA, leading to lethal cell damage (Wójcik et al., 2021). It has been shown that elevated ROS levels promote the activation of related enzymes involved in lipid metabolism such as lipoxygenases, phospholipases, cyclooxygenases, and cytochrome p450 (Liaras et al., 2018). Most importantly, oxidative stress leads to an increase in both oxidative fragmentation and oxidative cyclization of lipid hydrocarbon chains. (Wójcik et al., 2021). In the 1950s, the presence and extent of

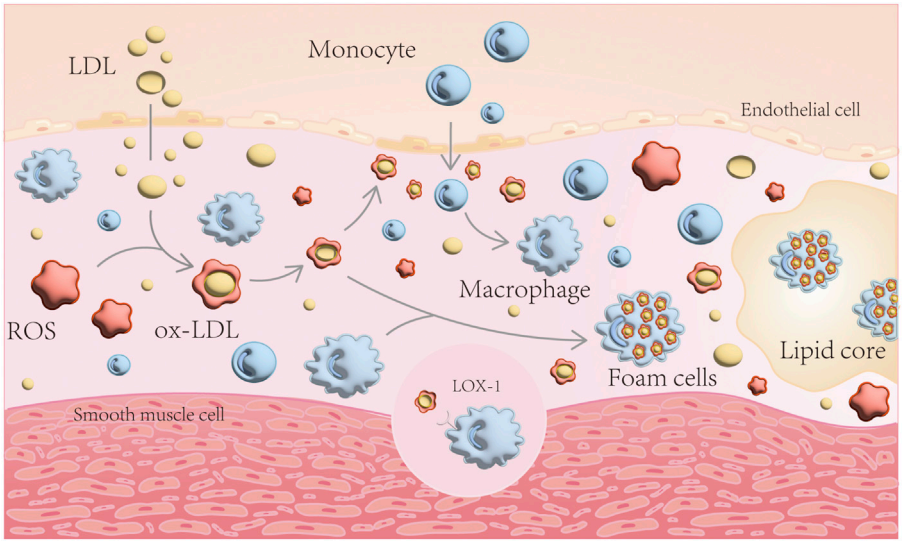


FIGURE 1
Schematic diagram of AS formation mechanism. LDL enters the subendothelium and undergoes ROS oxidation to become ox-LDL. ox-LDL damages the endothelium, allowing monocytes to enter the inner membrane and differentiate into macrophages, which engulf ox-LDL in large quantities, forming foam cells.

lipids and protein oxidation products and their relationship to the severity of atherosclerotic disease were first described in humans (Forstermann et al., 2017). From the above studies, it can be concluded that abnormal lipid metabolism and oxidative stress play an important role in the formation of the AS mechanism. Therefore, improving abnormal lipid metabolism and alleviating oxidative stress is vital in the prevention and treatment of AS.

Tea is a beverage with a long history. It has always been a hot topic for scholars because it can bring significant and positive health effects (Wang et al., 2020). Tea is not only a drink, but also a traditional Chinese medicine with a long medical history. For example, tea is used as medicine in Moshizi San in Taiping Shenghui Fang (Shuhui, 2015). The health benefits of tea are largely attributed to the effects of tea polyphenol. Green tea contains high levels of tea polyphenol, most of which are catechins. They are the main components of tea polyphenols and the main reason for their antioxidant activity (Koch et al., 2020). Existing data show that catechins have antioxidant, anti-tumor and anti-inflammatory effects, suggesting that catechins have great potential in the treatment of related diseases (Bernatoniene and Kopustinskiene, 2018). Catechins can be used as chain breaking antioxidants to eliminate lipid alkoxyl and peroxyl radicals to effectively inhibit lipid peroxidation (Lambert and Elias, 2010). This provides a good therapeutic method for relieving lipid accumulation and oxidative stress in AS.

The mechanism involving catechins regulating oxidative stress to improve abnormal lipid metabolism and thus prevent AS has not been systematically mapped. In this review, we summarized the preventive effect of catechin in AS, and emphasized that improving the antioxidant effect and lipid metabolism disorders of catechins is key to managing AS. We hope to provide reference for follow-up studies of catechins in oxidative stress and abnormal lipid metabolism diseases.

2 Relationship between oxidative stress and lipid metabolism in AS

Alterations in lipid metabolism may lead to it becoming a risk factor and feature of AS (Poznyak et al., 2020). Low-density lipoprotein cholesterol (LDL-C) is a high risk factor for ASCVD (Stone et al., 2014). Oxidative stress is an abnormal reaction state of the antioxidant system triggered by excess free radicals in the body (Kalyanaraman, 2013). It causes lipid peroxidation, which affects the structure, fluidity, integrity of membranes, ultimately leading to destruction of cell structure and function (Juan et al., 2021).

When vascular endothelial function is impaired, LDL enters the subendothelium and undergoes ROS oxidation to become ox-LDL. ox-LDL damages the endothelium, allowing monocytes to enter the inner membrane and differentiate into macrophages, which engulf ox-LDL in large quantities, forming foam cells, an important component of atherosclerotic plaques (Khatana et al., 2020) (Figure 1). Simultaneously, excessive accumulation of peroxidized lipids in the cell can cause endothelial dysfunction, VSMCs disorder - lipid deposition, macrophage dysfunction and foam cell formation (Hoseini et al., 2018; Cai et al., 2019; Marchio et al., 2019; Zhang et al., 2021). This chain reaction aggravates AS.

Both the lipid pathogenicity theory and the damage of endothelial cells by peroxide have confirmed the causal relationship between oxidative stress and lipid metabolism disorders in AS, and we tried to find therapeutic drugs that

improve lipid metabolism disorders by regulating oxidative stress.

3 Basic properties and regulatory mechanisms of catechins—Protective effect against atherosclerosis

Catechins are powerful antioxidants extracted from tea. The structure of catechin is the key determinant of its free-radical scavenging and metal chelating activities. Their antioxidant activity largely depends on the number and location of hydroxyl and other chemical groups. These allow catechins to act as metal ion chelators, providing them with the ability to reduce the level of lipid peroxidation biomarkers and improve lipid metabolism disorder caused by oxidative stress. Therefore, catechins have many advantages in preventing AS. A series of experimental results show that catechins act on all aspects of the formation and progression of AS and reduce the risk of AS. Relevant studies have shown that catechins may improve AS by mobilizing endogenous antioxidant networks, including regulating enzyme activity and signaling pathways.

3.1 Source of catechins

Catechins are widely distributed in many foods and herbs, including apples, broad beans, pears, chocolate wine and cocoa products (Isemura, 2019). Green tea is the most abundant in catechins and is considered the leading source of all dietary sources (Ahmad and Mukhtar, 1999), ahead of chocolate, red grapes, wine and apples (Cabrera et al., 2006). According to the data of the European Food Safety Agency (EFSA), there are 126 mg of catechins in every 100 mL of green tea (Prasanth et al., 2019). The fermentation of tea is carried out by the oxidation of its own oxidase. According to the degree of fermentation, we often classify tea into four major types: Non-fermented tea, semi-fermented tea, fully fermented tea and post-fermented tea (Kondo et al., 2015). According to existing documents, tea was first consumed as a drink or medicine by the Chinese around 2737 BC, and China is now a major tea producer as well (Vuong, 2014). People in Asia have been aware of the beneficial health effects of green tea for centuries (Shixian et al., 2006). Green tea is considered as a natural plant that can maintain cardiovascular health by reducing blood cholesterol and glucose levels, and inhibiting antioxidant effects (Hara, 1994; Basu and Lucas, 2007; Shapiro et al., 2009; Roychoudhury et al., 2017). Residents in Europe, mainly the United Kingdom, drink predominantly black tea and are the largest tea consumers per day (about 540 mL) (Gardner et al., 2007). Both green and black tea are made from the fresh leaves of the tea plant, but they are processed in different ways and their catechin content is altered. Green tea is produced by drying and steaming fresh leaves, which inactivates the enzyme polyphenol oxidase, thereby protecting most of the catechins in the tea (Bartoszek et al., 2018). In contrast, in the fermentation process of black tea, catechins are oxidized and condensed to produce theaflavins and thearugins, and their content is therefore reduced (Graham, 1992).

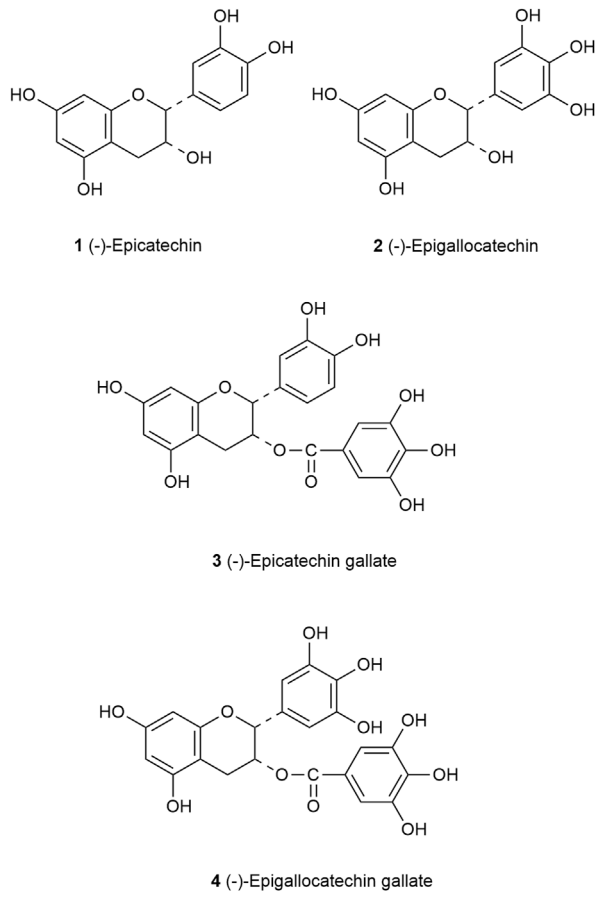


FIGURE 2
Structure of the principal catechins. EC (1) has an ortho-dihydroxyl group in the B-ring at carbons 3′and 4′and a hydroxyl group at carbon 3 on the C ring. EGC (2) has a trihydroxyl group at carbons 3′, 4′, and 5′on the B ring. ECG (3) has a gallate moiety esterified at carbon 3 of the C ring, while EGCG (4) has both a trihydroxyl group at carbons 3′, 4′, and 5′on the B ring and a gallate moiety esterified at carbon 3 on the C ring.

3.2 Chemical properties and pharmacological effects of catechins

Catechins are a major group of flavonoids with the molecular formula C15H14O6. Studies have shown that catechins have different stability in different pH environments, which was relatively stable at pH 4–6 and changed greatly when pH was less than 3 (Musial et al., 2020). According to the different types of carbon rings, catechins are mainly divided into four groups: (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin gallate (EGCG) (Peluso and Serafini, 2017). Catechins have a meta-5,7-dihydroxy group of the A ring, EC and ECG have an ortho-dihydroxyl group at carbon 3′and carbon 4′of the B ring, but EC has a hydroxyl group at carbon 2 of the C ring, ECG has a gallate moiety esterified at carbon 3 of the C ring, EGC and EGCG have a trihydroxyl group at carbon 3′, 4′and 5′of the B ring. However, EGCG has a gallate moiety esterified at carbon 3 of the C ring (Figure 2) (Higdon and Frei, 2003).

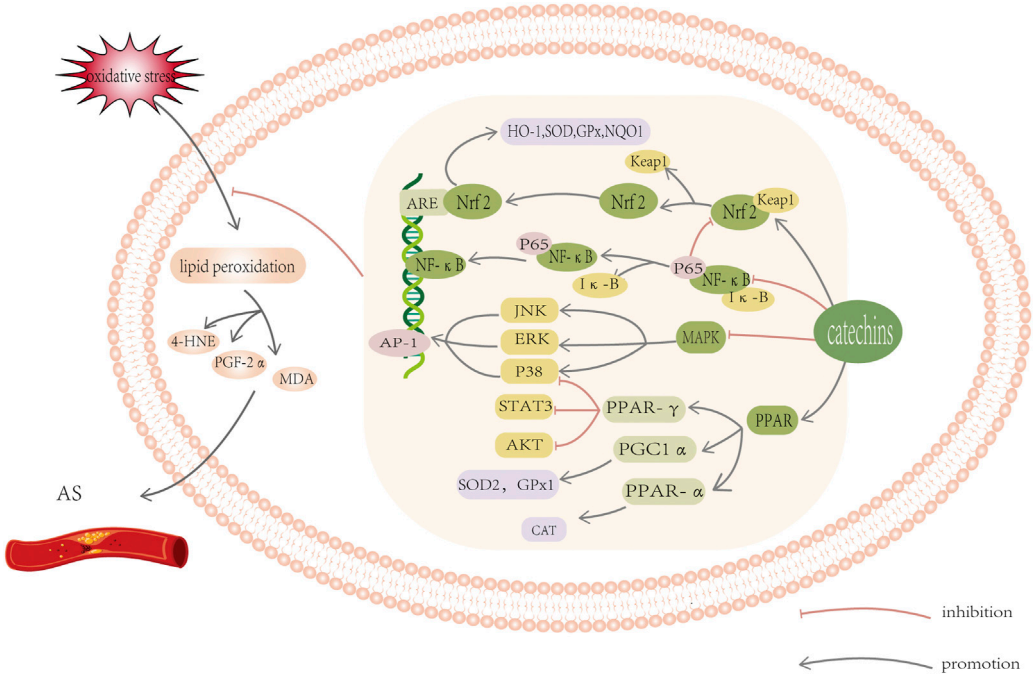


FIGURE 3
Schematic diagram of oxidative stress-related signal pathway. Catechins can induce the activation of Keap1/Nrf2/ARE signal pathway, inhibit the activation of MAPK/AP-1 pathway, and block the activation of transcription factor NF- κ B and increase PPAR γ, PGC1 *a* And PPAR *a* Protein level. These reactions all work together to help reduce oxidative stress and lipid peroxidation. For detailed explanation, please see text.

Catechins have been proved to have strong antioxidant activity. The existing literature data shows that the antioxidant activity of catechins is largely dependent on the structural of molecules, and the number and location of hydroxyl groups or their substituents (Leung et al., 2001; Chantre and Lairon, 2002). The distribution of hydroxyl groups is equally important too (Masek, 2017). The presence of one vicinal dihydroxyl group on the B ring and a galloyl group at the 3-position is essential to maintain the efficiency of the free radical scavenging capacity (Nanjo et al., 1999) In addition, Catechin chelates with metal ions to form an inactive complex, which can prevent such redox-active metal ions from catalyzing reactions and enhance their antioxidant effect. The catechol and pyrogallol groups in the B ring and the meta-5, 7-dihydroxy group in the A rings are required for the chelation of catechin with metal ions (Musial et al., 2020). EGCG rich green tea has been proven to have metal chelation properties (Thephinlap et al., 2007; Chan et al., 2016). The pyrogallol groups provide strong metal chelation of EGCG to transition metal ions that act as preventative antioxidants (Guo et al., 1996; Zhang et al., 2000).

Catechins can also effectively improve lipid peroxidation by reducing the levels of lipid peroxidation products such as malondialdehyde (MDA), 4-Hydroxynonenal (4-HNE), and F2 Isoprostane (PGF-2α). Experiments have shown that catechins can effectively reduce their level to alleviate lipid metabolism disorders caused by oxidative stress. Free radicals oxidation modifies lipids, and the final product of lipid peroxidation is MDA (Xiong et al., 2015; Zeng et al., 2021). EC can reduce the MDA content in erythrocytes in hypertensive patients (Kumar et al., 2010). An increase in Plasma MDA level was observed in N G-nitro-L-arginine methyl ester (L-NAME)-treated animals. However, after treatment with EC, MDA concentration was markedly reduced (Gomez-Guzman et al., 2011). PGF2- *a* is a recognized biomarker of oxidative stress *in vivo* and has been proved to be related to the increase of lipid peroxidation in animals and humans (Morrow et al., 1999). The 24 h urinary iso-PGF2a excretion was found to have increased after treatment with L-NAME, but excretion of iso-PGF2a returned to similar values to the control rats in the EC-treated L-NAME rats (Gomez-Guzman et al., 2011).8-Isopropane is a group of stable PGF2 *a* Isomers (Wang et al., 2007), GTE and its catechin constituents significantly reduce production of 8-iso-PGF2α after oxidative stress (Yang et al., 2016).4-HNE, an α, *ß*-unsaturated hydroxyalkenal, is a biomarker of oxidation stress (Keller et al., 2015) .4-HNE-protein adducts prompts macrophagic cells to engulf large amounts of LDL then leading to the formation of foam cells (Boléa et al., 2019). The catechins (EC, EGC, EGCG) found in white tea extracts can chelate peroxyl radicals that lead to the formation of 4-HNE (Espinosa et al., 2012). An experiment involving rats with oxidative stress induced through intraperitoneal injections of N-nitrosodimethylamine found that treatment with 0.2 mg EGCG/100 g body weight daily markedly reduced the expression of 4-HNE protein and/or mRNA levels (George et al., 2022).

3.3 Catechins prevent AS

Research have shown that catechins are powerful natural antioxidants (Kondo et al., 1999) that can mitigate antioxidative

TABLE 1 Experimental studies of catechins intervention on atherosclerosis.

Reference	Treatment	Subjects	Dose	Periods	Results	Potential molecular mechnisms
Inami et al. (2007)	Polyphenon 70 S	40 healthy adult volunteers (10 men, 30 women)	each subject orally ingested Polyphenon 70 S capsules containing 500 mg of catechin	4 weeks	The plasma Ox-LDL concentration decreased significantly in the catechin group; the Ox-LDL (mg/dL)/ LDL-C (U/mL) % ratio significantly decreased in the catechin group	The beneficial effect of green tea on coronary artery disease is thought to result partially from a decrease in circulating Ox-LDL.
Dower et al. (2015)	EC	37 healthy (pre) hypertensive men and women (40–80 years)	100 mg/d	4 weeks	The treatment effect of epicatechin supplementation could beneficially affect endothelial function and the development of atherosclerosis	The treatment effect of epicatechin supplementation was a significant decrease of plasma sE-selectin
Yu et al. (2021)	ECG	mices	different doses of ECGs (5, 25, and 50 mg kg−1 in w/v saline solution)	4 weeks	Serum TC, TG, LDL-C, and MDA levels were reduced; SOD activity increased	ECG reduced the progression of atherosclerosis by blocking the expression of NF-κB, and related proteins that activate the Nrf2 signaling pathway
Miltonprabu and Thangapandiyan (2015)	EGCG	rats	40 mg/kg.b.w/day	4 weeks	Pre-administration with EGCG significantly decreased the levels of plasma cholesterol, TG, FFA and PL, plasma LDL-C and VLDL-C with a significant increase in the level of HDL-C Pre-administration of EGCG along with F significantly decreased the levels of cardiac TBARS, LOOH, CD, and PC.	The ability of EGCG to prevent peroxidation of membrane phospholipids. Green tea catechins are effective freer adicals scavengers and exhibits antilipid peroxidative action through their free radical-scavenging activity
Tang et al. (2006)	EGCG	rats	10 or 50 mg kg-l, dissolved in saline, i.p. Once a day	5 days	EGCG significantly attenuated the impairment of endothelium-dependent vasodilation in isolated rat aortic rings induced by native LDI concomitantly with an elevation of NO release and a decreasein serum levels of ADMA.	It is probable that the decreased level of ADMA by EGCG may be related to reduction of lipid peroxidation; Another possibility responsible for EGCG in reducing the level of ADMA is the involvement of some cytokines such as TNF-a
Friedrich et al. (2012)	EGCG	mices	Mice were fed a semi-synthetic HF diet with dietary TEAVIGO supplementation (EGCG 0.5%, EGCG 1.0%; n ¼ 12 per group)	4 days	Plasma triglycerides were reduced dose dependently by EGCG.	EGCG treatment led to a downregulation of lipogenic genes: acetyl-CoA-carboxylase, fatty acid synthase, and stearoyl-CoA desaturase
Xu et al. (2014)	EGCG	rats	IP injection of EGCG dissolved in saline (100 mg/kg body weight) once daily	12 days	Rats treated with EGCG showed a significant decrease in TC, TG, bad cholesterols, and cardiac risk ratio values and a significant increase in the level of HDL cholesterol	Administration of EGCG perhaps acted by regulating the activities of these antioxidant enzymes, such as MDA, CAT, SOD, and GPx in cardiac tissue
Chen et al. (2016)	EGCG	rats	30 or 100 mg/kg	7 days	EGCG was able to decrease the oxidative stress in hippocampi of Rs; The decreased levels of GSH, SOD, and CAT caused by Reserpine were partially and completely restored by EGCG respectively	EGCG could exert its effecting by modifying NO pathway activity; It has been reported that NO can be produced by inducible nitric oxide synthase (iNOS) and neuronal nitric oxide synthase (nNOS)
Kawai et al. (2008)	ECG	THP-1 cells	100 μg/mL	4 h	The gene expression of CD36 was significantly inhibited by the treatment with ECG	Macrophages may be the potential target of ECG in the human atherosclerotic aorta

(Continued on following page)

TABLE 1 (*Continued*) Experimental studies of catechins intervention on atherosclerosis.

Reference	Treatment	Subjects	Dose	Periods	Results	Potential molecular mechnisms
Auger et al. (2010)	EGCG	porcine coronary arteries	100 μL	15 min	EGCG-induced concentration-dependent relaxations in porcine coronary artery	EGCG causes endothelium-dependent NO-mediated relaxations of coronary artery rings through the Akt-dependent activation of eNOS in endothelial cells
Chang et al. (2017)	catechins	Human umbilical vein endothelial cells line	50 μg/mL	4 h	Orally administrated catechins were shown to attenuate oxLDL- or PCOOH-induced vasoconstriction	Catechins decrease oxLDL- or PCOOH-induced endothelial cell apoptosis and vasoconstriction through H_2O_2 inhibition and eNOS restoration

reactions, reducing lipid metabolism abnormalities leading to AS. According to epidemiological studies, tea consumption reduces the risk of AS and is associated with all-cause mortality (Kishimoto et al., 2020; Wang et al., 2020). Several scholars have pointed out that tea polyphenols will alleviate AS in mice by altering endothelial function, plaque size, lipid metabolism, etc., (Minatti et al., 2012; Ding et al., 2017). The main active component of tea polyphenols is catechins, which have been shown to relax blood vessels, positively regulate dyslipidemia and oxidative damage (Bernatoniene and Kopustinskiene, 2018; Wu et al., 2020).

Ox-LDL can also cause AS by inducing the regulation of oxidative stress, lipid infiltration, inflammatory response, and vascular tone by influencing nitric oxide (NO)—A versatile signaling molecule involved in maintaining metabolism and cardiovascular homeostasis in the body (Chen et al., 2018). Asymmetric dimethylarginine (ADMA), a natural occurring compound found in plasma, can inhibit nitric oxide synthase activity and has a strong inverse relationship with HDL (Lorin et al., 2013). ADMA is also inversely proportional to LDL fatty acid oxidation, which means that ADMA can regulate lipid metabolism and influence the bioavailability of NO (Paiva et al., 2006). On this basis, the dose relationship between EGCG and ADMA bivalent effect is still worth exploring. CD36 is an important intermediate in the transformation of macrophages into foam cells, and low expression of CD36 effectively delayed the development of AS (Kawai et al., 2008). A series of experiments showed that catechins effectively reduced blood lipid levels, inhibit the formation of foam cell, and resist oxidative stress (Table 1).

3.3.1 In vivo

In an intervention experiment using a high-fat diet-induced $ApoE^{-/-}$ mice, serum TC, TG, LDL-C, and MDA levels were significantly reduced after taking ECG, while SOD activity increased. Pathological tests found that ECG reduced aortic atherosclerotic plaque size in mice (Yu et al., 2021). Animal studies have confirmed that EGCG can reduce plasma triglycerides dose dependently and inhibit cellular lipid uptake (Friedrich et al., 2012). In oxidative stress-mediated rat heart experiments, EGCG significantly reduced elevated serum cardiac markers and abnormal blood lipid metabolism caused by oxidative stress injury. At the same time, it inhibits lipid peroxidation and reduces the expression of TBARS, LOOH and CD (Miltonprabu and Thangapandiyan, 2015). EGCG also enables HFD-induced model rats to redistribute lipid levels and improve overall oxidative activity (Xu et al., 2014). Research on mice treated with reserpine to inudce excess NO and lipid peroxidation found that EGCG intervention counteracted these changes (Chen et al., 2016). EGCG can also improve vascular endothelial damage caused by LDL or ox-LDL by reducing ADMA levels (Tang et al., 2006).

3.3.2 In vitro

A study of atherosclerotic mice found that ECG accumulation in macrophages specifically inhibits genes encoding CD36, an important intermediate in the transformation of macrophages into foam cells (Kawai et al., 2008). Studies have shown that EGCG can increase endothelial cell NO activity by stimulating endothelial NO synthase expression (Auger et al., 2010). In endothelial cells damaged by ox-LDL and phosphatidylcholine (PCOOH), the main metabolite of ox-LDL, catechins can also improve endothelial cell dysfunction through the above pathways and inhibit oxidative stress (Chang et al., 2017).

3.3.3 Clinical trails

Studies have shown that EGCG can increase endothelial cell NO activity by stimulating endothelial NO synthase expression (Auger et al., 2010). A randomized, double blind, placebo-controlled crossover trial shows that EC may contribute to the AS protective effects through improvements in endothelial function (Dower et al., 2015).

3.4 Antioxidant mechanism of catechins—mobilizing endogenous antioxidant network

From the above statistics, we can conclude that oxidative stress can aggravate lipid metabolism disorder. Catechin, an exogenous

antioxidant, is an effective scavenger of a variety of lipid peroxidation products, and can regulate oxidative stress to improve the abnormality of lipid metabolism. A series of studies had shown that catechins have good preventative and therapeutic effects on AS. Therefore, it is important to explore the antioxidant mechanism of catechins in order to mitigate lipid metabolism abnormalities. Currently, there are many discussions on the antioxidant mechanism of catechins. Here, we systematically summarized the role catechins play against oxidative stress in the endogenous antioxidant system to improve the vascular endothelial state of AS, including influencing enzyme activity and regulating signal pathways (Figure 3).

3.4.1 ROS related enzymes

3.4.1.1 NADPH oxidases

NADPH (Nicotinamide adenine dinucleotide phosphate) oxidases are multisubunit enzyme complexes that include p22phox and a Nox homologue and cytosolic regulatory subunits (Forstermann et al., 2017). They can produce superoxide anions *via* superoxide radical formation and play an important role in the formation of endogenous H_2O_2 (Bedard and Krause, 2007; Drummond et al., 2011; Sies et al., 2017). NOX and p22phox form heterodimer, which together form NOX-p22 complex in the resting state. NOX will transfer electrons to generate O_2 , which is further converted into ROS. (Byrne et al., 2021). Endothelial NADPH oxidases are involved in proliferating and apoptosis through formation of capillary-like structures and angiogenesis (Cai, 2005). The high activity of NADPH oxidase is related to a series of proinflammatory and cytotoxic processes, which may lead to endothelial dysfunction (Steffen et al., 2008). Catechins can effectively inhibit the overexpression of NADPH oxidase. Research (Gomez-Guzman et al., 2011) found that (−)—Epicatechin treatment eliminates the increase of NADPH oxidase activity in L-NAME treated rats. The phase II metabolites of (−)—Epicatechin was believed to inhibit NADPH oxidase after observing that they prevented oxidative stress induced apoptosis of human fibroblast (Spencer et al., 2001).

3.4.1.2 Xanthine oxidase

Xanthine oxidase catalyzes the conversion of hypoxanthine and generates a large number of oxygen free radicals. (Schmidt et al., 2019). Xanthine oxidoreductase initially synthesizes xanthine dehydrogenase (XDH) and is proteolytically hydrolyzed to xanthine oxidase (XO). Due to different electron receptors, although XDH and XO catalyze the same substrate, the product with opposite biochemical action is obtained: XDH reduces NAD + to NADH. However, XO cannot reduce NAD+, but catalyzes the reduced molecular oxygen to produce superoxide. Guzik et al. found that compared with non-coronary artery disease, despite similar levels of XDH, the XO protein in the blood vessels of patients with coronary artery disease is significantly increased. This indicates that the increase of XO activity contributes to the production of vascular O_2 in coronary artery disease to a certain extent (Guzik et al., 2006). Studies have shown that catechins have inhibitory effects on XO. Lin et al. found that EGCG and tea xanthin inhibit XO to produce uric acid. Theaflavin-3,3′-digallate is the most effective XO inhibitor among a variety of tea polyphenol as a competitive inhibitor (Lin et al., 2000). Zhu et al. proved that treatment with high-dose EGCG significantly decreased the liver XO activity (Zhu et al., 2018).

3.4.1.3 Cyclooxygenase 2

Studies have shown that increases in vascular superoxide content and in plasma peroxides have been observed following cardiovascular application of COX2 selective inhibitors, so COX2 is considered to suppress the level of oxidative stress. (Li et al., 2008). The increased endothelium-dependent vasoconstriction induced by acetylcholine has previously been attributed to endothelial release of prostaglandins, such as PGH2 or thromboxane A2, which are COX-derived vasoconstrictors (Auch-Schwelk et al., 1990; Duarte et al., 2002). An increase in endothelium-dependent vasoconstriction induced by acetylcholine was observed in rats aorta treated with N- nitro -L-arginine methyl ester. However, rats treated with L-NAME plus (−)—Epicatechin showed the decreased vasoconstriction response to acetylcholine and COX-2, implying that (−) epicatechin may have altered the vascular endothelial state by down-regulating COX-2 to inhibit the release of COX-derived metabolites. (Gomez-Guzman et al., 2011).

3.4.1.4 Nitric oxide synthase

The indicator of endothelial dysfunction is the impairment of endothelium-dependent vasodilation mediated by NO (Augusti et al., 2008), which represents a key vasoprotective factor of the endothelium (Forstermann et al., 2017). L-arginine produce biologically active NO under that catalysis of nitric oxide synthase (NOS). Under pathological conditions, however, phagocytes are stimulated to produce excessive NO and O_2, which react rapidly *in vivo* to form OONO- and other NO-derived oxidants (Surh et al., 2001; Higdon and Frei, 2003). Under physiological conditions, activation of endothelial nitric oxide synthase (eNOS) (a subtype of NOS) typically generates NO(Forstermann et al., 2017). In the oxidative environment, eNOS no longer produces vasoprotective NO, but instead uncouples to produce vasoinjurious O_2 (Daiber et al., 2019). From the mechanism, deficiency of eNOS cofactor tetrahydrobiopterin (BH4) may be likely to be one of the main causes for the uncoupling of eNOS (Förstermann and Münzel, 2006; Li and Förstermann, 2013; Forstermann et al., 2017). NOX has a complex interrelationships with other ROS-producing oxidase systems. And there is more evidence that Nox-derived ROS affects the expression and activity of BH4, leading to the uncoupling of NOS(Griendling et al., 2021). Studies have found that catechins can improve phosphorylation of eNOS. When the vascular endothelium is damaged, platelets will undergo a series of activation reactions, which will lead to the production and release of pro-oxidation mediators to change the endothelial function. P-eNOS and NO bioavailability have been shown to be reduced in the activated platelet supernatant from patients with peripheral artery disease (PAD). In an experiment where human Umbilical Vein Endothelial Cells were incubated from patients with PAD and pretreated with standard epicatechin plus catechin, it was found that the bioavailability of p-eNOS and NO increased significantly. This resulted in a decrease in endothelial activation induced by activated platelets (Carnevale et al., 2014). Catechins may also improve the bioavailability of NO by reducing eNOS uncoupling. Studies have

shown that green tea can restore the reduction of BH4 levels, maintain the balance of the proportion of eNOS and BH4, and make eNOS in the coupled state. Therefore, green tea reduced ROS production, reduced oxidative stress, and improved endothelial function (Faria et al., 2012).

3.4.1.5 Antioxidants *in vivo*

To protect tissues from oxidation, biological systems have evolved to create multiple antioxidant systems for the removal of ROS inside cells (Parthasarathy et al., 2000). The anti-oxidation systems inherent in the human body are divided into enzymes and non-enzymes. Wherein that antioxidant enzymes comprise superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidases (GPxs), and the non-enzymatic antioxidants comprise glutathione (GSH). They inhibit oxidative stress by scavenging free radicals and inactivating ROS(Chen, 2021).

Some representative phase II detoxifying enzymes include glutathione S-transferase (GST) and NAD (P) H:quinone oxidoreductase 1 (NQO1). Nrf2 can regulate the expression of these enzymes through the antioxidant-response element (ARE) and significantly enhance their antioxidant response. This process can significantly improve their antioxidant response (Kong et al., 2001).

GSH is an endogenous antioxidant that exists in two forms in the human body, reduced thiol GSH and oxidized disulfide GSSG(Raza, 2011). Depletion of GSH usually destroys the redox homeostasis of cells, leading to accumulation of ROS, which in turn triggers cell damage or even death (Li X et al., 2019). GST is involved in protecting DNA damage from oxidative stress by catalyzing the covalent binding of glutathione with hydrophobic and electrophilic substrates (Hayes et al., 2005; Chatterjee and Gupta, 2018). The Alpha class GSTs can interrupt chain of lipid peroxidation reactions by reducing hydroperoxides and detoxifying the toxic end products of lipid peroxidation. (Sharma et al., 2004). The main function of SOD is to catalyze the dismutation of superoxide anion radical into O_2 and H_2O_2. They have a significant effect on the treatment of atherosclerosis by reducing the peroxidation caused by the accumulation of free radicals and maintain the metabolic balance of the body (Förstermann and Sessa, 2012; Li et al., 2014). The primary role of CAT is to catalyze the decomposition of H_2O_2 into H_2O and O_2, and protect cells from H_2O_2 poisoning (Wang Y et al., 2014),Overexpression of catalase reduced atherosclerosis in ApoEKO mice (Yang et al., 2004). GPx is a GSH-dependent enzyme that converts reduced GSH to oxidized GSH, and simultaneously reduces lipid hydroperoxide to the corresponding lipid alcohol or free hydrogen peroxide to water (Lubos et al., 2011). NQO1 is a homodimer flavin enzyme that catalyzes the reductions of quinones to hydroquinones through obligatory 2-electron reductions. This obligatory two-electron reduction prevents the formation of semiquinone and superoxide or H_2O_2 (Dinkova-Kostova and Talalay, 2010).

Reports have shown that EGCG can promote and mobilize the activities of a set of antioxidant enzymes *in vivo*, including GSH, SOD, CAT, GPX, and GST (Na and Surh, 2008). Ramesh et al., found that the activities of CAT, SOD, Gpx and GST in haemolysate and cardiac tissue samples increased significantly after being treated with EGCG (Ramesh et al., 2008). After treatment with acetaminophen (N-acetyl-p-aminophenol, APAP), EGCG increased the activities of GSH and NQO-1. In addition, the level of ROS, GSSG and TBARS in the liver decreased significantly. EGCG also increased GPxs activity, which might be responsible for the decreased ROS production during APAP metabolism (Yao et al., 2019). Polychlorinated biphenyls (PCB) can exacerbate oxidative stress in the body, and further induce inflammation of vascular endothelial cells. Studies showed that exposure of vascular endothelial cells to PCB 126 significantly increased superoxide. However, superoxide induced by PCB 126 was significantly reduced when primary vascular endothelial cells were pretreated with EGCG. Specifically, treatment of EGCG upregulated expression of antioxidant genes including GST and NQO1in a dose-dependent manner, all of which are controlled by NF-E2-related factor 2 (Nrf2) (Han et al., 2012).

3.4.2 Oxidative stress related signal pathway

3.4.2.1 Nrf2 pathways

The Keap1-Nrf2-ARE pathway represents one of the most important cellular defense mechanisms against oxidative stress (Bai et al., 2015; Orrù et al., 2020). Leucine zipper transcription factor (a basic region of Nrf2) can activate ARE and start a variety of antioxidant reactions to prevent oxidative stress. The Kelch-like ECH associated protein 1 (Keap1) is a receptor that affects the expression of Nrf2. Without electrophiles or oxidants, Nrf2 is located in the cytoplasm and binds to Keap1 (Kang et al., 2004). The binding of Keap1 to Nrf2 results in ubiquitin dependent proteasomal degradation under basal (reducing) conditions. Under oxidative stress, stable Nrf2 translocates to the cell nucleus and forms a heterodimer with Maf. It then interacts with ARE in target genes (Magesh et al., 2012), to drive the expression of antioxidant genes, such as NQO1, HO-1, SOD and GPx (Figuer 3) (Satoh et al., 2011; Patinen et al., 2019). Heme oxygenase 1 (HO-1) is a strong antioxidant (Araujo et al., 2012). It can increase the level of NO, reduce the level of inflammatory factors, reduce atherosclerotic plaque, and interfere with the formation and stability of plaque. In addition, HO-1 regulates cholesterol transport and plasma lipid peroxidation (Liu et al., 2012; Warner et al., 2018). Wu et al. found that after treatment with fixed concentration of 50 Amol EGCG, the level of HO-1 protein increased in a time-dependent manner. An experiment found that endothelial cell cultures cotreated with EGCG plus actinomycin D (AD) or cycloheximide (CHX) were able to completely block induction by EGCG. AD and CHX are transcriptional and translational inhibitors respectively, suggesting that EGCG most likely induced HO-1 *via de novo* RNA and protein synthesis (Wu et al., 2006). Some catechin derivatives can oxidize the cysteine thiols of Keap1, which will form disulfide bonds and release the Nfr2 (Na and Surh, 2008). For instance, under the influence of EGCG, the expression of Nrf2 decreased in cytoplasm and increased in the nucleus. Yu et al. found that ECG activated the Nrf2 and increased expression of HO-1 in ox-LDL induced VSMCs that previously had a very low expression of HO-1 and Nrf2 protein. This implies that ECG significantly ameliorated the atherosclerotic damage of VSMCs (Yu et al., 2021). Zheng et al. showed that after treatment with EGCG, nuclear accumulation of Nrf2 was significantly increased and the binding of Nrf2-ARE was also enhanced. (Zheng et al., 2012). Hence, we can conclude that EGCG can

influence the mRNA expression, activity and/or protein level of Nrf2 target genes (Wang et al., 2015).

3.4.2.2 PPAR pathways

Peroxisome proliferator-activated receptors (PPARs) are parts of nuclear receptor superfamily of ligand-activated transcription factors, including three member isoforms—α, β/, and γ. (Lee and Kim, 2015). PPAR- *a* is an important target for the treatment of lipid metabolism disorder, because it can regulate the expression of many lipid related genes, (Janssen et al., 2015; Kersten and Stienstra, 2017). PPAR- γ regulates target genes downstream involved in lipid production, and promotes fatty acid transport and deposition (Janani and Ranjitha Kumari, 2015; Xu et al., 2018). It is reported that pretreatment with PPAR-γ-specific antagonist saved the inhibition of activation and phosphorylation of AKT/STAT3/p38MAPK caused by PPAR-γ agonist. Therefore, PPAR -γ agonist can play an antioxidant role by means of the PPAR - γ -AKT/STAT3/p38 MAPK—Snail signaling pathway (Liu et al., 2020). After incubation with TNFα for 24 h, PPAR-γ protein levels decreased by 51% in lysates of 3T3-L1 adipocytes. EC attenuated the downregulation of PPARγ expression mediated by TNFα and reduced nuclear DNA binding (Vazquez-Prieto et al., 2012). Similarly, studies have shown that EGCG can also restore the down-regulation expression of PPAR- γ(Peng et al., 2011). Therefore, EC and EGCG may act as PPAR-γ agonists to exert antioxidant effects. In addition, PPAR-γ coactivator-1α (PGC-1α) regulates genes involved in lipid metabolism and oxidative stress (Bagattin et al., 2010; Katsouri et al., 2012; Wenz, 2013). It is also involved in the activation of PPARα Homologous. PGC1α and PPARα are key factors in antioxidant response (Fracassi et al., 2021). Research has proven that the activation of PPARα can trigger the activation of CAT, while PGC1α can regulate expression and localization of SOD2 and GPx1 (Figuer 3) (St-Pierre et al., 2006; Shin et al., 2016). The use of EC rescued the decrease in level of PGC-1α, and exhibited beneficial effects on obesity and decreased relevant cardiometabolic risk factors (Gutiérrez-Salmeán et al., 2014). Marinovic et al. demonstrated that EGC and EC can indirectly activate PPARα and reduce hepatic steatosis (Marinovic et al., 2022). Unfortunately, there are insufficient reports on the role of PPAR pathway in oxidative stress with catechins. Its role in ROS metabolism too has not been explored to a large extent.

3.4.2.3 MAPK pathways

The MAPK (mitogen-activated protein kinase) signaling cascades involving MAPKs ERK (extracellular signal regulated kinase), JNK (c-Jun N-terminal kinase) and p38 MAPK may play an important role in atherosclerosis and vascular restenosis (Muslin, 2008). Inhibition of the cascade is believed to protect cells from oxidative stress. Evidence suggests that when JNK, ERK, and p38 proteins are activated, ROS level increases, leading to oxidative stress and subsequently apoptosis (Kong et al., 2021). Specifically, the JNK pathway has been demonstrated to be part of oxidative stress responses in tumors, suggesting that inhibition of JNK signaling may be helpful to prevent several ROS-induced metabolic diseases (Li C et al., 2019). Activation of AP-1, a transcription factor, occurs through the MAPK pathway. Its activity is influenced by the intracellular redox environment, including the level of ROS and antioxidants (Figuer 3) (Higdon, J. V. and Frei, B., 2003; Nomura et al., 2000). EGCG can minimize the damage to endothelial cells and reduce IL-6 and TNF-α by inhibiting AP-1 activity (Riegsecker et al., 2013; Wang Z M et al., 2014). Catechins seem to inhibit AP-1 activity through inhibiting kinases in the MAPK pathway, such as JNK and Erks (Katiyar et al., 2001). EGCG was observed to significantly prevent thrombin-induced caspase 3 activation and apoptosis by suppressing JNK phosphorylation (He et al., 2015). It also inhibited the production of plasminogen activator inhibitor-1 mediated by TNFα and reduced ERK1/2 phosphorylation (Cao et al., 2013). Treatment with a standardized green tea polyphenol decoction containing 65% EGCG reduce the phosphorylation levels of c-Jun and Erk1/2 (Lu et al., 2006).

3.4.2.4 NF-κB pathways

The molecular signaling pathway regulated by catechins is responsible for its pro-apoptotic and anti-proliferative characteristics. One of which is the inhibition of a key oxidative stress-sensitive transcription factor -nuclear factor-κB (NF-κB) (Khan and Mukhtar, 2013; Musial et al., 2020). After exposure to oxidative and inflammatory stimuli, I κ B kinase (IKK) is activated, leading to IKK signalsome phosphorylation, which are subsequently degraded by the proteasome. Then NF-κB translocates to the nucleus, where it binds to specific promoter regions and initiates transcription. (Karin, 1999; Surh, 2003). In addition, NF-κB may aggravate oxidative stress by influencing the Nrf2 signaling pathway. Being a protein downstream of NF-κB, the research have shown that p65 may exert conflicting effects in the Nrf2 signaling pathway by accelerating peroxidation, leading to abnormal cell proliferation (Figuer 3) (Yang et al., 2020). Catechins, especially EGCG, can block the activation of NF-κB (Varilek et al., 2001) by many pro-inflammatory stimuli and inhibit the activity of I κ B kinase β (IKKβ, the key kinase for activating NF-kB pathway) (Youn et al., 2006). It was found that EGCG can reduce p65 expression induced by PCB (polychlorinated biphenyls) 126 and down-regulate the expression of NF-κB regulated genes, further suppressing endothelial cells inflammation (Liu et al., 2016). Another experiment discovered that ECG inhibited the phosphorylation of p65 in the NF-κB pathway, and reduce the lipid disorder and atherosclerotic lesions in ApoE$^{-/-}$ mice induced by high fat diet (Yu et al., 2021).

4 Potential problems of catechin application

Many studies have proven that catechins are protective against AS and are effective natural antioxidants. However, there are still a few limitations in place such as metabolite activity and low bioavailability.

Because catechins are rapidly and extensively metabolized, *in vitro* experiments data and the biological activity of catechins metabolites are often questioned. It is hence particularly important to demonstrate catechins antioxidant activity *in vivo*. Catechins have been found to experienced considerable biotransformation *in vivo*, and their main metabolic pathways are methylation, glucuronidation, sulfation and ring-fission metabolism. (Yang

et al., 2002; Feng, 2006). EGCG metabolites and metabolites produced from EC or ECG are proven to have stronger free radical scavenging power than parental catechins (Takagaki et al., 2011). The 30–and 40 -monomethyl ethers of EC can inhibit NADPH oxidase to increase NO in endothelial cells, thus reducing oxidative stress (Steffen et al., 2008). These evidence suggests that catechin metabolites can maintain the antioxidant capacity of their parent compounds. Another metabolic pathway includes the degradation of catechins. Liver and intestine are the backbone of the metabolization and absorption of catechins (Feng, 2006). Besides intestinal and liver metabolites, Sang et al. also found metabolites in colon bacteria (Sang et al., 2008). Investigation found that catechins not metabolized in the upper intestine were transported to the lower intestine through intestinal microflora (Roowi et al., 2010). Ottaviani et al. found that 70% of the ingested (¡)-epicatechin was absorbed by the lower intestinal after catabolism of intestinal microflora. (Ottaviani et al., 2016). Therefore, there is great research potential in intestinal microbiota to improve production and hence the bioavailability of catechin metabolites. It is also important to continue studying the antioxidant effect of metabolites to find the optimal condition for catechins to play an antioxidant role better in the local intestinal.

Tea polyphenols are susceptible to degradation under environmental stresses or digestive circumstances, such as alkaline pH and high temperature. In addition, the low bioavailability of catechins also due to degradation and metabolism in the gastrointestinal tract, poor membrane permeability, and pre-systemic hepatic clearance (Ye and Augustin, 2019; Sabaghi et al., 2021). The development of new agents, such as nanoparticles, may become an effective way to solve this problem in the future. Recently, studies found that nanomaterials based on carbon, nanozymes, and nanomedicine could improve stability of antioxidant treatments and further upgrade the antioxidant effect. For instance, nitrogen-doped carbon nanodots ionogels (Rizzo et al., 2018), Mn (3) O (4) nanozymes (Yao et al., 2018), and colloidal-stable nanotherapeutics made of bioadhesive chitosan materials that are suitable for oral delivery (Han et al., 2019). Green nanoparticles (GNPs) prepared by Yang et al. using TP in green tea as the monomer have strong free radical scavenging ability and oxidation resistance. The research provides a new green strategy for making safe and effective antioxidants. (Yang et al., 2021). It has been reported that synergistic effects of the combination of EGCG and fish oil. The presence of fish oil increased the bioavailability of EGCG (Giunta et al., 2010). Furthermore, using broccoli byproducts as the matrix for co-delivery of EGCG and fish oil could prevent the degradation of EGCG in the upper gastrointestinal tract can thus be metabolized by the microorganisms in the lower gut, leading to an increase in EGCG bioavailability (Shi et al., 2020). In addition, the combination of catechins with other drugs that show synergistic effects may be a promising approach, such as catechins showing good synergy with some conventional anticancer drugs (Cai et al., 2018).

Moreover, under certain conditions, catechins may have both prooxidative or toxic effects. The dual antioxidant and pro-oxidant functions of catechins depend primarily on the dose level and the biological context. In a safety study that examined genetic, acute, and short-term toxicity of EGCG, a no-adverse effect level (NOAEL) of 500 mg/kg/day of EGCG was determined (Isbrucker et al., 2006). Some European regulators have suggested that the tolerable upper intake level of EGCG should be 300 mg per day for humans (Yates et al., 2017). Tian et al. found that at 0.04%, TP promotes the oxidation of protein in emulsions with proteins at the interface, but still has a certain antioxidant effect on aqueous phase proteins. It is possible to optimize the TP level of foods or beverages based on emulsion to achieve the best antioxidant activity (Tian et al., 2022).

5 Conclusion

With the aging of the general population and the increase in chronic diseases such as hypertension and diabetes, the incidence rate of atherosclerosis further increase. Atherosclerosis has no obvious early symptoms. When the disease progresses to a higher stage with age, symptoms of atherosclerosis will appear. Therefore, it is very important to seek preventive diet or drugs, and the strategy of prevention before disease will greatly reduce hospital costs and other economic burdens of patients. The development of natural products to prevent AS has scientific significance and application value. At the same time, the discovery of lipid oxidation products implies that oxidative stress promotes the change of lipid metabolism, which provides a new idea for the treatment of diseases with abnormal lipid metabolism.

Tea, especially unfermented green tea, is rich in catechins, which have antioxidation and improve lipid metabolism disorders. The health benefits of tea are largely attributed to the effects of catechins. However, catechins correspond to a variety of targets and act through different signaling pathways. Due to the pleiotropic effects of catechins, more definitive studies on their biological functions and anti-atherosclerotic mechanisms are lacking before their clinical application. Current studies have not systematically revealed the mechanism of catechins in anti-oxidative stress to regulate abnormal lipid metabolism in AS. Therefore, we hope to clarify the therapeutic effect of catechin in AS by combing the mechanism of catechin regulating oxidative stress and improving abnormal lipid metabolism. This study will provide a reference for the subsequent development of catechin as AS adjuvant drugs.

Catechins play an antioxidant role in many ways, namely, by balancing enzyme activity and regulating signal pathways. They inhibit NADPH oxidase, XO, COX2, NOS, and other enzymes that produce ROS and activate antioxidants in the body, such as GSH, SOD, CAT, GPX, GST, NQO1, to significantly improve the antioxidant response. Concurrently, catechins induce the activation of Keap1/Nrf2/ARE signal pathway, inhibit the activation of MAPK/AP-1 pathway, and block the activation of transcription factor NF- κB and increase PPAR γ, PGC1 *a* And PPAR *a* Protein level. These reactions all work together to help reduce oxidative stress.

It is noteworthy to point out that there are still many limiting factors for the application of catechins, such as prooxidative and toxic effects under certain conditions, the dubious activity of its metabolites and low bioavailability. Determining the safe dose of catechin and finding the biological environment that can exert the best antioxidant activity of catechin are effective methods to

overcome the pro-oxidative side effects of catechin. Promoting the catabolism of catechins by intestinal flora can enhance the absorption and utilization of the host. Isolation and identification of microorganisms and microbial metabolites with the ability to catabolize the active catechins may be one of the methods to improve the utilization of catechins. The development of new preparations of catechins based on nanomaterials greatly improves their antioxidant stability. The combination of catechin with other bioactive dietary compounds and disease treatment drugs can play a synergistic effect of promoting the absorption and utilization of both sides. All these provides a new idea for solving the problem of low bioavailability of catechins.

Current research on catechins focuses on functional and metabolic studies. In the future research, the physiological function of catechins can be combined with their chemical structure and *in vivo* process. More clinical trials can be carried out to further verify the role of catechins in the prevention and treatment of AS. Studies on the pharmacokinetics and pharmacodynamics will be the focus of the application of catechins in AS. In order to improve the clinical application of catechins, the combination of catechins with existing AS drugs may become a direction of research on AS treatment. The potential combination of pharmaceutical and nutritional levels is able to establish a more effective treatment regimen.

More researches are needed to elucidate the antioxidant mechanism of catechins. Despite its limitations, we can effectively conclude that regular intake of an appropriate amount of tea can regulate the antioxidant capacity of the human body, improve lipid metabolism, and hence prevent atherosclerosis.

Author contributions

YuS, YiS, and YT lead the conception and design of the manuscript. YuS and YiS drafted the manuscript and figures. YuS, YiS, YY, and JW collected and interpreted the relevant literature. FZ, YL, YT, and YaS contributed to the provided guidance of the whole manuscript and reviewed the manuscript. All the authors of the article has made a contribution, and approved the version submitted.

Funding

We are grateful for funds supported from the National Natural Science Foundation of China (Grant No 82074325).

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

Ahmad, N., and Mukhtar, H. (1999). Green tea polyphenols and cancer: Biologic mechanisms and practical implications. *Nutr. Rev.* 57 (3), 78–83. doi:10.1111/j.1753-4887.1999.tb06927.x

Araujo, J. A., Zhang, M., and Yin, F. (2012). Heme oxygenase-1, oxidation, inflammation, and atherosclerosis. *Front. Pharmacol.* 3, 119. doi:10.3389/fphar.2012.00119

Auch-Schwelk, W., Katusic, Z. S., and Vanhoutte, P. M. (1990). Thromboxane A2 receptor antagonists inhibit endothelium-dependent contractions. *Hypertension* 15 (6), 699–703. doi:10.1161/01.hyp.15.6.699

Auger, C., Kim, J. H., Chabert, P., Chaabi, M., Anselm, E., Lanciaux, X., et al. (2010). The EGCg-induced redox-sensitive activation of endothelial nitric oxide synthase and relaxation are critically dependent on hydroxyl moieties. *Biochem. Biophys. Res. Commun.* 393 (1), 162–167. doi:10.1016/j.bbrc.2010.01.112

Augusti, P. R., Conterato, G. M., Somacal, S., Sobieski, R., Spohr, P. R., Torres, J. V., et al. (2008). Effect of astaxanthin on kidney function impairment and oxidative stress induced by mercuric chloride in rats. *Food Chem. Toxicol.* 46 (1), 212–219. doi:10.1016/j.fct.2007.08.001

Bagattin, A., Hugendubler, L., and Mueller, E. (2010). Transcriptional coactivator PGC-1alpha promotes peroxisomal remodeling and biogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 107 (47), 20376–20381. doi:10.1073/pnas.1009176107

Bai, Y., Wang, X., Zhao, S., Ma, C., Cui, J., and Zheng, Y. (2015). Sulforaphane protects against cardiovascular disease via Nrf2 activation. *Oxid. Med. Cell Longev.* 2015, 407580. doi:10.1155/2015/407580

Bartoszek, M., Polak, J., and Chorążewski, M. (2018). Comparison of antioxidant capacities of different types of tea using the spectroscopy methods and semi-empirical mathematical model. *Eur. Food Res. Technol.* 244 (4), 595–601. doi:10.1007/s00217-017-2986-z

Basu, A., and Lucas, E. A. (2007). Mechanisms and effects of green tea on cardiovascular health. *Nutr. Rev.* 65 (8), 361–375. doi:10.1301/nr.2007.aug.361-375

Bedard, K., and Krause, K. H. (2007). The NOX family of ROS-generating NADPH oxidases: Physiology and pathophysiology. *Physiol. Rev.* 87 (1), 245–313. doi:10.1152/physrev.00044.2005

Benzie, I. F. F., and Wachtel-Galor, S. (2011), Herbal medicine: Biomolecular and clinical aspects. In *CRC press/taylor & francis copyright © 2011*, (Taylor and Francis Group, LLC.: Boca Raton FL).

Bernatoniene, J., and Kopustinskiene, D. M. (2018). The role of catechins in cellular responses to oxidative stress. *Molecules* 23 (4), 965. doi:10.3390/molecules23040965

Boléa, G., Ginies, C., Vallier, M. J., and Dufour, C. (2019). Lipid protection by polyphenol-rich apple matrices is modulated by pH and pepsin in *in vitro* gastric digestion. *Food Funct.* 10 (7), 3942–3954. doi:10.1039/c9fo00705a

Byrne, N. J., Rajasekaran, N. S., Abel, E. D., and Bugger, H. (2021). Therapeutic potential of targeting oxidative stress in diabetic cardiomyopathy. *Free Radic. Biol. Med.* 169, 317–342. doi:10.1016/j.freeradbiomed.2021.03.046

Cabrera, C., Artacho, R., and Giménez, R. (2006). Beneficial effects of green tea--a review. *J. Am. Coll. Nutr.* 25 (2), 79–99. doi:10.1080/07315724.2006.10719518

Cai, C., Zhu, H., Ning, X., Li, L., Yang, B., Chen, S., et al. (2019). LncRNA ENST00000602558.1 regulates ABCG1 expression and cholesterol efflux from vascular smooth muscle cells through a p65-dependent pathway. *Atherosclerosis* 285, 31–39. doi:10.1016/j.atherosclerosis.2019.04.204

Cai, H. (2005). Hydrogen peroxide regulation of endothelial function: Origins, mechanisms, and consequences. *Cardiovasc Res.* 68 (1), 26–36. doi:10.1016/j.cardiores.2005.06.021

Cai, Z. Y., Li, X. M., Liang, J. P., Xiang, L. P., Wang, K. R., Shi, Y. L., et al. (2018). Bioavailability of tea catechins and its improvement. *Molecules* 23 (9), 2346. doi:10.3390/molecules23092346

Cao, Y., Wang, D., Wang, X., Zhang, J., Shan, Z., and Teng, W. (2013). (-)-Epigallocatechin gallate inhibits TNF-α-induced PAI-1 production in vascular

endothelial cells. *J. Cardiovasc Pharmacol.* 62 (5), 452–456. doi:10.1097/FJC.0b013e3182a18ba8

Carnevale, R., Loffredo, L., Nocella, C., Bartimoccia, S., Bucci, T., De Falco, E., et al. (2014). Epicatechin and catechin modulate endothelial activation induced by platelets of patients with peripheral artery disease. *Oxid. Med. Cell Longev.* 2014, 691015, doi:10.1155/2014/691015

Chan, S., Kantham, S., Rao, V. M., Palanivelu, M. K., Pham, H. L., Shaw, P. N., et al. (2016). Metal chelation, radical scavenging and inhibition of Aβ42 fibrillation by food constituents in relation to Alzheimer's disease. *Food Chem.* 199, 185–194. doi:10.1016/j.foodchem.2015.11.118

Chang, H. H., Chien, C. Y., Chen, K. H., Huang, S. C., and Chien, C. T. (2017). Catechins blunt the effects of oxLDL and its primary metabolite phosphatidylcholine hydroperoxide on endothelial dysfunction through inhibition of oxidative stress and restoration of eNOS in rats. *Kidney Blood Press Res.* 42 (5), 919–932. doi:10.1159/000485082

Chantre, P., and Lairon, D. (2002). Recent findings of green tea extract AR25 (Exolise) and its activity for the treatment of obesity. *Phytomedicine* 9 (1), 3–8. doi:10.1078/0944-7113-00078

Chatterjee, A., and Gupta, S. (2018). The multifaceted role of glutathione S-transferases in cancer. *Cancer Lett.* 433, 33–42. doi:10.1016/j.canlet.2018.06.028

Chen, C. N., Chang, K. C., Lin, R. F., Wang, M. H., Shih, R. L., Tseng, H. C., et al. (2016). Nitric oxide pathway activity modulation alters the protective effects of (-) Epigallocatechin-3-gallate on reserpine-induced impairment in rats. *Behav. Brain Res.* 305, 198–211. doi:10.1016/j.bbr.2016.02.038

Chen, J. Y., Ye, Z. X., Wang, X. F., Chang, J., Yang, M. W., Zhong, H. H., et al. (2018). Nitric oxide bioavailability dysfunction involves in atherosclerosis. *Biomed. Pharmacother.* 97, 423–428. doi:10.1016/j.biopha.2017.10.122

Chen, Q. M. (2021). Nrf2 for cardiac protection: Pharmacological options against oxidative stress. *Trends Pharmacol. Sci.* 42 (9), 729–744. doi:10.1016/j.tips.2021.06.005

Daiber, A., Xia, N., Steven, S., Oelze, M., Hanf, A., Kröller-Schön, S., et al. (2019). New therapeutic implications of endothelial nitric oxide synthase (eNOS) function/dysfunction in cardiovascular disease. *Int. J. Mol. Sci.* 20 (1), 187. doi:10.3390/ijms20010187

Ding, S., Jiang, J., Yu, P., Zhang, G., Zhang, G., and Liu, X. (2017). Green tea polyphenol treatment attenuates atherosclerosis in high-fat diet-fed apolipoprotein E-knockout mice via alleviating dyslipidemia and up-regulating autophagy. *PLoS One* 12 (8), e0181666. doi:10.1371/journal.pone.0181666

Dinkova-Kostova, A. T., and Talalay, P. (2010). NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1), a multifunctional antioxidant enzyme and exceptionally versatile cytoprotector. *Arch. Biochem. Biophys.* 501 (1), 116–123. doi:10.1016/j.abb.2010.03.019

Dower, J. I., Geleijnse, J. M., Gijsbers, L., Schalkwijk, C., Kromhout, D., and Hollman, P. C. (2015). Supplementation of the pure flavonoids epicatechin and quercetin affects some biomarkers of endothelial dysfunction and inflammation in (Pre)Hypertensive adults: A randomized double-blind, placebo-controlled, crossover trial. *J. Nutr.* 145 (7), 1459–1463. doi:10.3945/jn.115.211888

Drummond, G. R., Selemidis, S., Griendling, K. K., and Sobey, C. G. (2011). Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets. *Nat. Rev. Drug Discov.* 10 (6), 453–471. doi:10.1038/nrd3403

Duarte, J., Jiménez, R., O'Valle, F., Galisteo, M., Pérez-Palencia, R., Vargas, F., et al. (2002). Protective effects of the flavonoid quercetin in chronic nitric oxide deficient rats. *J. Hypertens.* 20 (9), 1843–1854. doi:10.1097/00004872-200209000-00031

Espinosa, C., López-Jiménez, J., Cabrera, L., Larqué, E., Almajano, M. P., Arnao, M. B., et al. (2012). Protective effect of white tea extract against acute oxidative injury caused by adriamycin in different tissues. *Food Chem.* 134 (4), 1780–1785. doi:10.1016/j.foodchem.2012.03.083

Faria, A. M., Papadimitriou, A., Silva, K. C., Lopes de Faria, J. M., and Lopes de Faria, J. B. (2012). Uncoupling endothelial nitric oxide synthase is ameliorated by green tea in experimental diabetes by re-establishing tetrahydrobiopterin levels. *Diabetes* 61 (7), 1838–1847. doi:10.2337/db11-1241

Feng, W. Y. (2006). Metabolism of green tea catechins: An overview. *Curr. Drug Metab.* 7 (7), 755–809. doi:10.2174/138920006778520552

Förstermann, U., and Münzel, T. (2006). Endothelial nitric oxide synthase in vascular disease: From marvel to menace. *Circulation* 113 (13), 1708–1714. doi:10.1161/CIRCULATIONAHA.105.602532

Förstermann, U., and Sessa, W. C., 2012. Nitric oxide synthases: Regulation and function. *Eur. Heart J.* 33(7) 837a-837d. doi:10.1093/eurheartj/ehr304

Forstermann, U., Xia, N., and Li, H. (2017). Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. *Circ. Res.* 120 (4), 713–735. doi:10.1161/CIRCRESAHA.116.309326

Fracassi, A., Marcatti, M., Zolochevska, O., Tabor, N., Woltjer, R., Moreno, S., et al. (2021). Oxidative damage and antioxidant response in frontal cortex of demented and nondemented individuals with alzheimer's neuropathology. *J. Neurosci.* 41 (3), 538–554. doi:10.1523/JNEUROSCI.0295-20.2020

Friedrich, M., Petzke, K. J., Raederstorff, D., Wolfram, S., and Klaus, S. (2012). Acute effects of epigallocatechin gallate from green tea on oxidation and tissue incorporation of dietary lipids in mice fed a high-fat diet. *Int. J. Obes. (Lond)* 36 (5), 735–743. doi:10.1038/ijo.2011.136

Gardner, E. J., Ruxton, C. H., and Leeds, A. R. (2007). Black tea--helpful or harmful? A review of the evidence. *Eur. J. Clin. Nutr.* 61 (1), 3–18. doi:10.1038/sj.ejcn.1602489

George, J., Tsuchishima, M., and Tsutsumi, M. (2022). Epigallocatechin-3-gallate inhibits osteopontin expression and prevents experimentally induced hepatic fibrosis. *Biomed. Pharmacother.* 151, 113111. doi:10.1016/j.biopha.2022.113111

Giunta, B., Hou, H., Zhu, Y., Salemi, J., Ruscin, A., Shytle, R. D., et al. (2010). Fish oil enhances anti-amyloidogenic properties of green tea EGCG in Tg2576 mice. *Neurosci. Lett.* 471 (3), 134–138. doi:10.1016/j.neulet.2010.01.026

Gomez-Guzman, M., Jimenez, R., Sanchez, M., Romero, M., O'Valle, F., Lopez-Sepulveda, R., et al. (2011). Chronic (-)-epicatechin improves vascular oxidative and inflammatory status but not hypertension in chronic nitric oxide-deficient rats. *Br. J. Nutr.* 106 (9), 1337–1348. doi:10.1017/S0007114511004314

Graham, H. N. (1992). Green tea composition, consumption, and polyphenol chemistry. *Prev. Med.* 21 (3), 334–350. doi:10.1016/0091-7435(92)90041-f

Griendling, K. K., Camargo, L. L., Rios, F. J., Alves-Lopes, R., Montezano, A. C., and Touyz, R. M. (2021). Oxidative stress and hypertension. *Circ. Res.* 128 (7), 993–1020. doi:10.1161/CIRCRESAHA.121.318063

Guo, Q., Zhao, B., Li, M., Shen, S., and Xin, W. (1996). Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes. *Biochim. Biophys. Acta* 1304 (3), 210–222. doi:10.1016/s0005-2760(96)00122-1

Gupta, M., Blumenthal, C., Chatterjee, S., Bandyopadhyay, D., Jain, V., Lavie, C. J., et al. (2020). Novel emerging therapies in atherosclerosis targeting lipid metabolism. *Expert Opin. Investig. Drugs* 29 (6), 611–622. doi:10.1080/13543784.2020.1764937

Gutiérrez-Salmeán, G., Ortiz-Vilchis, P., Vacaseydel, C. M., Garduño-Siciliano, L., Chamorro-Cevallos, G., Meaney, E., et al. (2014). Effects of (-)-epicatechin on a diet-induced rat model of cardiometabolic risk factors. *Eur. J. Pharmacol.* 728, 24–30. doi:10.1016/j.ejphar.2014.01.053

Guzik, T. J., Sadowski, J., Guzik, B., Jopek, A., Kapelak, B., Przybylowski, P., et al. (2006). Coronary artery superoxide production and nox isoform expression in human coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* 26 (2), 333–339. doi:10.1161/01.ATV.0000196651.64776.51

Han, S. G., Han, S. S., Toborek, M., and Hennig, B. (2012). EGCG protects endothelial cells against PCB 126-induced inflammation through inhibition of AhR and induction of Nrf2-regulated genes. *Toxicol. Appl. Pharmacol.* 261 (2), 181–188. doi:10.1016/j.taap.2012.03.024

Han, W., Xie, B., Li, Y., Shi, L., Wan, J., Chen, X., et al. (2019). Orally deliverable nanotherapeutics for the synergistic treatment of colitis-associated colorectal cancer. *Theranostics* 9 (24), 7458–7473. doi:10.7150/thno.38081

Hara, Y. (1994). Physiological functions of tea polyphenols: Part 2. *Am. Biotechnol. Lab.* 12 (9), 18.

Hayes, J. D., Flanagan, J. U., and Jowsey, I. R. (2005). Glutathione transferases. *Annu. Rev. Pharmacol. Toxicol.* 45, 51–88. doi:10.1146/annurev.pharmtox.45.120403.095857

He, Q., Bao, L., Zimering, J., Zan, K., Zhang, Z., Shi, H., et al. (2015). The protective role of (-)-epigallocatechin-3-gallate in thrombin-induced neuronal cell apoptosis and JNK-MAPK activation. *Neuroreport* 26 (7), 416–423. doi:10.1097/WNR.0000000000000363

Higdon, J. V., and Frei, B. (2003). Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. *Crit. Rev. Food Sci. Nutr.* 43 (1), 89–143. doi:10.1080/10408690390826464

Hoseini, Z., Sepahvand, F., Rashidi, B., Sahebkar, A., Masoudifar, A., and Mirzaei, H. (2018). NLRP3 inflammasome: Its regulation and involvement in atherosclerosis. *J. Cell Physiol.* 233 (3), 2116–2132. doi:10.1002/jcp.25930

Hu, L., Tian, K., Zhang, T., Fan, C. H., Zhou, P., Zeng, D., et al. (2019). Cyanate induces oxidative stress injury and abnormal lipid metabolism in liver through Nrf2/HO-1. *Molecules* 24 (18), 3231. doi:10.3390/molecules24183231

Inami, S., Takano, M., Yamamoto, M., Murakami, D., Tajika, K., Yodogawa, K., et al. (2007). Tea catechin consumption reduces circulating oxidized low-density lipoprotein. *Int. Heart J.* 48 (6), 725–732. doi:10.1536/ihj.48.725

Isbrucker, R. A., Edwards, J. A., Wolz, E., Davidovich, A., and Bausch, J. (2006). Safety studies on epigallocatechin gallate (EGCG) preparations. Part 2: Dermal, acute and short-term toxicity studies. *Food Chem. Toxicol.* 44 (5), 636–650. doi:10.1016/j.fct.2005.11.003

Isemura, M. (2019). Catechin in human health and disease. *Molecules* 24 (3), 528. doi:10.3390/molecules24030528

Janani, C., and Ranjitha Kumari, B. D. (2015). PPAR gamma gene--a review. *Diabetes Metab. Syndr.* 9 (1), 46–50. doi:10.1016/j.dsx.2014.09.015

Janssen, A. W., Betzel, B., Stoopen, G., Berends, F. J., Janssen, I. M., Peijnenburg, A. A., et al. (2015). The impact of PPARα activation on whole genome gene expression in human precision cut liver slices. *BMC Genomics* 16, 760. doi:10.1186/s12864-015-1969-3

Juan, C. A., Perez de la Lastra, J. M., Plou, F. J., and Perez-Lebena, E. (2021). The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *Int. J. Mol. Sci.* 22 (9), 4642. doi:10.3390/ijms22094642

Kalyanaraman, B. (2013). Teaching the basics of redox biology to medical and graduate students: Oxidants, antioxidants and disease mechanisms. *Redox Biol.* 1 (1), 244–257. doi:10.1016/j.redox.2013.01.014

Kang, M. I., Kobayashi, A., Wakabayashi, N., Kim, S. G., and Yamamoto, M. (2004). Scaffolding of Keap1 to the actin cytoskeleton controls the function of Nrf2 as key regulator of cytoprotective phase 2 genes. *Proc. Natl. Acad. Sci. U. S. A.* 101 (7), 2046–2051. doi:10.1073/pnas.0308347100

Karin, M. (1999). How NF-kappaB is activated: The role of the IkappaB kinase (IKK) complex. *Oncogene* 18 (49), 6867–6874. doi:10.1038/sj.onc.1203219

Katiyar, S. K., Afaq, F., Azizuddin, K., and Mukhtar, H. (2001). Inhibition of UVB-induced oxidative stress-mediated phosphorylation of mitogen-activated protein kinase signaling pathways in cultured human epidermal keratinocytes by green tea polyphenol (-)-epigallocatechin-3-gallate. *Toxicol. Appl. Pharmacol.* 176 (2), 110–117. doi:10.1006/taap.2001.9276

Katsouri, L., Blondrath, K., and Sastre, M. (2012). Peroxisome proliferator-activated receptor-γ cofactors in neurodegeneration. *IUBMB Life* 64 (12), 958–964. doi:10.1002/iub.1097

Kawai, Y., Tanaka, H., Murota, K., Naito, M., and Terao, J. (2008). (-)-Epicatechin gallate accumulates in foamy macrophages in human atherosclerotic aorta: Implication in the anti-atherosclerotic actions of tea catechins. *Biochem. Biophys. Res. Commun.* 374 (3), 527–532. doi:10.1016/j.bbrc.2008.07.086

Keller, J., Baradat, M., Jouanin, I., Debrauwer, L., and Guéraud, F. (2015). Twin peaks": Searching for 4-hydroxynonenal urinary metabolites after oral administration in rats. *Redox Biol.* 4, 136–148. doi:10.1016/j.redox.2014.12.016

Kersten, S., and Stienstra, R. (2017). The role and regulation of the peroxisome proliferator activated receptor alpha in human liver. *Biochimie* 136, 75–84. doi:10.1016/j.biochi.2016.12.019

Khan, N., and Mukhtar, H. (2013). Tea and health: Studies in humans. *Curr. Pharm. Des.* 19 (34), 6141–6147. doi:10.2174/1381612811319340008

Khatana, C., Saini, N. K., Chakrabarti, S., Saini, V., Sharma, A., Saini, R. V., et al. (2020). Mechanistic insights into the oxidized low-density lipoprotein-induced atherosclerosis. *Oxid. Med. Cell Longev.* 2020, 5245308. doi:10.1155/2020/5245308

Kishimoto, Y., Saita, E., Taguchi, C., Aoyama, M., Ikegami, Y., Ohmori, R., et al. (2020). Associations between green tea consumption and coffee consumption and the prevalence of coronary artery disease. *J. Nutr. Sci. Vitaminol. (Tokyo)* 66 (3), 237–245. doi:10.3177/jnsv.66.237

Koch, W., Kukuła-Koch, W., Czop, M., Helon, P., and Gumbarewicz, E. (2020). The role of extracting solvents in the recovery of polyphenols from green tea and its antiradical activity supported by principal component analysis. *Molecules* 25 (9)

Kondo, K., Kurihara, M., Miyata, N., Suzuki, T., and Toyoda, M. (1999). Scavenging mechanisms of (-)-epigallocatechin gallate and (-)-epicatechin gallate on peroxyl radicals and formation of superoxide during the inhibitory action. *Free Radic. Biol. Med.* 27 (7-8), 855–863. doi:10.1016/s0891-5849(99)00133-1

Kondo, M., Nishi, K., and Sugahara, T. (2015). Ishizuchi dark tea suppresses IgE-mediated degranulation of RBL-2H3 cells and nasal rubbing behavior of pollinosis in mice. *J. Funct. Foods* 14, 659–669. doi:10.1016/j.jff.2015.02.045

Kong, A. N., Owuor, E., Yu, R., Hebbar, V., Chen, C., Hu, R., et al. (2001). Induction of xenobiotic enzymes by the MAP kinase pathway and the antioxidant or electrophile response element (ARE/EpRE). *Drug Metab. Rev.* 33 (3-4), 255–271. doi:10.1081/dmr-120000652

Kong, L., Zhu, L., Yi, X., Huang, Y., Zhao, H., Chen, Y., et al. (2021). Betulinic acid alleviates spleen oxidative damage induced by acute intraperitoneal exposure to T-2 toxin by activating Nrf2 and inhibiting MAPK signaling pathways. *Antioxidants (Basel)* 10 (2), 158. doi:10.3390/antiox10020158

Kumar, N., Kant, R., and Maurya, P. K. (2010). Concentration-dependent effect of (-) epicatechin in hypertensive patients. *Phytother. Res.* 24 (10), 1433–1436. doi:10.1002/ptr.3119

Lambert, J. D., and Elias, R. J. (2010). The antioxidant and pro-oxidant activities of green tea polyphenols: A role in cancer prevention. *Arch. Biochem. Biophys.* 501 (1), 65–72. doi:10.1016/j.abb.2010.06.013

Lee, W. S., and Kim, J. (2015). Peroxisome proliferator-activated receptors and the heart: Lessons from the past and future directions. *PPAR Res.* 2015, 271983. doi:10.1155/2015/271983

Leung, L. K., Su, Y., Chen, R., Zhang, Z., Huang, Y., and Chen, Z. Y. (2001). Theaflavins in black tea and catechins in green tea are equally effective antioxidants. *J. Nutr.* 131 (9), 2248–2251. doi:10.1093/jn/131.9.2248

Li, C., Miao, X., Li, F., Adhikari, B. K., Liu, Y., Sun, J., et al. (2019). Curcuminoids: Implication for inflammation and oxidative stress in cardiovascular diseases. *Phytother. Res.* 33 (5), 1302–1317. doi:10.1002/ptr.6324

Li, H., and Förstermann, U. (2013). Uncoupling of endothelial NO synthase in atherosclerosis and vascular disease. *Curr. Opin. Pharmacol.* 13 (2), 161–167. doi:10.1016/j.coph.2013.01.006

Li, H., Horke, S., and Förstermann, U. (2014). Vascular oxidative stress, nitric oxide and atherosclerosis. *Atherosclerosis* 237 (1), 208–219. doi:10.1016/j.atherosclerosis.2014.09.001

Li, H., Hortmann, M., Daiber, A., Oelze, M., Ostad, M. A., Schwarz, P. M., et al. (2008). Cyclooxygenase 2-selective and nonselective nonsteroidal anti-inflammatory drugs induce oxidative stress by up-regulating vascular NADPH oxidases. *J. Pharmacol. Exp. Ther.* 326 (3), 745–753. doi:10.1124/jpet.108.139030

Li, X., Sun, R., and Liu, R. (2019). Natural products in licorice for the therapy of liver diseases: Progress and future opportunities. *Pharmacol. Res.* 144, 210–226. doi:10.1016/j.phrs.2019.04.025

Liaras, K., Fesatidou, M., and Geronikaki, A. (2018). Thiazoles and thiazolidinones as COX/LOX inhibitors. *Molecules* 23 (3), 685. doi:10.3390/molecules23030685

Libby, P., Buring, J. E., Badimon, L., Hansson, G. K., Deanfield, J., Bittencourt, M. S., et al. (2019). Atherosclerosis. *Nat. Rev. Dis. Prim.* 5 (1), 56. doi:10.1038/s41572-019-0106-z

Lin, J. K., Chen, P. C., Ho, C. T., and Lin-Shiau, S. Y. (2000). Inhibition of xanthine oxidase and suppression of intracellular reactive oxygen species in HL-60 cells by theaflavin-3,3'-digallate, (-)-epigallocatechin-3-gallate, and propyl gallate. *J. Agric. Food Chem.* 48 (7), 2736–2743. doi:10.1021/jf000066d

Liu, D., He, Z., Wu, L., and Fang, Y. (2012). Effects of induction/inhibition of endogenous heme oxygenase-1 on lipid metabolism, endothelial function, and atherosclerosis in rabbits on a high fat diet. *J. Pharmacol. Sci.* 118 (1), 14–24. doi:10.1254/jphs.11071fp

Liu, D., Perkins, J. T., and Hennig, B. (2016). EGCG prevents PCB-126-induced endothelial cell inflammation via epigenetic modifications of NF-κB target genes in human endothelial cells. *J. Nutr. Biochem.* 28, 164–170. doi:10.1016/j.jnutbio.2015.10.003

Liu, Y., Chen, S., Liu, J., Jin, Y., Yu, S., and An, R. (2020). Telmisartan inhibits oxalate and calcium oxalate crystal-induced epithelial-mesenchymal transformation via PPAR-γ-AKT/STAT3/p38 MAPK-Snail pathway. *Life Sci.* 241, 117108. doi:10.1016/j.lfs.2019.117108

Lorin, J., Guilland, J. C., Korandji, C., Touzery, C., Bichat, F., Chagnon, A., et al. (2013). High levels of asymmetric dimethylarginine are strongly associated with low HDL in patients with acute myocardial infarction. *PLoS One* 8 (6), e64796. doi:10.1371/journal.pone.0064796

Lu, G., Liao, J., Yang, G., Reuhl, K. R., Hao, X., and Yang, C. S. (2006). Inhibition of adenoma progression to adenocarcinoma in a 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis model in A/J mice by tea polyphenols and caffeine. *Cancer Res.* 66 (23), 11494–11501. doi:10.1158/0008-5472.CAN-06-1497

Lubos, E., Loscalzo, J., and Handy, D. E. (2011). Glutathione peroxidase-1 in health and disease: From molecular mechanisms to therapeutic opportunities. *Antioxid. Redox Signal* 15 (7), 1957–1997. doi:10.1089/ars.2010.3586

Magesh, S., Chen, Y., and Hu, L. (2012). Small molecule modulators of Keap1-Nrf2-ARE pathway as potential preventive and therapeutic agents. *Med. Res. Rev.* 32 (4), 687–726. doi:10.1002/med.21257

Marchio, P., Guerra-Ojeda, S., Vila, J. M., Aldasoro, M., Victor, V. M., and Mauricio, M. D. (2019). Targeting early atherosclerosis: A focus on oxidative stress and inflammation. *Oxid. Med. Cell Longev.* 2019, 8563845. doi:10.1155/2019/8563845

Marinovic, M. P., Sousa-Filho, C. P. B., Batista, F. A. H., Avelino, T. M., Cogliati, B., Figueira, A. C. M., et al. (2022). Green tea extract increases adiponectin and PPAR α levels to improve hepatic steatosis. *J. Nutr. Biochem.* 103, 108957. doi:10.1016/j.jnutbio.2022.108957

Masek, A. (2017). Antioxidant and antiradical properties of green tea extract compounds. *Int. J. Electrochem. Sci.*, 6600–6610. doi:10.20964/2017.07.06

Miltonprabu, S., and Thangapandiyan, S. (2015). Epigallocatechin gallate potentially attenuates Fluoride induced oxidative stress mediated cardiotoxicity and dyslipidemia in rats. *J. Trace Elem. Med. Biol.* 29, 321–335. doi:10.1016/j.jtemb.2014.08.015

Minatti, J., Wazlawik, E., Hort, M. A., Zaleski, F. L., Ribeiro-do-Valle, R. M., Maraschin, M., et al. (2012). Green tea extract reverses endothelial dysfunction and reduces atherosclerosis progression in homozygous knockout low-density lipoprotein receptor mice. *Nutr. Res.* 32 (9), 684–693. doi:10.1016/j.nutres.2012.08.003

Morrow, J. D., Chen, Y., Brame, C. J., Yang, J., Sanchez, S. C., Xu, J., et al. (1999). The isoprostanes: Unique prostaglandin-like products of free-radical-initiated lipid peroxidation. *Drug Metab. Rev.* 31 (1), 117–139. doi:10.1081/dmr-100101910

Musial, C., Kuban-Jankowska, A., and Gorska-Ponikowska, M. (2020). Beneficial properties of green tea catechins. *Int. J. Mol. Sci.* 21 (5), 1744. doi:10.3390/ijms21051744

Muslin, A. J. (2008). MAPK signalling in cardiovascular health and disease: Molecular mechanisms and therapeutic targets. *Clin. Sci. (Lond)* 115 (7), 203–218. doi:10.1042/CS20070430

Na, H. K., and Surh, Y. J. (2008). Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG. *Food Chem. Toxicol.* 46 (4), 1271–1278. doi:10.1016/j.fct.2007.10.006

Nanjo, F., Mori, M., Goto, K., and Hara, Y. (1999). Radical scavenging activity of tea catechins and their related compounds. *Biosci. Biotechnol. Biochem.* 63 (9), 1621–1623. doi:10.1271/bbb.63.1621

Nomura, M., Ma, W. Y., Huang, C., Yang, C. S., Bowden, G. T., Miyamoto, K., et al. (2000). Inhibition of ultraviolet B-induced AP-1 activation by theaflavins from black tea. *Mol. Carcinog.* 28 (3), 148–155. doi:10.1002/1098-2744(200007)28:3<148::aid-mc3>3.0.co;2-q

Orrù, C., Giordano, S., and Columbano, A. (2020). Nrf2 in neoplastic and non-neoplastic liver diseases. *Cancers (Basel)* 12 (10), 2932. doi:10.3390/cancers12102932

Ottaviani, J. I., Borges, G., Momma, T. Y., Spencer, J. P., Keen, C. L., Crozier, A., et al. (2016). The metabolome of [2-(14)C](-)-epicatechin in humans: Implications for the assessment of efficacy, safety, and mechanisms of action of polyphenolic bioactives. *Sci. Rep.* 6, 29034. doi:10.1038/srep29034

Paiva, H., Laakso, J., Ruokonen, I., Luomala, M., Saarela, M., Solakivi, T., et al. (2006). Plasma asymmetric dimethylarginine (ADMA), nitrate and the indices of low-density lipoprotein oxidation. *Clin. Chim. Acta* 371 (1-2), 97–101. doi:10.1016/j.cca.2006.02.028

Parthasarathy, S., Santanam, N., Ramachandran, S., and Meilhac, O. (2000). Potential role of oxidized lipids and lipoproteins in antioxidant defense. *Free Radic. Res.* 33 (3), 197–215. doi:10.1080/10715760000301381

Patinen, T., Adinolfi, S., Cortés, C. C., Härkönen, J., Jawahar Deen, A., and Levonen, A. L. (2019). Regulation of stress signaling pathways by protein lipoxidation. *Redox Biol.* 23, 101114. doi:10.1016/j.redox.2019.101114

Peluso, I., and Serafini, M. (2017). Antioxidants from black and green tea: From dietary modulation of oxidative stress to pharmacological mechanisms. *Br. J. Pharmacol.* 174 (11), 1195–1208. doi:10.1111/bph.13649

Peng, A., Ye, T., Rakheja, D., Tu, Y., Wang, T., Du, Y., et al. (2011). The green tea polyphenol (-)-epigallocatechin-3-gallate ameliorates experimental immune-mediated glomerulonephritis. *Kidney Int.* 80 (6), 601–611. doi:10.1038/ki.2011.121

Poznyak, A., Grechko, A. V., Poggio, P., Myasoedova, V. A., Alfieri, V., and Orekhov, A. N. (2020). The diabetes mellitus-atherosclerosis connection: The role of lipid and glucose metabolism and chronic inflammation. *Int. J. Mol. Sci.* 21 (5), 1835. doi:10.3390/ijms21051835

Prasanth, M. I., Sivamaruthi, B. S., Chaiyasut, C., and Tencomnao, T. (2019). A review of the role of green tea (camellia sinensis) in antiphotoaging, stress resistance, neuroprotection, and autophagy. *Nutrients* 11 (2), 474. doi:10.3390/nu11020474

Ramesh, E., Elanchezhian, R., Sakthivel, M., Jayakumar, T., Senthil Kumar, R. S., Geraldine, P., et al. (2008). Epigallocatechin gallate improves serum lipid profile and erythrocyte and cardiac tissue antioxidant parameters in Wistar rats fed an atherogenic diet. *Fundam. Clin. Pharmacol.* 22 (3), 275–284. doi:10.1111/j.1472-8206.2008.00585.x

Raza, H. (2011). Dual localization of glutathione S-transferase in the cytosol and mitochondria: Implications in oxidative stress, toxicity and disease. *Febs J.* 278 (22), 4243–4251. doi:10.1111/j.1742-4658.2011.08358.x

Riegsecker, S., Wiczynski, D., Kaplan, M. J., and Ahmed, S. (2013). Potential benefits of green tea polyphenol EGCG in the prevention and treatment of vascular inflammation in rheumatoid arthritis. *Life Sci.* 93 (8), 307–312. doi:10.1016/j.lfs.2013.07.006

Rizzo, C., Arcudi, F., Đorđević, L., Dintcheva, N. T., Noto, R., D'Anna, F., et al. (2018). Nitrogen-doped carbon nanodots-ionogels: Preparation, characterization, and radical scavenging activity. *ACS Nano* 12 (2), 1296–1305. doi:10.1021/acsnano.7b07529

Roowi, S., Stalmach, A., Mullen, W., Lean, M. E., Edwards, C. A., and Crozier, A. (2010). Green tea flavan-3-ols: Colonic degradation and urinary excretion of catabolites by humans. *J. Agric. Food Chem.* 58 (2), 1296–1304. doi:10.1021/jf9032975

Roychoudhury, S., Agarwal, A., Virk, G., and Cho, C. L. (2017). Potential role of green tea catechins in the management of oxidative stress-associated infertility. *Reprod. Biomed. Online* 34 (5), 487–498. doi:10.1016/j.rbmo.2017.02.006

Sabaghi, M., Hoseyni, S. Z., Tavasoli, S., Mozafari, M. R., and Katouzian, I. (2021). Strategies of confining green tea catechin compounds in nano-biopolymeric matrices: A review. *Colloids Surf. B Biointerfaces* 204, 111781. doi:10.1016/j.colsurfb.2021.111781

Sang, S., Lee, M. J., Yang, I., Buckley, B., and Yang, C. S. (2008). Human urinary metabolite profile of tea polyphenols analyzed by liquid chromatography/electrospray ionization tandem mass spectrometry with data-dependent acquisition. *Rapid Commun. Mass Spectrom.* 22 (10), 1567–1578. doi:10.1002/rcm.3546

Satoh, T., Rezaie, T., Seki, M., Sunico, C. R., Tabuchi, T., Kitagawa, T., et al. (2011). Dual neuroprotective pathways of a pro-electrophilic compound via HSF-1-activated heat-shock proteins and Nrf2-activated phase 2 antioxidant response enzymes. *J. Neurochem.* 119 (3), 569–578. doi:10.1111/j.1471-4159.2011.07449.x

Schmidt, H. M., Kelley, E. E., and Straub, A. C. (2019). The impact of xanthine oxidase (XO) on hemolytic diseases. *Redox Biol.* 21, 101072. doi:10.1016/j.redox.2018.101072

Shapiro, H., Lev, S., Cohen, J., and Singer, P. (2009). Polyphenols in the prevention and treatment of sepsis syndromes: Rationale and pre-clinical evidence. *Nutrition* 25 (10), 981–997. doi:10.1016/j.nut.2009.02.010

Sharma, R., Yang, Y., Sharma, A., Awasthi, S., and Awasthi, Y. C. (2004). Antioxidant role of glutathione S-transferases: Protection against oxidant toxicity and regulation of stress-mediated apoptosis. *Antioxid. Redox Signal* 6 (2), 289–300. doi:10.1089/152308604322899350

Shi, M., Ying, D. Y., Ye, J. H., Sanguansri, L., and Augustin, M. A. (2020). Broccoli byproducts for protection and co-delivery of EGCG and tuna oil. *Food Chem.* 326, 126963. doi:10.1016/j.foodchem.2020.126963

Shin, M. H., Lee, S. R., Kim, M. K., Shin, C. Y., Lee, D. H., and Chung, J. H. (2016). Activation of peroxisome proliferator-activated receptor alpha improves aged and UV-irradiated skin by catalase induction. *PLoS One* 11 (9), e0162628. doi:10.1371/journal.pone.0162628

Shixian, Q., VanCrey, B., Shi, J., Kakuda, Y., and Jiang, Y. (2006). Green tea extract thermogenesis-induced weight loss by epigallocatechin gallate inhibition of catechol-O-methyltransferase. *J. Med. Food* 9 (4), 451–458. doi:10.1089/jmf.2006.9.451

Shuhui, G. (2015). *Literature research of the medicinal camellia sinensis*. Jinan, Shandong, China: Shandong University of Traditional Chinese Medicine.

Sies, H., Berndt, C., and Jones, D. P. (2017). Oxidative stress. *Annu. Rev. Biochem.* 86, 715–748. doi:10.1146/annurev-biochem-061516-045037

Spencer, J. P., Schroeter, H., Kuhnle, G., Srai, S. K., Tyrrell, R. M., Hahn, U., et al. (2001). Epicatechin and its *in vivo* metabolite, 3'-O-methyl epicatechin, protect human fibroblasts from oxidative-stress-induced cell death involving caspase-3 activation. *Biochem. J.* 354 (3), 493–500. doi:10.1042/0264-6021:3540493

St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J. M., Rhee, J., Jäger, S., et al. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* 127 (2), 397–408. doi:10.1016/j.cell.2006.09.024

Steffen, Y., Gruber, C., Schewe, T., and Sies, H. (2008). Mono-O-methylated flavanols and other flavonoids as inhibitors of endothelial NADPH oxidase. *Arch. Biochem. Biophys.* 469 (2), 209–219. doi:10.1016/j.abb.2007.10.012

Stone, N. J., Robinson, J. G., Lichtenstein, A. H., Bairey Merz, C. N., Blum, C. B., Eckel, R. H., et al. (2014). 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: A report of the American college of cardiology/American heart association task force on practice guidelines. *J. Am. Coll. Cardiol.* 63 (25), 2889–2934. doi:10.1016/j.jacc.2013.11.002

Surh, Y. J. (2003). Cancer chemoprevention with dietary phytochemicals. *Nat. Rev. Cancer* 3 (10), 768–780. doi:10.1038/nrc1189

Surh, Y. J., Chun, K. S., Cha, H. H., Han, S. S., Keum, Y. S., Park, K. K., et al. (2001). Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: Down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. *Mutat. Res.* 480-481, 243–268. doi:10.1016/s0027-5107(01)00183-x

Takagaki, A., Otani, S., and Nanjo, F. (2011). Antioxidative activity of microbial metabolites of (-)-epigallocatechin gallate produced in rat intestines. *Biosci. Biotechnol. Biochem.* 75 (3), 582–585. doi:10.1271/bbb.100683

Tang, W. J., Hu, C. P., Chen, M. F., Deng, P. Y., and Li, Y. J. (2006). Epigallocatechin gallate preserves endothelial function by reducing the endogenous nitric oxide synthase inhibitor level. *Can. J. Physiol. Pharmacol.* 84 (2), 163–171. doi:10.1139/y05-156

Thephinlap, C., Ounjaijean, S., Khansuwan, U., Fucharoen, S., Porter, J. B., and Srichairatanakool, S. (2007). Epigallocatechin-3-gallate and epicatechin-3-gallate from green tea decrease plasma non-transferrin bound iron and erythrocyte oxidative stress. *Med. Chem.* 3 (3), 289–296. doi:10.2174/157340607780620608

Tian, L., Zhang, S., Yi, J., Zhu, Z., Cui, L., Andrew Decker, E., et al. (2022). Antioxidant and prooxidant activities of tea polyphenols in oil-in-water emulsions depend on the level used and the location of proteins. *Food Chem.* 375, 131672. doi:10.1016/j.foodchem.2021.131672

Varilek, G. W., Yang, F., Lee, E. Y., deVilliers, W. J., Zhong, J., Oz, H. S., et al. (2001). Green tea polyphenol extract attenuates inflammation in interleukin-2-deficient mice, a model of autoimmunity. *J. Nutr.* 131 (7), 2034–2039. doi:10.1093/jn/131.7.2034

Vazquez-Prieto, M. A., Bettaieb, A., Haj, F. G., Fraga, C. G., and Oteiza, P. I. (2012). (-)-Epicatechin prevents TNFα-induced activation of signaling cascades involved in inflammation and insulin sensitivity in 3T3-L1 adipocytes. *Arch. Biochem. Biophys.* 527 (2), 113–118. doi:10.1016/j.abb.2012.02.019

Vuong, Q. V. (2014). Epidemiological evidence linking tea consumption to human health: A review. *Crit. Rev. Food Sci. Nutr.* 54 (4), 523–536. doi:10.1080/10408398.2011.594184

Wang, C. C., Chu, K. O., Chong, W. S., Li, W. Y., Pang, C. P., Shum, A. S., et al. (2007). Tea epigallocatechin-3-gallate increases 8-isoprostane level and induces caudal regression in developing rat embryos. *Free Radic. Biol. Med.* 43 (4), 519–527. doi:10.1016/j.freeradbiomed.2007.04.034

Wang, D., Wang, Y., Wan, X., Yang, C. S., and Zhang, J. (2015). Green tea polyphenol (-)-epigallocatechin-3-gallate triggered hepatotoxicity in mice: Responses of major antioxidant enzymes and the Nrf2 rescue pathway. *Toxicol. Appl. Pharmacol.* 283 (1), 65–74. doi:10.1016/j.taap.2014.12.018

Wang, X., Liu, F., Li, J., Yang, X., Chen, J., Cao, J., et al. (2020). Tea consumption and the risk of atherosclerotic cardiovascular disease and all-cause mortality: The China-PAR project. *Eur. J. Prev. Cardiol.* 27 (18), 1956–1963. doi:10.1177/2047487319894685

Wang, Y., Wang, G. Z., Rabinovitch, P. S., and Tabas, I. (2014). Macrophage mitochondrial oxidative stress promotes atherosclerosis and nuclear factor-κB-mediated inflammation in macrophages. *Circ. Res.* 114 (3), 421–433. doi:10.1161/CIRCRESAHA.114.302153

Wang, Z. M., Gao, W., Wang, H., Zhao, D., Nie, Z. L., Shi, J. Q., et al. (2014). Green tea polyphenol epigallocatechin-3-gallate inhibits TNF-alpha-induced production of

monocyte chemoattractant protein-1 in human umbilical vein endothelial cells. *Cell Physiol. Biochem.* 33 (5), 1349–1358. doi:10.1159/000358702

Warner, E. F., Rodriguez-Ramiro, I., O'Connell, M. A., and Kay, C. D. (2018). Cardiovascular mechanisms of action of anthocyanins may Be associated with the impact of microbial metabolites on heme oxygenase-1 in vascular smooth muscle cells. *Molecules* 23 (4), 898. doi:10.3390/molecules23040898

Wenz, T. (2013). Regulation of mitochondrial biogenesis and PGC-1α under cellular stress. *Mitochondrion* 13 (2), 134–142. doi:10.1016/j.mito.2013.01.006

Wójcik, P., Biernacki, M., Wroński, A., Łuczaj, W., Waeg, G., Žarković, N., et al. (2019). Altered lipid metabolism in blood mononuclear cells of psoriatic patients indicates differential changes in psoriasis vulgaris and psoriatic arthritis. *Int. J. Mol. Sci.* 20 (17), 4249. doi:10.3390/ijms20174249

Wójcik, P., Gęgotek, A., Žarković, N., and Skrzydlewska, E. (2021). Oxidative stress and lipid mediators modulate immune cell functions in autoimmune diseases. *Int. J. Mol. Sci.* 22 (2), 723. doi:10.3390/ijms22020723

Wu, C. C., Hsu, M. C., Hsieh, C. W., Lin, J. B., Lai, P. H., and Wung, B. S. (2006). Upregulation of heme oxygenase-1 by Epigallocatechin-3-gallate via the phosphatidylinositol 3-kinase/Akt and ERK pathways. *Life Sci.* 78 (25), 2889–2897. doi:10.1016/j.lfs.2005.11.013

Wu, Y., Sun, H., Yi, R., Tan, F., and Zhao, X. (2020). Anti-obesity effect of Liupao tea extract by modulating lipid metabolism and oxidative stress in high-fat-diet-induced obese mice. *J. Food Sci.* 86 (1), 215–227. doi:10.1111/1750-3841.15551

Xiong, M., Jia, C., Cui, J., Wang, P., Du, X., Yang, Q., et al. (2015). Shexiang Tongxin dropping pill attenuates atherosclerotic lesions in ApoE deficient mouse model. *J. Ethnopharmacol.* 159, 84–92. doi:10.1016/j.jep.2014.11.013

Xu, L., Ma, X., Verma, N. K., Wang, D., Gavrilova, O., Proia, R. L., et al. (2018). Ablation of PPARγ in subcutaneous fat exacerbates age-associated obesity and metabolic decline. *Aging Cell* 17 (2), e12721. doi:10.1111/acel.12721

Xu, X., Pan, J., and Zhou, X. (2014). Amelioration of lipid profile and level of antioxidant activities by epigallocatechin-gallate in a rat model of atherogenesis. *Heart Lung Circ.* 23 (12), 1194–1201. doi:10.1016/j.hlc.2014.05.013

Yang, C. S., Maliakal, P., and Meng, X. (2002). Inhibition of carcinogenesis by tea. *Annu. Rev. Pharmacol. Toxicol.* 42, 25–54. doi:10.1146/annurev.pharmtox.42.082101.154309

Yang, H., Roberts, L. J., Shi, M. J., Zhou, L. C., Ballard, B. R., Richardson, A., et al. (2004). Retardation of atherosclerosis by overexpression of catalase or both Cu/Zn-superoxide dismutase and catalase in mice lacking apolipoprotein E. *Circ. Res.* 95 (11), 1075–1081. doi:10.1161/01.RES.0000149564.49410.0d

Yang, P., Zhang, J., Xiang, S., Jin, Z., Zhu, F., Wang, T., et al. (2021). Green nanoparticle scavengers against oxidative stress. *ACS Appl. Mater Interfaces* 13 (33), 39126–39134. doi:10.1021/acsami.1c12176

Yang, S., Chu, S., Ai, Q., Zhang, Z., Gao, Y., Lin, M., et al. (2020). Anti-inflammatory effects of higenamine (Hig) on LPS-activated mouse microglia (BV2) through NF-κB and Nrf2/HO-1 signaling pathways. *Int. Immunopharmacol.* 85, 106629. doi:10.1016/j.intimp.2020.106629

Yang, Y., Qin, Y. J., Yip, Y. W., Chan, K. P., Chu, K. O., Chu, W. K., et al. (2016). Green tea catechins are potent anti-oxidants that ameliorate sodium iodate-induced retinal degeneration in rats. *Sci. Rep.* 6, 29546. doi:10.1038/srep29546

Yao, H. T., Li, C. C., and Chang, C. H. (2019). Epigallocatechin-3-Gallate reduces hepatic oxidative stress and lowers CYP-mediated bioactivation and toxicity of acetaminophen in rats. *Nutrients* 11 (8), 1862. doi:10.3390/nu11081862

Yao, J., Cheng, Y., Zhou, M., Zhao, S., Lin, S., Wang, X., et al. (2018). ROS scavenging Mn(3)O(4) nanozymes for *in vivo* anti-inflammation. *Chem. Sci.* 9 (11), 2927–2933. doi:10.1039/c7sc05476a

Yates, A. A., Erdman, J. W., Jr., Shao, A., Dolan, L. C., and Griffiths, J. C. (2017). Bioactive nutrients - time for tolerable upper intake levels to address safety. *Regul. Toxicol. Pharmacol.* 84, 94–101. doi:10.1016/j.yrtph.2017.01.002

Ye, J. H., and Augustin, M. A. (2019). Nano- and micro-particles for delivery of catechins: Physical and biological performance. *Crit. Rev. Food Sci. Nutr.* 59 (10), 1563–1579. doi:10.1080/10408398.2017.1422110

Youn, H. S., Lee, J. Y., Saitoh, S. I., Miyake, K., Kang, K. W., Choi, Y. J., et al. (2006). Suppression of MyD88-and TRIF-dependent signaling pathways of Toll-like receptor by (-)-epigallocatechin-3-gallate, a polyphenol component of green tea. *Biochem. Pharmacol.* 72 (7), 850–859. doi:10.1016/j.bcp.2006.06.021

Yu, J., Li, W., Xiao, X., Huang, Q., Yu, J., Yang, Y., et al. (2021). (-)-Epicatechin gallate blocks the development of atherosclerosis by regulating oxidative stress *in vivo* and *in vitro*. *Food Funct.* 12 (18), 8715–8727. doi:10.1039/d1fo00846c

Zeng, J., Deng, Z., Zou, Y., Liu, C., Fu, H., Gu, Y., et al. (2021). Theaflavin alleviates oxidative injury and atherosclerosis progress via activating microRNA-24-mediated Nrf2/HO-1 signal. *Phytother. Res.* 35 (6), 3418–3427. doi:10.1002/ptr.7064

Zhang, G., Miura, Y., and Yagasaki, K. (2000). Suppression of adhesion and invasion of hepatoma cells in culture by tea compounds through antioxidative activity. *Cancer Lett.* 159 (2), 169–173. doi:10.1016/s0304-3835(00)00545-0

Zhang, S., Li, L., Chen, W., Xu, S., Feng, X., and Zhang, L. (2021). Natural products: The role and mechanism in low-density lipoprotein oxidation and atherosclerosis. *Phytother. Res.* 35 (6), 2945–2967. doi:10.1002/ptr.7002

Zheng, Y., Morris, A., Sunkara, M., Layne, J., Toborek, M., and Hennig, B. (2012). Epigallocatechin-gallate stimulates NF-E2-related factor and heme oxygenase-1 via caveolin-1 displacement. *J. Nutr. Biochem.* 23 (2), 163–168. doi:10.1016/j.jnutbio.2010.12.002

Zhu, C., Xu, Y., Liu, Z. H., Wan, X. C., Li, D. X., and Tai, L. L. (2018). The anti-hyperuricemic effect of epigallocatechin-3-gallate (EGCG) on hyperuricemic mice. *Biomed. Pharmacother.* 97, 168–173. doi:10.1016/j.biopha.2017.10.013

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 31 March 2023
DOI 10.3389/fphar.2023.1161657

OPEN ACCESS

EDITED BY
Xiaofei Zhang,
Shaanxi University of Chinese Medicine, China

REVIEWED BY
Boxun Zhang,
Guang'anmen Hospital (CACMS), China
Yongqing Cai,
Daping Hospital, China

*CORRESPONDENCE
Hong Su,
shcdmm@126.com
Lisha He,
helisha@cdutcm.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Ethnopharmacology, a section of the journal Frontiers in Pharmacology

RECEIVED 08 February 2023
ACCEPTED 22 March 2023
PUBLISHED 31 March 2023

CITATION
Duan H, Song P, Li R, Su H and He L (2023), Attenuating lipid metabolism in atherosclerosis: The potential role of Anti-oxidative effects on low-density lipoprotein of herbal medicines. *Front. Pharmacol.* 14:1161657. doi: 10.3389/fphar.2023.1161657

Attenuating lipid metabolism in atherosclerosis: The potential role of Anti-oxidative effects on low-density lipoprotein of herbal medicines

Huxinyue Duan[1†], Pan Song[2†], Ruolan Li[1], Hong Su[2*] and Lisha He[1*]

[1]School of Pharmacy, School of Basic Medicine, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]Chengdu Integrated TCM and Western Medicine Hospital, Chengdu, China

Atherosclerosis (AS) is a multifactorial chronic disease with great harm to the health of human being, which is a basic pathogenesis of many cardiovascular diseases and ultimately threatens human life. Abnormal blood lipid level is one of the most common diagnostic indicators of AS in clinic, and lipid metabolism disorder is often observed in patients with AS. Cholesterol is an important lipid in the human body, which is of great significance for maintaining normal life activities. Generally, cholesterol is transported to peripheral tissues by low-density lipoprotein (LDL), and then transported to the liver by high-density lipoprotein (HDL) *via* its cholesterol reverse transport function, and finally discharged. Under oxidative stress condition, LDL is commonly oxidized to the form ox-LDL, which is ingested by macrophages in large quantities and further forms foam cells, disrupting the normal metabolic process of cholesterol. Importantly, the foam cells are involved in forming atherosclerotic plaques, whose rupture may lead to ischemic heart disease or stroke. Furthermore, ox-LDL could also promote the development of AS by damaging vascular endothelium, promoting the migration and proliferation of smooth muscle cells, and activating platelets. Therefore, inhibiting LDL oxidation may be an effective way to improve lipid metabolism and prevent AS. In recent years, increasing studies have shown that herbal medicines have great potentiality in inhibiting LDL oxidation and reducing ox-LDL induced foam cell formation. Accordingly, this paper summarized current research on the inhibitory effects of herbal medicines against LDL oxidation and foam cell formation, and made a brief description of the role of cholesterol and LDL in lipid metabolism disorder and AS pathogenesis. Importantly, it is suggested that herbal medicines could inhibit LDL oxidation and regulate cholesterol homeostasis *via* downregulation of CD36 and SR-A, whereas upregulation of ABCA1 and ABCG1.

KEYWORDS

atherosclerosis, oxidative stress, lipid metabolism, low-density lipoprotein, herbal medicines

1 Introduction

Atherosclerosis (AS) is a common chronic inflammatory disease characterized by the accumulation of fatty substances under the vascular subintimal layer, and which is the basis of various cardiovascular diseases, such as myocardial infarction and cerebral infarction, causing a large number of deaths worldwide (Gupta et al., 2020). The pathogenesis of AS is complex and has not yet been fully elucidated. Accumulating studies have shown that abnormal lipid metabolism, oxidative stress, injury and dysfunction of endothelial cell, and hyperproliferation and migration of smooth muscle cell are involved in the development of AS (Witztum, 1994; Weber and Noels, 2011). Based on basic and clinical research, there are several popular theories about the pathogenesis of AS, including fatty infiltration, endothelial cell injury, platelet aggregation, and the oxidation hypothesis, etc. In addition, abnormal lipid metabolism is an independent risk factor for AS, mainly manifested as hyperlipidemia, which plays a vital role in the occurrence and development of AS (Gupta et al., 2020). The relationship between cholesterol (which is an important lipid in the human body) and AS has been aroused considerable concerning for a long time. Goldstein and Brown, two American scientists, who won the Nobel Prize in Medicine and Physiology in 1985, discovered the mechanism of LDL receptor, proving that plasma LDL is the direct main pathogenic factor of AS. Otherwise, an American scientist named Steinberg reported oxidation of LDL plays a crucial role in the development of AS (Steinberg, 1983).

LDL is the main carrier of lipids in plasma (Itabe, 1998), and appears as a spherical particle with a particle size of about 220 nm, a mass of about 3,000 kDa, and a density ranging from 1.019 to 1.063 g/mL (Zmysłowski and Szterk, 2017). And an individual LDL particle consists of a hydrophobic region (containing about 170 triglycerides and 1,500 cholesterol esters) and a hydrophilic region (containing approximately 700 phospholipid molecules, 500 cholesterol molecules, and apolipoproteins, which is mostly Apo B) (Esterbauer et al., 1992) (Figure 1A). Importantly, it has been clarified that LDL is converted from VLDL. Briefly, CM, consisted mainly of dietary fat and Apo B48, is hydrolyzed by lipoprotein lipase and then produces a chylomicron remnant, which can be taken up by the liver. Subsequently, lipids components obtained from chylomicron remnant are recombined with Apo B, which is synthesized in liver, to form VLDLs. Then, VLDLs are released into circulation. After interaction with lipases, TG in VLDLs is partially hydrolyzed, leading to decreasing in TG and loss of Apo C and Apo E in VLDLs. As a result, the density, diameter, and composition of VLDLs are changed to convert to LDL (Matsuura et al., 2006) (Figure 1B).

Under normal physiological conditions, the human body maintains redox homeostasis. However, under the stimulation of some exogenous or endogenous factors, the production of ROS increases, resulting in redox imbalance and oxidative stress (Winterbourn, 2008; D'Autréaux and Toledano, 2007). Lipids, especially phospholipids and cholesterol esters in LDL, are prone to be oxidized under the existence of ROS, and finally generate oxidized LDL (ox-LDL). Ox-LDL is closely involved into the development of AS in many ways, such as damaging vascular endothelial cells, participating in the formation of foam cells, promoting the migration and proliferation of vascular smooth muscle cells, and activating platelets (Khatana et al., 2020). It is reported that LDL without oxidation modification does not promote

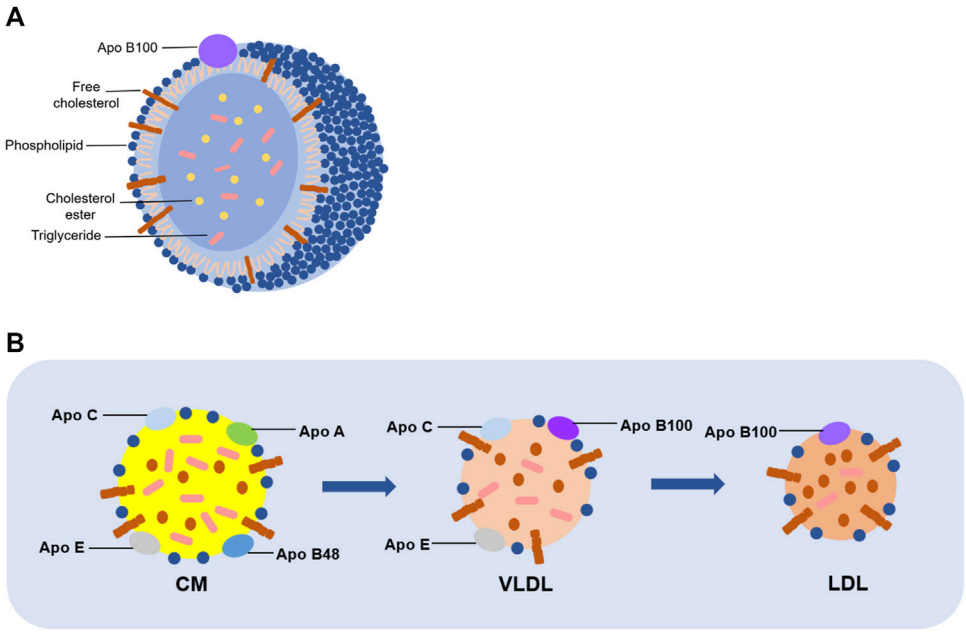


FIGURE 1
Structure and formation of LDL. **(A)** A single LDL particle is composed of phospholipids, unesterified cholesterol and Apo B in the outer layer, and cholesterol esters and triglycerides in the inner layer. **(B)** CM mainly consists of dietary fat and several kinds of apolipoprotein, including Apo B48, Apo A, Apo C, and Apo E. It is generated on the intestinal mucosa, and then hydrolyzed into chyle particles in the intestine, which will form VLDL with Apo B100. Consequently, TG in VLDL is partially hydrolyzed, as well as losing Apo C and Apo E to be converted into LDL.

the occurrence of AS, suggesting that ox-LDL is the direct risk factor (Quinn et al., 1985). The formation of ox-LDL has a significant impact on the metabolism of cholesterol. Generally, cholesterol in LDL can be absorbed by peripheral cells, and then transported by HDL to the liver for metabolism and LDL can be finally excreted to keep the normal level of cholesterol (Altmann et al., 2004; Duffy and Rader, 2009; Goldstein and Brown, 2009). This process is of great significance to ensure normal physiological activities of human being. However, once ox-LDL is formed in large quantities, excessive ox-LDL will be ingested by macrophages, resulting in ox-LDL accumulated in macrophages and could not be excreted. As a result, a large number of foam cells are formed and accumulated in the blood vessels, accelerating the development of AS (Kume et al., 2000). Therefore, inhibiting LDL oxidation is a feasible way to inhibit the formation of foam cells and improve cholesterol metabolism.

Herbal medicines have been used for treatment and prevention of various diseases for thousands of years worldwide. Accumulating evidence has shown that many herbal medicines, as complementary and alternative therapies, have beneficial effects on AS, such as red sage root (*Radix Salvia miltiorrhiza*), garlic, celery, and ginkgo (*Ginkgo biloba* L.), which exhibit the hypolipidemic effect (Hao et al., 2017). In addition, some active compounds isolated from herbal medicines, such as quercetin, resveratrol, lycopene and epigalactin-3-gallate (Zhang et al., 2021), have also been proved to have inhibitory effects on LDL oxidation. However, there is lack of systematic review on the anti-atherosclerotic effects of herbal medicines *via* regulating lipid metabolism and inhibiting LDL oxidation. Therefore, this review summarized herbal medicines used to protect AS by inhibiting LDL oxidation and lipid metabolism to provide a reference for the research of AS prevention and treatment.

2 Lipid metabolism disorder and lipoprotein in atherosclerosis

It is well known that normal lipid metabolism is important for humans' health, and when lipid metabolism is abnormal, the body will undergo pathological changes. At present, lipid metabolism disorders have been regarded as one of the independent risk factors for cardiovascular diseases and have attracted great attention (Kimura et al., 2010). What is lipid metabolism disorder? It means processes of synthesis, breakdown, digestion, absorption, and transport of lipids in the body do not proceed normally. As a result, abnormal levels of lipids and their metabolites in the blood or organs usually cause hyperlipidemia, which is one of the pathogenic factors of AS (Hurtubise et al., 2016). Lipids in blood include cholesterol, triglycerides, and phospholipids, which are hydrophobic substances that must bind to apolipoproteins to form lipoproteins to be transported and metabolized. Lipoproteins include chylomicrons (CM), very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (Segrest et al., 2001; Afonso and Spickett, 2019). Among them, abnormal HDL and LDL levels are characteristic of dyslipidemia, which is clinically manifested by elevated low-density lipoprotein cholesterol levels and decreased high-density lipoprotein cholesterol levels (Gupta et al., 2020).

HDL is often considered atheroprotective because of its reverse cholesterol transport (RCT) role. It lowers plasma cholesterol levels by transporting peripheral cholesterol to the liver, where cholesterol is excreted into the bile as a prototype or converted to bile acids, which are partially reabsorbed in the intestine and the remainder excreted in the feces (Duffy and Rader, 2009). In contrast, LDL transports cholesterol to peripheral tissues, and the level of cholesterol it carries is positively associated with the development of cardiovascular disease. Briefly, cholesterol ingested from food is absorbed in the intestine mediated by the Niemann-Pick type C1-like 1 (NPC1L1) protein and then released as CMs, and the cholesterol is subsequently taken up by the liver. Upon arrival in the liver, cholesterol is released into the blood as VLDLs, then converted to LDLs, which are taken up by peripheral cells mediate (Altmann et al., 2004; Goldstein and Brown, 2009) (Figure 2). Due to its small particle size, cholesterol-rich LDL can pass through vascular endothelial cells and bind to subendothelial glycoproteins to deposit on the vessel wall. The higher the concentration of LDL in the blood, the faster it deposits (Weber and Noels, 2011). Meanwhile, under the effect of oxidative stress, LDL bound to glycoproteins is easily modified to ox-LDL, which is an important step in the development of AS (Braamskamp et al., 2015). In conclusion, both HDL and LDL play an important role in lipid metabolism. The former contributes to reverse transport of cholesterol to reduce the cholesterol level in plasma. At the same time, the latter mediates transport of cholesterol to peripheral tissues, and it is easy to pass through the damaged vascular endothelium to promote the occurrence and development of AS.

3 Oxidative stress and oxidation of LDL

3.1 Oxidative stress and reactive oxygen species (ROS)

Under the stimulation of external chemical or physical factors or the effects of endogenous enzymes, the imbalance between oxidation and reduction may occur, resulting in increasing production of ROS and damage to proteins, nucleic acids, and lipids (Winterbourn, 2008; D'Autréaux and Toledano, 2007). This state in which the balance between antioxidant capacity and active species is disturbed is known as oxidative stress. ROS are derivatives of oxygen molecules produced during metabolism and are continuously produced during normal physiological processes. There are two types of ROS, called free radical ROS and non-free radical ROS(Dröge, 2002). Generally, free radical ROS are unstable in nature and include superoxide (O^{2-}) and hydroxyl radical (OH), which can be produced under mediation of various enzyme systems. In contrast, non-free radical ROS have more stable properties and include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and peroxynitrite (ONOO-) (Martin-Ventura et al., 2007; Duan et al., 2021). Normal levels of ROS have an important physiological function in transmitting signaling, while the production of excess ROS causes harm to organisms (Li et al., 2014).

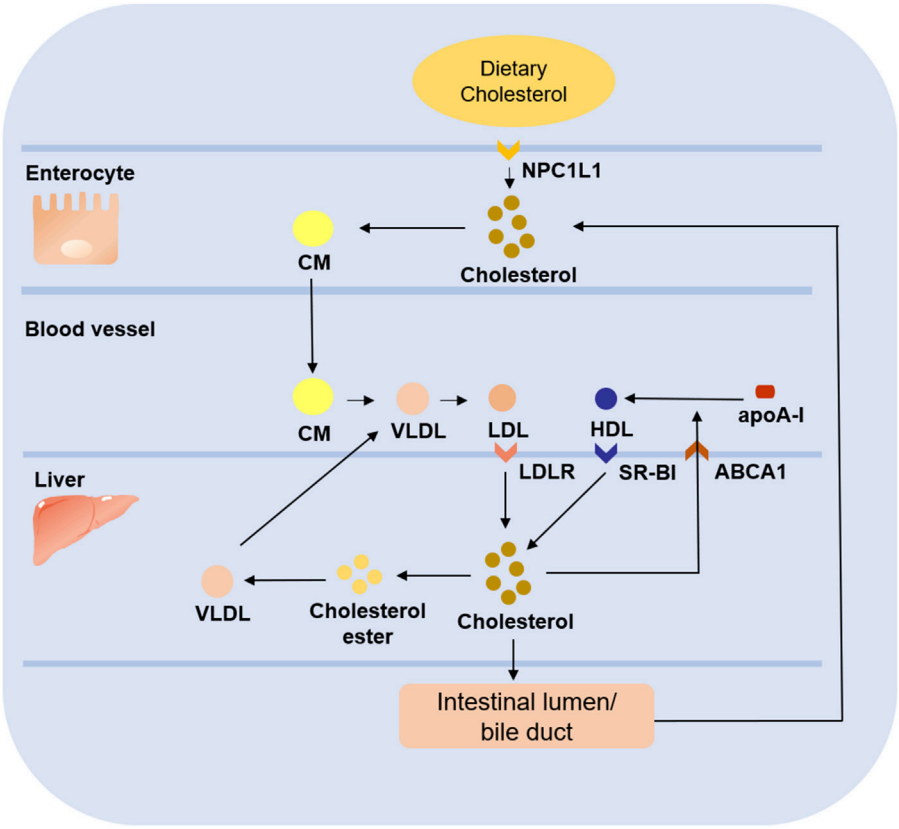


FIGURE 2
Role of LDL and HDL in cholesterol metabolism. Dietary cholesterol ingested from food enters enterocyte under the mediation of NPC1L1 and forms CM. After being released into blood, CM is gradually converted into VLDL, and then further converted into LDL, which is absorbed by liver under the mediation of LDLR. Some of the cholesterol in the liver is esterified to form cholesterol ester to form VLDL which will be released into blood, while the other part enters intestinal lumen/bile duct to be reabsorbed into the circulation or excreted from the body. In addition, the free cholesterol transferred to plasma under the mediation of ABCA1 combines with apoA-I to form nascent HDL, which is absorbed by liver through SR-BI to complete the reverse cholesterol transport.

3.2 Oxidative modification of LDL

It has been reported that oxidative stress is a prerequisite for pathogenesis of AS (Yang et al., 2017). Under pathological state of oxidative stress, the vascular endothelium is damaged, allowing LDL to cross it and adhere to the intima (Kruth et al., 2002). LDL attached to the intima of blood vessels is susceptible to oxidative modification by ROS to produce ox-LDL (Frei et al., 1988; Stocker and Keaney, 2004). And there has been increasing evidence suggesting that ox-LDL, but not LDL, has a role in promoting the development of AS.

Due to the presence of polyunsaturated fatty acid moieties, phospholipids in the outer layer of LDL are oxidized first in oxidative modification process under the presence of ROS. Then cholesterol esters in the core are oxidized gradually (Steinbrecher et al., 1990). Among them, the oxidation rate of esterified fatty acids in cholesterol esters is as high as 90% (Brown et al., 2000). In the process of LDL oxidation, a variety of bioactive molecules are formed, such as oxidized sterols, oxidized fatty acids, and some small molecules (Witztum, 1994). As a result, a large number of bioactive molecules are produced, triggering a series of subsequent reactions that cause further damage to the body. For example, cholesteryl hydroperoxy-octadecadienoate (Chol-HPODE), one of the major oxidation products of LDL, inactivates platelet-derived growth factor (Kritharides et al., 1993). In addition, Apo B100 on the surface of LDL is also highly susceptible to ROS modification and degradation to produce peptides ranging from 14 kDa to 500 kDa (Fong et al., 1987) (Figure 3). Ox-LDL adhering to the vascular endothelium recruits monocytes, which subsequently differentiate into macrophages. The CD36 receptors on the surface of macrophages recognize ox-LDL and allow it to enter macrophages. Then the retention of large amounts of ox-LDL makes macrophages form foam cells and accelerates the development of AS (Hofmann et al., 2017).

4 Ox-LDL in the formation of foam cells and other atherogenic ways

4.1 Formation of foam cells

Macrophages play an important role in cholesterol homeostasis during lipid metabolism. Under the induction of inflammatory factors and chemokines, monocytes migrate to subendothelial space through the damaged endothelium. Then, the expression of

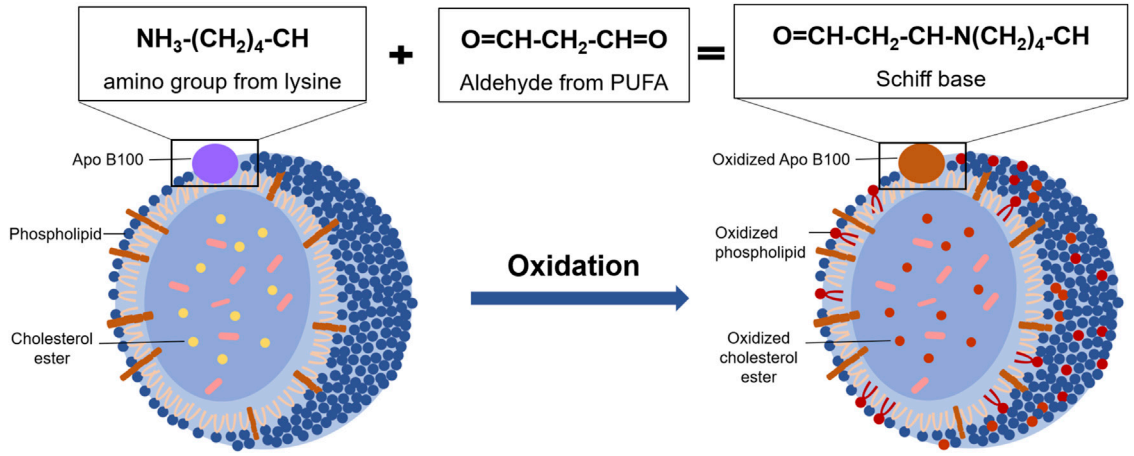


FIGURE 3
The oxidative modification of LDL. Under oxidative stress condition, phospholipids and Apo B100 in the outer layer of LDL are oxidized, and the lysine residues of Apo B100 reacted with reactive aldehydes from oxidative phospholipids to form Schiff base. Then, the cholesterol ester in the inner layer of LDL is gradually oxidized with the oxidation rate is as high as 90%.

scavenger receptors and toll-like receptors on the surface of these monocytes is upregulated, marking transformation of monocytes into macrophages. There are many scavenger receptors on the surface of macrophages that mediate the uptake of lipids, such as scavenger receptor B2 (CD36), scavenger receptor class A (SR-A), and lectin-like ox-LDL receptor-1 (LOX-1). Among them, LOX-1 in macrophages is upregulated under stimulation of a variety of atherogenic stimuli, such as ox-LDL, pro-inflammatory cytokines, and advanced glycation end products (AGEs) (Kume et al., 2000; Pirillo et al., 2013). In addition, macrophages express a variety of receptors that mediate reverse cholesterol transport, including ATP-binding cassette transporter A1 (ABCA1), ATP-binding cassette protein G1 (ABCG1), and scavenger receptor class B type 1 (SR-B1) (Chistiakov et al., 2017). It means that there are different receptors that mediate lipid uptake and reverse transport of cholesterol on the surface of macrophages, and the former would be upregulated by atherogenic stimuli.

In the early stage of AS, foam cells play an important role in reducing lipid accumulation, clearing apoptotic cells, and attenuating inflammation. At this stage, phagocytosis and ejection of lipids by macrophages are balanced. In brief, lipids in macrophages first react with lysosomal acid lipase (LAL) to digest cholesterol esters and produce free cholesterol, which will then be converted into cholesterol ester after treatment with acetyl-CoA acetyltransferase (ACAT1). Consequently, on the endoplasmic reticulum, these cholesterol esters will react with neutral cholesterol ester hydrolase (NCHH) to produce free cholesterol, which can be discharged by cholesterol transporters (Chistiakov et al., 2017). However, this balance will be destroyed under the influence of multiple AS risk factors. It is observed that the expression of LOX-1 is increased, and the expression of ABCA1 and ABCG1 is decreased on the surface of macrophages after the development of AS. In addition, the expression of ACAT1 is increased, while the expression of NCEH is reduced. As a result, the uptake of ox-LDL is increased. At the same time, ejection of lipids is decreased, leading to excessive accumulation of cholesterol in macrophages, which gradually makes formation of foam cells, and then accumulates into lipid stripes (Figure 4). More importantly, continuous uptake of ox-LDL by macrophages aggravates inflammation, as well as drives matrix metalloproteinase (MMP) to degrade the fibrous cap on the outer layer of the lipid cores, making AS plaque unstable or broken. Interestingly, unmodified LDL does not cause excessive accumulation of cholesterol esters by macrophages. In addition, although macrophages produce most foam cells, a few foam cells are produced by endothelial cells and vascular smooth muscle cells (Xu et al., 2021).

4.2 Other atherogenic ways

4.2.1 Endothelial dysfunction

As a barrier between blood and the underlying layer of the vascular wall, vascular endothelium plays an important role in maintaining vascular homeostasis. Because of being exposed to circulating blood, vascular endothelium is easily susceptible to various atherogenic stimuli. Normal blood vessels regulate vascular tension to maintain stability by secreting a set of vasoactive molecules, including nitric oxide (NO), prostacyclin (PGI2), endothelium-dependent hyperpolarization factor (EDRF), and endothelin-1 (ET-1) (Xu et al., 2021). However, ox-LDL is taken up by endothelial cells under mediation of the lectin-like low-density lipoprotein receptor-1 (LOX-1), and then activates adhesion molecules in the surface of endothelial cells. As a result, monocytes migrate into the sub-endothelial layers, and then differentiate into macrophages, engulfing large amounts of ox-LDL to generate foam cells (Wang et al., 2019). What's more, ox-LDL reduces the production of eNOS-derived NO, which is a vasodilator with protective effects on vascular endothelium (Förstermann and Sessa, 2012; Fujimura et al., 2012; Das et al., 2021). In addition, ox-LDL damages vascular endothelium in several ways, including increasing production of ROS, causing inflammation, and causing endoplasmic reticulum (ER) stress

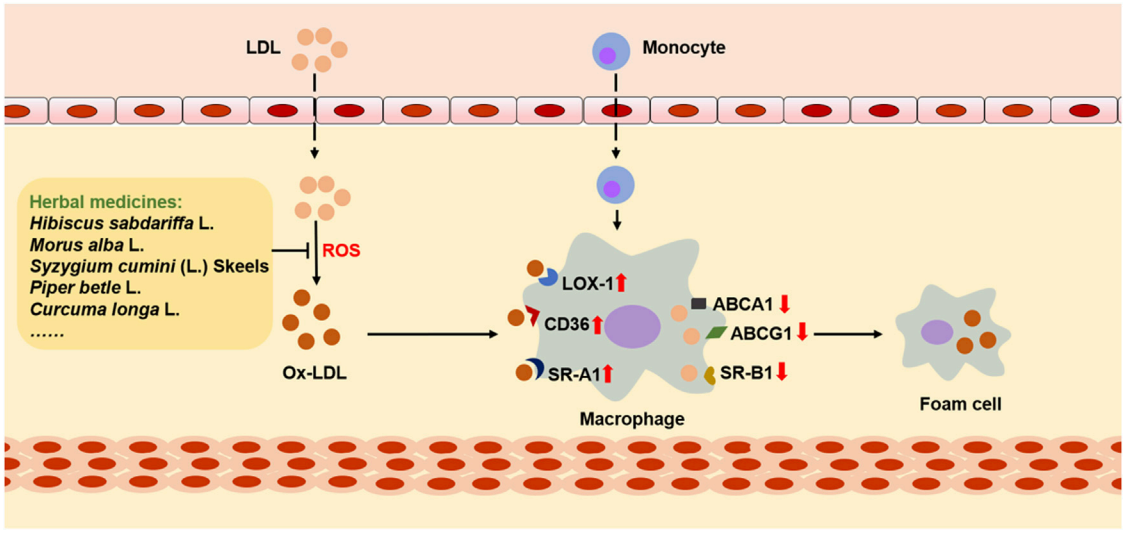


FIGURE 4
The role of ox-LDL in cholesterol metabolism and foam cell formation. LDL passes through the damaged endothelium, where it is oxidized by ROS and then converts to ox-LDL, interacting with macrophage-derived monocytes. During this process, the expression of CD36, SR-A1, and LOX-1 on the surface of macrophages is upregulated, while the expression of receptors that mediate cholesterol reverse transport is downregulated, including ABCA1, ABCG1, and SR-B1. As a result, cholesterol in macrophages accumulates and eventually foam cells are formed. However, many herbal medicines can reduce the accumulation of cholesterol and inhibit the formation of foam cells by inhibiting the oxidative modification of LDL.

(Jiang et al., 2022). Under persistent injury, endothelial cells may experience cell death through apoptosis, necrosis, and ferroptosis, which impairs the integrity of endothelial cells, and thus aggravating endothelial dysfunction.

4.2.2 Migration and proliferation of vascular smooth muscle cells

In the occurrence and development of atherosclerotic lesions, the proliferation, migration, and death of vascular smooth muscle cells (VSMCs) are involved to the formation, growth, rupture of plaque, and to vascular remodeling and occurrence of hemangioma (Alexander and Owens, 2012). Under stimulation of ox-LDL, VSMCs migrate into the subendothelial space, and then change from the contractile to the synthetic phenotype to proliferate excessively (Yang et al., 2018). It has been reported that ox-LDL-stimulation promotes VSMC proliferation by increasing the release of growth factors, including the insulin-like growth factor (IGF-1), the platelet-derived growth factor (PDGF), and epidermal growth factor (EGF), while inhibiting the expression of miR-141 (Eto et al., 2006; Sun and Chen, 2011; Goyal et al., 2012). Then, VSMCs proliferate excessively, secrete extracellular matrix proteins and synthesize collagen to form the fibrous cap for the atherosclerotic plaque. Moreover, the stability of atherosclerotic plaque is determined by the thickness of the fibrous cap. However, ox-LDL-stimulated apoptosis of VSMCs and local inflammatory reaction lead to activation of matrix metalloproteinases (MMPS), which leads to the thinning of the fibrous cap, and eventually leading to rupture of atherosclerotic plaque, which causes thrombosis in the blood vessel (Bennett et al., 2016).

4.2.3 Activation of platelet

Platelets play an important role in the development of AS, with the involvement of ox-LDL. Under the stimulation of ox-LDL, platelets are rapidly activated *via* Src kinases and Rho kinase-signaling pathways (Wraith et al., 2013). In addition, the expression of P-selectin and activity of integrin $\alpha_{IIb}\beta_3$ are increased through interaction between ox-LDL and CD36 in resting platelets, and then the expression of LOX-1 is increased (Podrez et al., 2007). As a result, LOX-1 and CD36 promote platelet adhesion to endothelial cells and increase the composition of AS plaques (Khatana et al., 2020). Additionally, chemokines released by activated platelets accelerate the development of AS by aggravating endothelial dysfunction and promoting foam cell production (Curtiss et al., 1987; Siegel-Axel et al., 2008).

5 Herbal medicines that inhibited oxidation of LDL and formation of foam cells

Mixtures and monomers extracted from herbal medicines are often studied. Here, aqueous extract, methanol extract, whole extract, and some monomers will be discussed (Table 1).

5.1 Aqueous extract

5.1.1 *Morus alba* L

Mulberry, the fruit of *Morus alba* L., is rich in anthocyanins, which have been reported to possess many potentially therapeutic

TABLE 1 Inhibition LDL oxidation and foam cell formation effects of herbal medicines in Atherosclerosis.

Botanical drugs	Part of plant used	Main components	Animal/cells	Effects	Related targets	Refs
Aqueous extract						
Morus alba L. [Moraceae; *Mori Folium*]	Leaf	Gallocatechin, Gallocatechin gallate, Naringenin	ox-LDL-induced J774A.1 cells	Inhibiting LDL oxidation; Inhibiting foam cell formation	PPARγ↓, CD36↓, SR-A↓, SOD-1↑, GPx↑	Yang et al. (2011)
Syzygium cumini (L.) Skeels [Myrtaceae; *Fructus Syzygii Cumini*]	Seed	Quercetin, Rutin, Kaempferol	ox-LDL-induced RAW264.7 cells	Inhibiting LDL oxidation; Inhibiting foam cell formation	N/A	Jadeja et al. (2012)
			Atherogenic diet-induced *Charles foster* rats	Reducing TBARS and CD contents in LDL	VCAM-1↓, P-selectin↓	
Piper betle L. [Piperaceae]	Leaf	3-ρ-coumaroylquinic acid, 4-ρ-coumaroylquinic acid	ox-LDL-induced J774A.1 cells	Inhibiting LDL oxidation; Inhibiting foam cell formation; Activating the reverse cholesterol transport	ABCA1↑, LXR↑	Ma et al. (2013)
Methanol extract						
Hibiscus sabdariffa L. [Malvaceae]	Flower	Cyanidin, Delphinidin	ox-LDL-induced RAW264.7 cells	Inhibiting LDL oxidation; Reducing macrophages apoptosis	Caspase-3↓	Chang et al. (2006)
	Leaf	Catechin, ECG, Ellagic acid	ox-LDL-induced J774A.1 cells	Inhibiting LDL oxidation; Inhibiting foam cell formation	LXRα↑, ABCA1↑, CD36↓, PPAR-c↓	Chen et al. (2013)
Scoparia dulcis L. [Plantaginaceae; *Scopariae Herba*]	Leaf	Myricetin, Rutin	ox-LDL-induced RAW264.7 cells	Inhibiting LDL oxidation; Inhibiting foam cell formation; Decreasing inflammation	N/A	Nambiar et al. (2014)
Phyllanthus emblica L. [Phyllanthaceae; *Phyllanthi Fructus*]	Fruit	Kaempferol, Rutin, Coumaric acid	ox-LDL-induced RAW264.7 cells	Inhibiting LDL oxidation; Inhibiting foam cell formation	N/A	Nambiar and Shetty (2015)
Whole extract (aqueous and ethanol extract)						
Morus alba L. [Moraceae; *Mori Fructus*]	Fruit	Cyanidine-3-glucoside, Cyanidine-3-rutinoside, Pelargonidin-3-glucoside, Pelargonidine-3-rutinoside	ox-LDL-induced J774A.1 cells	Inhibiting LDL oxidation; Reducing macrophages apoptosis; Inhibiting foam cell formation	N/A	Liu et al. (2008)
Trachyspermum ammi (L.) Sprague [Apiaceae]	Seed	2-(4-Methyl-1H-1,2,3-triazol-1-yl) ethan-1-amine, 1,3-Dioxolan-2-one, 4,5-bis (methylene)-, N-methylene-n-ctadecylamine	ox-LDL-induced RAW264.7 cells	Inhibiting LDL oxidation; Inhibiting foam cell formation	N/A	Priyaa and More (2022)
Others						
Pinus morrisonicola Hayata [Pinaceae]	Needle	1-Docosene, Neophytadiene, Methyl abietate	ox-LDL-induced RAW264.7 cells	Inhibiting lipid peroxidation; Inhibiting foam cell formation	N/A	Cheng et al. (2015)
Citrus × aurantium f. deliciosa (Ten.) M.Hiroe [Rutaceae]	Peel	Limonene	ox-LDL-induced RAW264.7 cells	Inhibiting LDL oxidation; Inhibiting foam cell formation; Decreasing cholesterol synthesis	CD36↓	Castro et al. (2020)
Monomers						
	N/A	Curcumin			CD36↓, MDA↑	

(Continued on following page)

TABLE 1 (*Continued*) Inhibition LDL oxidation and foam cell formation effects of herbal medicines in Atherosclerosis.

Botanical drugs	Part of plant used	Main components	Animal/cells	Effects	Related targets	Refs
Aqueous extract						
Curcuma longa L. [Zingiberaceae; *Curcumae Longae Rhizoma*]			ox-LDL-induced RAW264.7 cells	Inhibiting LDL oxidation; Inhibiting foam cell formation		Kou et al. (2013), Min et al. (2013)
Vitex rotundifolia L.f. [Lamiaceae]	N/A	(3R)-5-Hydroxymellein	ox-LDL-induced RAW264.7 cells	Inhibiting LDL oxidation; Inhibiting foam cell formation	N/A	Kim et al. (2020)
N/A	N/A	Maslinic acid	ox-LDL-induced THP-1 monocytes	Inhibiting LDL oxidation; Inhibiting foam cell formation; Reducing monocytes adhesion; Enhancing cholesterol efflux	ABCA1↑, ABCG1↑, VCAM-1↓, MCP-1↓, CD36↓, SR-A↓	Phang et al. (2020)
Dendrobium venustum Teijsm. & Binn. [Orchidaceae]	N/A	Lusianthridin	ox-LDL-induced RAW264.7 cells	Inhibiting LDL oxidation; Inhibiting foam cell formation	N/A	Thant et al. (2021)

benefits, such as anti-oxidation, anti-inflammation, and anti-tumor (Dai et al., 2007). In 2008, Liu et al. (Liu et al., 2008) investigated the effect of mulberry anthocyanin-rich extracts (MACs) on Cu^{2+}-induced LDL and ox-LDL-induced macrophages. The results showed that MACs (0.1 mg/mL) could suppress the REM, Apo B fragmentation, and TBARS formation, which meant MACs had the ability to inhibit the oxidation of LDL. Then, the picture of Oil Red O staining suggested MACs reduced macrophage-derived foam cell formation. All these results suggested that MACs had an anti-oxidative effect on preventing or treating AS. Moreover, cyanidine-3-glucoside, cyanidine-3-rutinoside, pelargonidin-3-glucoside, and pelargonidine-3-rutinoside were identified from mulberry anthocyanin-rich extracts by HPLC/ESI/MS/MS.

5.1.2 *Syzygium cumini* (L.) skeels

Syzygium cumini (L.) Skeels is a plant of the Myrtaceae family, and the flavonoid-rich fraction of its seed has been reported to have many pharmacological effects, such as lowering blood lipids, anti-diabetes, and preventing cardiac and hepatic oxidative stress. Researchers (Sharma et al., 2008; Jadeja et al., 2012) studied the effect of flavonoid-rich *S. cumini* (L.) Skeels seed extract (SSE) on $Cu2^{+}$-induced LDL, ox-LDL-induced RAW264.7 cells and atherogenic diet (ATH)-induced Charles foster rats, and found that the main components of SSE are quercetin, rutin, and kaempferol. SSE could attenuate the oxidation of LDL *in vitro*, reduce foam cells formation, and reducing TBARS and CD contents in LDL isolated from rats. In addition, SSE ameliorated histoarchitectural changes of the thoracic aorta in ATH-induced rats and upregulated the expression of adhesion molecules *viz.* VCAM-1 and P-selectin. These results indicated that SSE might prevent AS through its anti-oxidative effects.

5.1.3 *Piper betle* L

Piper betel L., a plant of the Piperaceae family, has been shown to have anti-oxidative activity with low toxicity *in vitro* and *in vivo* (Choudhary and Kale, 2002), but its effects on LDL oxidation and foam cell formation are unclear. Ma et al. (Ma et al., 2013) studied the influence of the extract of *Piper betel* L. leaves (PBLs) on $Cu2^{+}$-induced LDL and ox-LDL-induced J774A.1 cells, and explored underlying molecular mechanisms. Their results showed that the main components of PBLs are 3-ρ-coumaroylquinic acid and 4-ρ-coumaroylquinic acid, and PBLs (0.1, 0.5, and 1.0 mg/mL) inhibited the oxidation of LDL, and further reduced the uptake of ox-LDL by macrophages and, in turn, prevented the formation of foam cells. In addition, PBLs reduced the lipid accumulation in macrophages *via* increasing levels of the class A and class B scavenger receptors, ABCA1, and Liver X receptor (LXR) in ox-LDL-induced J774A.1 cells, suggesting that PBLs activated the reverse cholesterol transport to prevent both lipid accumulation and foam cell formation.

5.2 Methanol extract

5.2.1 *Hibiscus sabdariffa* L

Due to a variety of pharmacological effects, *Hibiscus sabdariffa* L. is considered to be beneficial for health, and it possesses antioxidant components, including vitamin E, β-carotene and anthocyanins (Tsuda et al., 1996; Tsuda et al., 2000; Ramirez-Tortosa et al., 2001). In 2006, Chang et al. (Chang et al., 2006) studied the antioxidant effect of *H. sabdariffa* L. (HS), extracted from the dried flower *H. sabdariffa* L., on LDL *in vitro*. In their study, relative electrophoretic mobility (REM), fragmentation of Apo B, and thiobarbituric acid reaction substances (TBARS) assay were used to determine whether HAs could suppress oxidation of LDL stimulated by Cu^{2+}, and ox-LDL-induced RAW 264.7 cells were used to research the protective of HS on RAW 264.7 cells. The results showed that HS (1, 1.5, 2 mg/mL) effectively inhibited the oxidation of LDL, and reduced apoptosis of RAW 264.7 cells. Besides, two

components were identified by HPLC, namely, cyanidin and delphinidin.

Hibiscus sabdariffa L. leaves, the edible part of *H. sabdariffa* L., consist of multiple polyphenols, and possess a variety of pharmacological functions, including anti-hyperglycaemia, anti-hyperlipidemia, and anti-oxidation. In 2013, Chen et al. (Chen et al., 2013) studied whether *H. sabdariffa* L. leaf polyphenolic extract (HLP) could inhibit the formation of foam cells. The ability to inhibit LDL oxidation of HLP (mainly including catechin, (−)-epicatechin gallate, ellagic acid, ferulic acid, and quercetin) was measured by TBARS analysis, agarose gel electrophoresis and electrophoresis of Apo B fragmentation assay. The results showed that HLP could inhibit LDL oxidation. Subsequently, the Oil Red O staining assay was utilized to study the effect of HLP on foam cell formation, and the result indicated the amount of foam cells was increased with treatments of HLP. In addition, the results of WB indicated that the underlying mechanism was up-regulating LXRa/ABCA1 pathway and decreasing the expressions of CD36 and PPAR-γ.

5.2.2 *Scoparia dulcis* L

Due to the ability of anti-diabetes, anti-hypertension, anti-hyperlipidemia, and anti-tumor, *Scoparia dulcis* L., an edible ethnomedicinal folklore botanical drug, has attracted the interest of researchers. It has been reported that *S. dulcis* L. could reduce levels of LDL cholesterol in diabetic rats with its anti-oxidative and anti-inflammatory effects, so that *S. dulcis* L. might have an anti-atherosclerotic effect (Pari and Latha, 2006). In 2014, Nambiar et al. (Nambiar et al., 2014) found that foliar methanol extract of *S. dulcis* effectively inhibited lipid peroxidation and LDL oxidation, indicating that *S. dulcis* L. might own strongly anti-oxidative effect. In addition, *S. dulcis* L. inhibited the formation of ox-LDL, as shown in the result of the Oil red O staining. Moreover, *S. dulcis* L. improved human erythrocyte membrane stability, suggesting anti-inflammatory effects of *S. dulcis* L. Besides, myricetin and rutin, identified by HPLC, might be the main components of *S. dulcis* L.

5.2.3 *Phyllanthus emblica* L

Phyllanthus emblica L., belonging to the Phyllanthaceae family, is an edible fruit distributed in many countries, and is also used as a medicinal fruit to prevent diseases. Traditionally, *P. emblica* L. is believed to have antioxidant and anti-aging effects. And then, the anti-inflammatory, hypoglycemic, hypolipidemic, and immunomodulatory effects of *P. emblica* L. were proved by modern research. It has been reported that *P. emblica* L. could decrease cholesterol levels in cholesterol-fed rats, showing anti-atherosclerotic effects (Kim et al., 2005). Then, Nambiar et al. (Nambiar and Shetty, 2015) studied the effects of *P. emblica* L. on LDL oxidation and foam cell formation, and found that *P. emblica* L. on the concentration of 50 μg/mL effectively inhibited the oxidation of LDL induced by Cu^{2+}, and reduced uptake of ox-LDL in RAW264.7 cells to inhibit foam cell formation. In addition, the results of HPLC analysis showed that phenolic and flavonoid are the main component groups of *P. emblica* L., while kaempferol, rutin, and coumaric acid are the three components with the highest content.

5.3 Whole extract (aqueous and ethanol extract)

5.3.1 *Morus alba* L

Mulberry leaf, the leaf of *M. alba* L., is a traditional Chinese medicine that has been used for anti-diabetes, anti-hyperlipidemia, and prevention of coronary artery disease (CAD) for many years (Andallu et al., 2001). Yang et al. (Yang et al., 2011). Studied the effect of mulberry leaf polyphenolic extracts (MLPE) on LDL oxidation and foam cell formation, and found that MLPE could attenuate the oxidation and lipid peroxidation of LDL, reduce ox-LDL-generated ROS, and elevate the pressions of SOD-1 and GPx in macrophages, suggesting that MLPE had a strongly anti-oxidative effect. Furthermore, MLPE effectively inhibited the foam cell formation and decreased the amount of TG and cholesterol, and the underlying mechanism might be downregulating expression of expression of PPARγ, CD36, and SR-A, implying that MLPE reduced the uptake of ox-LDL. According to the results of HPLC analysis, gallocatechin, gallocatechin gallate, and naringenin are the main components.

5.3.2 *Trachyspermum ammi* (L.) sprague

Trachyspermum ammi (L.) Sprague (*Trachyspermum ammi*) is a traditional Chinese medicine and a very famous spice, which is usually used in respiratory ailments, bronchial pneumonia, and stomach disorders. In recent years, it has been reported that *T. ammi* has antioxidant, antihyperlipidemic, and antidiabetic effects. In addition, honey, a natural sweetener used in many countries, is beneficial in preventing cardiovascular diseases, including AS. However, the anti-atherosclerotic effects of the combination of *T. ammi* and honey had not been demonstrated. Therefore, Priyaa et al. studied how the extracts of *T. ammi* and honey work on AS, especially on antioxidation and lipid metabolism (Priyaa and More, 2022). In their results, tannin methanol extract (TME) showed the highest activity to inhibit LDL oxidation and further inhibited ox-LDL-induced foam cell formation, apoptosis, and proliferation. Moreover, the analysis of TEM compositions *via* HPLC and GC-MS showed 2-(4-Methyl-1H-1,2,3-triazol-1-yl) ethan-1-amine, 1,3-Dioxolan-2-one, 4,5-bis (methylene)-, N-methylene-n-octadecylamine are the three ingredients with the highest content.

5.4 Others

5.4.1 *Pinus morrisonicola* hayata

Pinus morrisonicola Hayata, a plant of the Pinaceae family, has a variety of pharmacological effects, including anti-oxidation, anti-mutagenicity, and anti-inflammation, and has been used as a folk medicine for anti-hypertension in Asia for a long time. In 2008, Yen et al. (Yen et al., 2008) reported that *P. morrisonicola* Hayata could inhibit Cu^{2+}-mediated LDL oxidation and increase NO production in cells, showing an anti-atherosclerotic effect. Considering the important role of ox-LDL-induced foam cells in AS development, in 2015, Cheng et al. (Cheng et al., 2015) studied the effects of essential oil extracted from *P. morrisonicola* Hayata (PME) on ox-LDL-induced RAW264.7 cells. The results showed

that PME contained 1-docosene, neophytadiene, and methyl abietate, and could inhibit lipid peroxidation and foam cell formation *in vitro*. Therefore, it seems that PME has an anti-atherosclerotic effect through its anti-oxidative capacity.

5.4.2 *Citrus* × *aurantium f. deliciosa* (ten.) M.Hiroe

Recently, accumulating evidence has suggested that medicinal essential oils usually own properties of anti-oxidation and lowering blood lipid, but the molecular mechanism still has been unclear. Mandarin [*Citrus* × *aurantium f. deliciosa* (Ten.) M. Hiroe] peel oil (MPO) is recognized to be safe and has an anti-atherosclerotic effect (Chung et al., 2007; Chung et al., 2008). In 2020, Castro et al. (Castro et al., 2020) studied the effects of MPO on cholesterol metabolism and lipid synthesis, and its antioxidant capacity. The results showed that MPO (mainly includes limonene) decreased cholesterol synthesis *via* inhibiting post-squalene reaction of the mevalonate pathway, while inhibited LDL oxidation under oxidative stress. In addition, MPO inhibited foam cell formation and significantly decreased lipid quantity in foam cells, and downregulated the expression of CD36. In a word, the above results suggest that MPO may be a potential candidate for preventing AS.

5.5 Monomers

5.5.1 Curcumin

Curcumin is a bioactive compound derived from the rhizomes of *Curcuma longa* L., and has been reported to possess a variety of activities, such as anti-tumor and anti-inflammatory. In 2013, Kou et al. (Kou et al., 2013) studied the effect of curcumin on Cu^{2+}-mediated LDL oxidation in a cell-free system and foam cell formation, and found that curcumin (10 μM) could increase the level of MDA and dose-dependently attenuate LDL oxidation. Later, Min et al. (Min et al., 2013) studied the potential molecular mechanism of curcumin to inhibit foam cell formation, and their results showed that the expression of CD36 and PPAR-γ was downregulated, and the phosphorylation of p38 MAPK was inhibited in ox-LDL-induced RAW 264.7 cells, suggesting that curcumin reduced the formation of ox-LDL *via* inhibiting the phosphorylation of p38 MAPK.

5.5.2 (3R)-5-hydroxymellein

(3R)-5-Hydroxymellein is a secondary metabolite from a plant called *Vitex rotundifolia* L. f. From the first time (3R)-5-Hydroxymellein was isolated and identified in 1990 until now, it has been reported to have a variety of pharmacological activities, such as antifungal, antibacterial, and antioxidant effects (Wedge and Nagle, 2000). In 2020, Kim et al. (Kim et al., 2020) first illustrated that (3R)-5-Hydroxymellein might have the ability to reduce the risk of AS. In their research, the production of conjugated dienes and malondialdehyde, the amount of hyperchromicity and carbonyl content, and anti-LDL oxidation were used to demonstrate the oxidation of LDL and HDL, while the Oil red O staining was used to demonstrate the inhibition of foam cell formation. The results suggested that (3R)-5-Hydroxymellein could inhibit the oxidation of LDL and HDL, as well as inhibit foam cell formation.

5.5.3 Maslinic acid

Maslinic acid (MA) is a natural pentacyclic triterpene and can be isolated from many natural sources, including herbal remedies, vegetables, and fruits. MA has various beneficial properties, such as antioxidant, anti-inflammatory, antitumoral, and cardioprotective effects (Liu et al., 2011). Thus, Phang et al. (Phang et al., 2020) hypothesized that MA could inhibit foam cell formation, and verified it by a series of experiments *in vitro*. In detail, the Oil Red O staining and flow cytometric analysis were utilized to explore the formation of foam cells, while Cu^{2+}-stimulated LDL was utilized to explore LDL oxidation, and the results showed that MA (20 μM) inhibited LDL oxidation and foam cell formation. In addition, MA reduced THP-1monocyte adhesion to HUVEC cells *via* downregulated the expression of VCAM-1 and MCP-1, and enhanced cholesterol efflux *via* downregulated the expression of SR-A and CD36, while upregulated the expression of ABCA1 and ABCG1. In conclusion, MA showed a strong ability for AS prevention.

5.5.4 Lusianthridin

Lusianthridin (LST) is a phenanthrene compound isolated from *Dendrobium venustum* Teijsm. & Binn., which belongs to the family Orchidaceae. As reported in a research article, LST was a potential antioxidant. In thalassemia patients, an oxidant called hemin can be found, which causes oxidative stress and LDL oxidation, and is a risk factor for AS. In 2021, Thant et al. (Thant et al., 2021) verified whether LST could attenuate hemin-induced ox-LDL and foam cell formation. In their study, results of TBAR assay and REM assay suggested that LST inhibited LDL oxidation, while the result of the Oil Red O staining assay suggested that LST inhibited ox-LDL-induced foam cell formation. Otherwise, LST improved the level of cholesteryl arachidonate and cholesteryl linoleate.

6 Conclusion and perspectives

AS is a chronic disease with complex pathogenesis, and lipid metabolism disorder is one of the important mechanisms of AS pathogenesis, which makes great contributions to occurrence and development of AS. It is found that metabolic disorder of cholesterol, an important lipid component in the human body, is closely related to oxidative stress (Khatana et al., 2020). As mentioned above, fat-soluble cholesterol cannot be transported in the blood alone. Therefore, it must combine with apolipoprotein and other components to form plasma lipoprotein. LDL, composed of cholesterol ester, triglyceride, phospholipid, and apolipoprotein, is the plasma lipoprotein with the highest cholesterol content and plays an important role in cholesterol metabolism (Itabe, 1998). Under normal physiological conditions, cholesterol is transported to peripheral tissues by LDL, and then is reversely transported to the liver by HDL. Finally, part of the cholesterol is reabsorbed, while the other part is discharged from the body. However, under the condition of oxidative stress, LDL containing a large number of cholesterol esters is oxidized to ox-LDL, which causes macrophages to engulf a large number of ox-LDL and eventually form foam cells, promoting the development of AS (Braamskamp et al., 2015). In addition, ox-LDL can promote the development of AS in many ways, including causing endothelial dysfunction, promoting smooth

muscle cell migration and proliferation, and activating platelets. Therefore, inhibiting the oxidative modification of LDL is of great significance for the prevention and treatment of AS.

It has been a long time since herbal medicines to be used to prevent and treat diseases, and herbal medicines have made great contributions to human health. And there are accumulating studies have reported that botanical drugs and their extracts have significant effects on AS, including protecting vascular endothelial cells, improving lipid metabolism disorder, reducing foam cell formation, and so on (Zhang et al., 2021). In recent years, more and more studies have reported that botanical drugs and their extracts can significantly inhibit the oxidation of LDL, thereby reducing the production of foam cells to play an anti-atherosclerosis role. In these researches, copper ions have been used to oxidize the separated LDL *in vitro*, while REM, fragmentation of Apo B, and TBARS assay are used to measure whether LDL is oxidized. In addition, RAW 264.7 cells induced by ox-LDL have been used for Oil red O staining to judge the generation of foam cells. Moreover, PCR and WB experiments have been used to reveal the potential molecular mechanism of herbal medicines to treat AS. The results showed that herbal medicines mentioned in this review had significant antioxidative effects and could effectively inhibit the oxidation of LDL. In addition, these herbal medicines regulated cholesterol homeostasis by reducing cholesterol intake and increasing its efflux, and the molecular mechanism was related to down-regulating the expression of scavenger receptor CD36 and SR-A, and up-regulating the expression of ABCA1 and ABCG1. Interestingly, most of the effective ingredients reported in the articles collected in this review are phenols and polyphenols extract of herbal medicines. Phenols/polyphenols are compounds with one aromatic ring attached to one or more hydroxyl functional groups in structure, and widely exist in fruits, vegetables, botanical drugs, and tea. It has been reported that phenols/polyphenols have strong antioxidant activity, which may improve cardiovascular diseases and neurodegenerative diseases (Cheng et al., 2017). This review summarized the antioxidation of LDL and inhibition of foam cell formation effects of phenols/polyphenols extracts from mulberry leaf, *H. sabdariffa* leaf, *Eugenia jambolana* seed, mulberry anthocyanin, *S. dulcis*, *Emplica officinalis*, lusianthridin, and *T. ammi*.

Although a large number of studies have clarified to some extent that herbal medicines can prevent and cure AS by inhibiting LDL oxidation, some deficiencies still exist. First, most of current studies measured *in vitro* experiments, therefore, the beneficial effects and safety *in vivo* should be clarified. Especially, the change of LDL and ox-LDL levels after treatment with herbal medicines should be determined *in vivo*. Second, existing research has not revealed the molecular mechanisms of these herbal medicines, thus, in-depth research should be carried out to find underlying molecular mechanisms. Third, current research mostly used mixed extracts of herbal medicines, and individual compounds that play pharmacological roles were not clear, limiting the further development of these herbal medicines. In addition, some active compounds are found in very low levels in plants, while sources of these active compounds have not been systematically developed. So, it is necessary to find more sources. Fourth, toxicity studies on these herbal medicines are still lacking, so a lot of subsequent work is needed to determine target organs, toxic reactions and mechanisms, and to explore detoxification methods to ensure the safety of these herbal medicines. Fifth, due to complex components and unexplained mechanisms, the promotion of herbal medicines is limited. Although herbal medicines have gained widespread clinical applications in some Asian countries, they are rarely used in many western countries worldwide. Therefore, more clinical trials need to be measured to verify the safety and effectiveness of herbal medicines. In future research, these problems need to be solved. Sixth, current research on these herbal medicines is focused on pharmacodynamic studies, and appropriate drug delivery system should be developed.

In conclusion, this review described the effects of lipid metabolism disorder related to LDL oxidation on AS and the beneficial role of herbal medicines in this process. We hope that this review will provide ideas for the development of medicines for the prevention and treatment of AS from herbal medicines.

Author contributions

HS and LH conception and design of the review, HD and PS contributed to data analysis and write the manuscript. RL contributed to write sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China (Grant Nos. 82004242).

Acknowledgments

We thank all the people involved with the related work.

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

Afonso, C. B., and Spickett, C. M. (2019). Lipoproteins as targets and markers of lipoxidation. *Redox Biol.* 23, 101066. doi:10.1016/j.redox.2018.101066

Alexander, M. R., and Owens, G. K. (2012). Epigenetic control of smooth muscle cell differentiation and phenotypic switching in vascular development and disease. *Annu. Rev. Physiol.* 74, 13–40. doi:10.1146/annurev-physiol-012110-142315

Altmann, S. W., Davis, H. R.,, JR., Zhu, L. J., Yao, X., Hoos, L. M., Tetzloff, G., et al. (2004). Niemann-Pick C1 like 1 protein is critical for intestinal cholesterol absorption. *Science* 303, 1201–1204. doi:10.1126/science.1093131

Andallu, B., Suryakantham, V., Lakshmi Srikanthi, B., and Reddy, G. K. (2001). Effect of mulberry (Morus indica L.) therapy on plasma and erythrocyte membrane lipids in patients with type 2 diabetes. *Clin. Chim. Acta* 314, 47–53. doi:10.1016/s0009-8981(01)00632-5

Bennett, M. R., Sinha, S., and Owens, G. K. (2016). Vascular smooth muscle cells in atherosclerosis. *Circ. Res.* 118, 692–702. doi:10.1161/CIRCRESAHA.115.306361

Braamskamp, M. J., Stefanutti, C., Langslet, G., Drogari, E., Wiegman, A., Hounslow, N., et al. (2015). Efficacy and safety of pitavastatin in children and adolescents at high future cardiovascular risk. *J. Pediatr.* 167, 338–343.e5. doi:10.1016/j.jpeds.2015.05.006

Brown, A. J., Mander, E. L., Gelissen, I. C., Kritharides, L., Dean, R. T., and Jessup, W. (2000). Cholesterol and oxysterol metabolism and subcellular distribution in macrophage foam cells. Accumulation of oxidized esters in lysosomes. *J. Lipid Res.* 41, 226–236. doi:10.1016/S0022-2275(20)32056-3

Castro, M. A., Llanos, M. A., Rodenak-Kladniew, B. E., Gavernet, L., Galle, M. E., and Crespo, R. (2020). Citrus reticulata peel oil as an antiatherogenic agent: Hypolipogenic effect in hepatic cells, lipid storage decrease in foam cells, and prevention of LDL oxidation. *Nutr. Metab. Cardiovasc Dis.* 30, 1590–1599. doi:10.1016/j.numecd.2020.04.033

Chang, Y. C., Huang, K. X., Huang, A. C., Ho, Y. C., and Wang, C. J. (2006). Hibiscus anthocyanins-rich extract inhibited LDL oxidation and oxLDL-mediated macrophages apoptosis. *Food Chem. Toxicol.* 44, 1015–1023. doi:10.1016/j.fct.2005.12.006

Chen, J. H., Wang, C. J., Wang, C. P., Sheu, J. Y., Lin, C. L., and Lin, H. H. (2013). Hibiscus sabdariffa leaf polyphenolic extract inhibits LDL oxidation and foam cell formation involving up-regulation of LXRα/ABCA1 pathway. *Food Chem.* 141, 397–406. doi:10.1016/j.foodchem.2013.03.026

Cheng, M. C., Chang, W. H., Chen, C. W., Li, W. W., Tseng, C. Y., and Song, T. Y. (2015). Antioxidant properties of essential oil extracted from Pinus morrisonicola hay needles by supercritical fluid and identification of possible active compounds by GC/MS. *Molecules* 20, 19051–19065. doi:10.3390/molecules201019051

Cheng, Y. C., Sheen, J. M., Hu, W. L., and Hung, Y. C. (2017). Polyphenols and oxidative stress in atherosclerosis-related ischemic heart disease and stroke. *Oxid. Med. Cell. Longev.* 2017, 8526438. doi:10.1155/2017/8526438

Chistiakov, D. A., Melnichenko, A. A., Myasoedova, V. A., Grechko, A. V., and Orekhov, A. N. (2017). Mechanisms of foam cell formation in atherosclerosis. *J. Mol. Med. Berl.* 95, 1153–1165. doi:10.1007/s00109-017-1575-8

Choudhary, D., and Kale, R. K. (2002). Antioxidant and non-toxic properties of piper betle leaf extract: *In vitro* and *in vivo* studies. *Phytother. Res.* 16, 461–466. doi:10.1002/ptr.1015

Chung, M. J., Kang, A. Y., Park, S. O., Park, K. W., Jun, H. J., and Lee, S. J. (2007). The effect of essential oils of dietary wormwood (Artemisia princeps), with and without added vitamin E, on oxidative stress and some genes involved in cholesterol metabolism. *Food Chem. Toxicol.* 45, 1400–1409. doi:10.1016/j.fct.2007.01.021

Chung, M. J., Park, K. W., Kim, K. H., Kim, C. T., Baek, J. P., Bang, K. H., et al. (2008). Asian plantain (Plantago asiatica) essential oils suppress 3-hydroxy-3-methyl-glutaryl-co-enzyme A reductase expression *in vitro* and *in vivo* and show hypocholesterolaemic properties in mice. *Br. J. Nutr.* 99, 67–75. doi:10.1017/S0007114507798926

Curtiss, L. K., Black, A. S., Takagi, Y., and Plow, E. F. (1987). New mechanism for foam cell generation in atherosclerotic lesions. *J. Clin. Invest.* 80, 367–373. doi:10.1172/JCI113081

D'AutréAUX, B., and Toledano, M. B. (2007). ROS as signalling molecules: Mechanisms that generate specificity in ROS homeostasis. *Nat. Rev. Mol. Cell. Biol.* 8, 813–824. doi:10.1038/nrm2256

Dai, J., Patel, J. D., and Mumper, R. J. (2007). Characterization of blackberry extract and its antiproliferative and anti-inflammatory properties. *J. Med. Food* 10, 258–265. doi:10.1089/jmf.2006.238

Das, M., Devi, K. P., Belwal, T., Devkota, H. P., Tewari, D., Sahebnasagh, A., et al. (2021). Harnessing polyphenol power by targeting eNOS for vascular diseases. *Crit. Rev. Food Sci. Nutr.* 1–26. doi:10.1080/10408398.2021.1971153

DröGE, W. (2002). Free radicals in the physiological control of cell function. *Physiol. Rev.* 82, 47–95. doi:10.1152/physrev.00018.2001

Duan, H., Zhang, Q., Liu, J., Li, R., Wang, D., Peng, W., et al. (2021). Suppression of apoptosis in vascular endothelial cell, the promising way for natural medicines to treat atherosclerosis. *Pharmacol. Res.* 168, 105599. doi:10.1016/j.phrs.2021.105599

Duffy, D., and Rader, D. J. (2009). Update on strategies to increase HDL quantity and function. *Nat. Rev. Cardiol.* 6, 455–463. doi:10.1038/nrcardio.2009.94

Eto, H., Miyata, M., Kume, N., Minami, M., Itabe, H., Orihara, K., et al. (2006). Expression of lectin-like oxidized LDL receptor-1 in smooth muscle cells after vascular injury. *Biochem. Biophys. Res. Commun.* 341, 591–598. doi:10.1016/j.bbrc.2005.12.211

Fong, L. G., Parthasarathy, S., Witztum, J. L., and Steinberg, D. (1987). Nonenzymatic oxidative cleavage of peptide bonds in apoprotein B-100. *J. Lipid Res.* 28, 1466–1477. doi:10.1016/S0022-2275(20)38576-X

FöRSTERMANN, U., and Sessa, W. C. (2012). Nitric oxide synthases: Regulation and function. *Eur. Heart J.* 33 (829-37), 829–837d. doi:10.1093/eurheartj/ehr304

Frei, B., Stocker, R., and Ames, B. N. (1988). Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc. Natl. Acad. Sci. U. S. A.* 85, 9748–9752. doi:10.1073/pnas.85.24.9748

Fujimura, N., Jitsuiki, D., Maruhashi, T., Mikami, S., Iwamoto, Y., Kajikawa, M., et al. (2012). Geranylgeranylacetone, heat shock protein 90/AMP-activated protein kinase/endothelial nitric oxide synthase/nitric oxide pathway, and endothelial function in humans. *Arterioscler. Thromb. Vasc. Biol.* 32, 153–160. doi:10.1161/ATVBAHA.111.237263

Goldstein, J. L., and Brown, M. S. (2009). The LDL receptor. *Arterioscler. Thromb. Vasc. Biol.* 29, 431–438. doi:10.1161/ATVBAHA.108.179564

Goyal, T., Mitra, S., Khaidakov, M., Wang, X., Singla, S., Ding, Z., et al. (2012). Current concepts of the role of oxidized LDL receptors in atherosclerosis. *Curr. Atheroscler. Rep.* 14, 150–159. doi:10.1007/s11883-012-0228-1

Gupta, M., Blumenthal, C., Chatterjee, S., Bandyopadhyay, D., Jain, V., Lavie, C. J., et al. (2020). Novel emerging therapies in atherosclerosis targeting lipid metabolism. *Expert Opin. Investig. Drugs* 29, 611–622. doi:10.1080/13543784.2020.1764937

Hao, P., Jiang, F., Cheng, J., Ma, L., Zhang, Y., and Zhao, Y. (2017). Traditional Chinese medicine for cardiovascular disease: Evidence and potential mechanisms. *J. Am. Coll. Cardiol.* 69, 2952–2966. doi:10.1016/j.jacc.2017.04.041

Hofmann, A., Brunssen, C., and Morawietz, H. (2017). Contribution of lectin-like oxidized low-density lipoprotein receptor-1 and LOX-1 modulating compounds to vascular diseases. *Vasc. Pharmacol.* 107, 1–11. doi:10.1016/j.vph.2017.10.002

Hurtubise, J., Mclellan, K., Durr, K., Onasanya, O., Nwabuko, D., and Ndisang, J. F. (2016). The different facets of dyslipidemia and hypertension in atherosclerosis. *Curr. Atheroscler. Rep.* 18, 82. doi:10.1007/s11883-016-0632-z

Itabe, H. (1998). Oxidized phospholipids as a new landmark in atherosclerosis. *Prog. Lipid Res.* 37, 181–207. doi:10.1016/s0163-7827(98)00009-5

Jadeja, R. N., Thouaojam, M. C., Sankhari, J. M., Jain, M., Devkar, R. V., and Ramachandran, A. V. (2012). Standardized flavonoid-rich Eugenia jambolana seed extract retards *in vitro* and *in vivo* LDL oxidation and expression of VCAM-1 and P-selectin in atherogenic rats. *Cardiovasc Toxicol.* 12, 73–82. doi:10.1007/s12012-011-9140-0

Jiang, H., Zhou, Y., Nabavi, S. M., Sahebkar, A., Little, P. J., Xu, S., et al. (2022). Mechanisms of oxidized LDL-mediated endothelial dysfunction and its consequences for the development of atherosclerosis. *Front. Cardiovasc Med.* 9, 925923. doi:10.3389/fcvm.2022.925923

Khatana, C., Saini, N. K., Chakrabarti, S., Saini, V., Sharma, A., Saini, R. V., et al. (2020). Mechanistic insights into the oxidized low-density lipoprotein-induced atherosclerosis. *Oxid. Med. Cell. Longev.* 2020, 5245308. doi:10.1155/2020/5245308

Kim, H. J., Yokozawa, T., Kim, H. Y., Tohda, C., Rao, T. P., and Juneja, L. R. (2005). Influence of amla (Emblica officinalis Gaertn.) on hypercholesterolemia and lipid peroxidation in cholesterol-fed rats. *J. Nutr. Sci. Vitaminol. (Tokyo)* 51, 413–418. doi:10.3177/jnsv.51.413

Kim, J. Y., Kim, S., and Shim, S. H. (2020). Anti-atherosclerotic activity of (3r)-5-hydroxymellein from an endophytic fungus neofusicoccum parvum JS-0968 derived from Vitex rotundifolia through the inhibition of lipoproteins oxidation and foam cell formation. *Biomolecules* 10, 715. doi:10.3390/biom10050715

Kimura, T., Itoh, T., Fusazaki, T., Matsui, H., Sugawara, S., Ogino, Y., et al. (2010). Low-density lipoprotein-cholesterol/high-density lipoprotein-cholesterol ratio predicts lipid-rich coronary plaque in patients with coronary artery disease--integrated-backscatter intravascular ultrasound study. *Circ. J.* 74, 1392–1398. doi:10.1253/circj.cj-09-0849

Kou, M. C., Chiou, S. Y., Weng, C. Y., Wang, L., Ho, C. T., and Wu, M. J. (2013). Curcuminoids distinctly exhibit antioxidant activities and regulate expression of scavenger receptors and heme oxygenase-1. *Mol. Nutr. Food Res.* 57, 1598–1610. doi:10.1002/mnfr.201200227

Kritharides, L., Jessup, W., Gifford, J., and Dean, R. T. (1993). A method for defining the stages of low-density lipoprotein oxidation by the separation of cholesterol- and cholesteryl ester-oxidation products using HPLC. *Anal. Biochem.* 213, 79–89. doi:10.1006/abio.1993.1389

Kruth, H. S., Huang, W., Ishii, I., and Zhang, W. Y. (2002). Macrophage foam cell formation with native low density lipoprotein. *J. Biol. Chem.* 277, 34573–34580. doi:10.1074/jbc.M205059200

Kume, N., Moriwaki, H., Kataoka, H., Minami, M., Murase, T., Sawamura, T., et al. (2000). Inducible expression of LOX-1, a novel receptor for oxidized LDL, in

macrophages and vascular smooth muscle cells. *Ann. N. Y. Acad. Sci.* 902, 323–327. doi:10.1111/j.1749-6632.2000.tb06332.x

Li, H., Horke, S., and FöRSTERMANN, U. (2014). Vascular oxidative stress, nitric oxide and atherosclerosis. *Atherosclerosis* 237, 208–219. doi:10.1016/j.atherosclerosis.2014.09.001

Liu, J., Sun, H., Shang, J., Yong, Y., and Zhang, L. (2011). Effect of olive pomace extracts on hyperlipidaemia. *Nat. Prod. Res.* 25, 1190–1194. doi:10.1080/14786419.2010.521750

Liu, L. K., Lee, H. J., Shih, Y. W., Chyau, C. C., and Wang, C. J. (2008). Mulberry anthocyanin extracts inhibit LDL oxidation and macrophage-derived foam cell formation induced by oxidative LDL. *J. Food Sci.* 73, H113–H121. doi:10.1111/j.1750-3841.2008.00801.x

Ma, G. C., Wu, P. F., Tseng, H. C., Chyau, C. C., Lu, H. C., and Chou, F. P. (2013). Inhibitory effect of Piper betel leaf extracts on copper-mediated LDL oxidation and oxLDL-induced lipid accumulation via inducing reverse cholesterol transport in macrophages. *Food Chem.* 141, 3703–3713. doi:10.1016/j.foodchem.2013.06.003

Martin-Ventura, J. L., Leclercq, A., Blanco-Colio, L. M., Egido, J., Rossignol, P., Meilhac, O., et al. (2007). Low plasma levels of HSP70 in patients with carotid atherosclerosis are associated with increased levels of proteolytic markers of neutrophil activation. *Atherosclerosis* 194, 334–341. doi:10.1016/j.atherosclerosis.2006.10.030

Min, K. J., Um, H. J., Cho, K. H., and Kwon, T. K. (2013). Curcumin inhibits oxLDL-induced CD36 expression and foam cell formation through the inhibition of p38 MAPK phosphorylation. *Food Chem. Toxicol.* 58, 77–85. doi:10.1016/j.fct.2013.04.008

Nambiar, S. S., Shetty, N. P., Bhatt, P., and Neelwarne, B. (2014). Inhibition of LDL oxidation and oxidized LDL-induced foam cell formation in RAW 264.7 cells show anti-atherogenic properties of a foliar methanol extract of Scoparia dulcis. *Pharmacogn. Mag.* 10, S240–S248. doi:10.4103/0973-1296.133241

Nambiar, S. S., and Shetty, N. P. (2015). Phytochemical profiling and assessment of low-density lipoprotein oxidation, foam cell-preventing ability and antioxidant activity of commercial products of emblica officinalis fruit. *J. Food Biochem.* 39, 218–229. doi:10.1111/jfbc.12122

Pari, L., and Latha, M. (2006). Antihyperlipidemic effect of Scoparia dulcis (sweet broomweed) in streptozotocin diabetic rats. *J. Med. Food* 9, 102–107. doi:10.1089/jmf.2006.9.102

Phang, S. W., Ooi, B. K., Ahemad, N., and Yap, W. H. (2020). Maslinic acid suppresses macrophage foam cells formation: Regulation of monocyte recruitment and macrophage lipids homeostasis. *Vasc. Pharmacol.* 128-129, 106675. doi:10.1016/j.vph.2020.106675

Pirillo, A., Norata, G. D., and Catapano, A. L. (2013). LOX-1, OxLDL, and atherosclerosis. *Mediat. Inflamm.* 2013, 152786. doi:10.1155/2013/152786

Podrez, E. A., Byzova, T. V., Febbraio, M., Salomon, R. G., Ma, Y., Valiyaveettil, M., et al. (2007). Platelet CD36 links hyperlipidemia, oxidant stress and a prothrombotic phenotype. *Nat. Med.* 13, 1086–1095. doi:10.1038/nm1626

Priyaa, H. G., and More, S. S. (2022). Antioxidant, anti-proliferative, and anti-atherosclerotic effect of phytochemicals isolated from Trachyspermum ammi with honey in RAW 264.7 and THP-1 cells. *Pharmacogn. Mag.* 18, 143–151. doi:10.4103/pm.pm_436_21

Quinn, M. T., Parthasarathy, S., and Steinberg, D. (1985). Endothelial cell-derived chemotactic activity for mouse peritoneal macrophages and the effects of modified forms of low density lipoprotein. *Proc. Natl. Acad. Sci. U. S. A.* 82, 5949–5953. doi:10.1073/pnas.82.17.5949

Ramirez-Tortosa, C., Andersen Ø, M., Cabrita, L., Gardner, P. T., Morrice, P. C., Wood, S. G., et al. (2001). Anthocyanin-rich extract decreases indices of lipid peroxidation and DNA damage in vitamin E-depleted rats. *Free Radic. Biol. Med.* 31, 1033–1037. doi:10.1016/s0891-5849(01)00618-9

Segrest, J. P., Jones, M. K., de Loof, H., and Dashti, N. (2001). Structure of apolipoprotein B-100 in low density lipoproteins. *J. Lipid Res.* 42, 1346–1367. doi:10.1016/s0022-2275(20)30267-4

Sharma, B., Balomajumder, C., and Roy, P. (2008). Hypoglycemic and hypolipidemic effects of flavonoid rich extract from Eugenia jambolana seeds on streptozotocin induced diabetic rats. *Food Chem. Toxicol.* 46, 2376–2383. doi:10.1016/j.fct.2008.03.020

Siegel-Axel, D., Daub, K., Seizer, P., Lindemann, S., and Gawaz, M. (2008). Platelet lipoprotein interplay: Trigger of foam cell formation and driver of atherosclerosis. *Cardiovasc Res.* 78, 8–17. doi:10.1093/cvr/cvn015

Steinberg, D. (1983). Lipoproteins and atherosclerosis. A look back and a look ahead. *Arteriosclerosis* 3, 283–301. doi:10.1161/01.atv.3.4.283

Steinbrecher, U. P., Zhang, H. F., and Lougheed, M. (1990). Role of oxidatively modified LDL in atherosclerosis. *Free Radic. Biol. Med.* 9, 155–168. doi:10.1016/0891-5849(90)90119-4

Stocker, R., and Keaney, J. F., JR. (2004). Role of oxidative modifications in atherosclerosis. *Physiol. Rev.* 84, 1381–1478. doi:10.1152/physrev.00047.2003

Sun, Y., and Chen, X. (2011). Ox-LDL-induced LOX-1 expression in vascular smooth muscle cells: Role of reactive oxygen species. *Fundam. Clin. Pharmacol.* 25, 572–579. doi:10.1111/j.1472-8206.2010.00885.x

Thant, S. W., Morales, N. P., Buranasudja, V., Sritularak, B., and Luechapudiporn, R. (2021). Protective effect of lusianthridin on hemin-induced low-density lipoprotein oxidation. *Pharm. (Basel)* 14, 567. doi:10.3390/ph14060567

Tsuda, T., Kato, Y., and Osawa, T. (2000). Mechanism for the peroxynitrite scavenging activity by anthocyanins. *FEBS Lett.* 484, 207–210. doi:10.1016/s0014-5793(00)02150-5

Tsuda, T., Shiga, K., Ohshima, K., Kawakishi, S., and Osawa, T. (1996). Inhibition of lipid peroxidation and the active oxygen radical scavenging effect of anthocyanin pigments isolated from Phaseolus vulgaris L. *Biochem. Pharmacol.* 52, 1033–1039. doi:10.1016/0006-2952(96)00421-2

Wang, D., Yang, Y., Lei, Y., Tzvetkov, N. T., Liu, X., Yeung, A. W. K., et al. (2019). Targeting foam cell formation in atherosclerosis: Therapeutic potential of natural products. *Pharmacol. Rev.* 71, 596–670. doi:10.1124/pr.118.017178

Weber, C., and Noels, H. (2011). Atherosclerosis: Current pathogenesis and therapeutic options. *Nat. Med.* 17, 1410–1422. doi:10.1038/nm.2538

Wedge, D. E., and Nagle, D. G. (2000). A new 2D-TLC bioautography method for the discovery of novel antifungal agents to control plant pathogens. *J. Nat. Prod.* 63, 1050–1054. doi:10.1021/np990628r

Winterbourn, C. C. (2008). Reconciling the chemistry and biology of reactive oxygen species. *Nat. Chem. Biol.* 4, 278–286. doi:10.1038/nchembio.85

Witztum, J. L., Kendall, M., and Cramb, R. (1994). The oxidation hypothesis of atherosclerosis. *Lancet* 344, 1363–1364. doi:10.1016/S0140-6736(94)90721-8

Wraith, K. S., Magwenzi, S., Aburima, A., Wen, Y., Leake, D., and Naseem, K. M. (2013). Oxidized low-density lipoproteins induce rapid platelet activation and shape change through tyrosine kinase and Rho kinase-signaling pathways. *Blood* 122, 580–589. doi:10.1182/blood-2013-04-491688

Xu, S., Ilyas, I., Little, P. J., Li, H., Kamato, D., Zheng, X., et al. (2021). Endothelial dysfunction in atherosclerotic cardiovascular diseases and beyond: From mechanism to pharmacotherapies. *Pharmacol. Rev.* 73, 924–967. doi:10.1124/pharmrev.120.000096

Yang, F., Chen, Q., He, S., Yang, M., Maguire, E. M., An, W., et al. (2018). miR-22 is a novel mediator of vascular smooth muscle cell phenotypic modulation and neointima formation. *Circulation* 137, 1824–1841. doi:10.1161/CIRCULATIONAHA.117.027799

Yang, M. Y., Huang, C. N., Chan, K. C., Yang, Y. S., Peng, C. H., and Wang, C. J. (2011). Mulberry leaf polyphenols possess antiatherogenesis effect via inhibiting LDL oxidation and foam cell formation. *J. Agric. Food Chem.* 59, 1985–1995. doi:10.1021/jf103661v

Yang, X., Li, Y., Li, Y., Ren, X., Zhang, X., Hu, D., et al. (2017). Oxidative stress-mediated atherosclerosis: Mechanisms and therapies. *Front. Physiol.* 8, 600. doi:10.3389/fphys.2017.00600

Yen, G. C., Duh, P. D., Huang, D. W., Hsu, C. L., and Fu, T. Y. (2008). Protective effect of pine (Pinus morrisonicola Hay.) needle on LDL oxidation and its anti-inflammatory action by modulation of iNOS and COX-2 expression in LPS-stimulated RAW 264.7 macrophages. *Food Chem. Toxicol.* 46, 175–185. doi:10.1016/j.fct.2007.07.012

Zhang, S., Li, L., Chen, W., Xu, S., Feng, X., and Zhang, L. (2021). Natural products: The role and mechanism in low-density lipoprotein oxidation and atherosclerosis. *Phytother. Res.* 35, 2945–2967. doi:10.1002/ptr.7002

Glossary

ABCA1 ATP-binding cassette transporter A1
ABCG1 ATP-binding cassette protein G1
ACAT1 acetyl-CoA acetyltransferase
AGEs advanced glycation end products
AS atherosclerosis
ATH atherogenic diet
CAD coronary artery disease
CD36 scavenger receptor B2
Chol-HPODE cholesteryl hydroperoxy-octadecadienoate
CM chylomicrons
EDRF endothelium-dependent hyperpolarization factor
EGF epidermal growth factor
ER endoplasmic reticulum
ET-1 endothelin-1
HDL high-density lipoprotein
HLP *H. sabdariffa* leaf polyphenolic extract
HS *Hibiscus sabdariffa* L.
IDL intermediate-density lipoprotein
IGF-1 insulin-like growth factor
LAL lysosomal acid lipase
LDL low density lipoprotein
LOX-1 lectin-like ox-LDL receptor-1
LST lusianthridin
LXR liver X receptor
MA maslinic acid
MACs mulberry anthocyanin-rich extracts
MLPE mulberry leaf polyphenolic extracts
mmLDL minimally modified LDL
MMP matrix metalloproteinase
MPO mandarin (*Citrus reticulata*) peel oil
NADPH nicotinamide adenine dinucleotide phosphate
NCHH neutral cholesterol ester hydrolase
NPC1L1 Niemann-Pick type C1-like 1
NO nitric oxide
PBLs piper betel leaves
PDGF platelet derived growth factor
PGI2 prostacyclin
RCT reverse cholesterol transport
REM relative electrophoretic mobility
ROS reactive oxygen species
SR-A scavenger receptor class A
SR-B1 scavenger receptor class B type 1
SSE *Syzygium cumini* (L.) Skeels seed extract
TBARS thiobarbituric acid reaction substances
TME tannin methanol extract
VLDL very low-density lipoprotein
VSMCs vascular smooth muscle cells

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 28 April 2023
DOI 10.3389/fphar.2023.1166898

OPEN ACCESS

EDITED BY
Xiaofei Zhang,
Shaanxi University of Chinese Medicine, China

REVIEWED BY
Chao Liu,
Jinzhou Medical University, China
Shilin Xia,
Juntendo University, Japan

*CORRESPONDENCE
Yang Song,
songyang1989@cdutcm.edu.cn
Quanyu Du,
quanydu@163.com

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

RECEIVED 15 February 2023
ACCEPTED 17 April 2023
PUBLISHED 28 April 2023

CITATION
Jin W, Li C, Yang S, Song S, Hou W, Song Y and Du Q (2023), Hypolipidemic effect and molecular mechanism of ginsenosides: a review based on oxidative stress.
Front. Pharmacol. 14:1166898.
doi: 10.3389/fphar.2023.1166898

Hypolipidemic effect and molecular mechanism of ginsenosides: a review based on oxidative stress

Wei Jin[1†], Chunrun Li[2†], Shihui Yang[2], Shiyi Song[2], Weiwei Hou[1], Yang Song[1*] and Quanyu Du[3*]

[1]Emergency Department, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]School of Clinical Medicine, Chengdu University of Traditional Chinese Medicine, Sichuan, China, [3]Endocrinology Department, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China

Hyperlipidemia is considered a risk factor for cardiovascular and endocrine diseases. However, effective approaches for treating this common metabolic disorder remain limited. Ginseng has traditionally been used as a natural medicine for invigorating energy or "Qi" and has been demonstrated to possess antioxidative, anti-apoptotic, and anti-inflammatory properties. A large number of studies have shown that ginsenosides, the main active ingredient of ginseng, have lipid-lowering effects. However, there remains a lack of systematic reviews detailing the molecular mechanisms by which ginsenosides reduce blood lipid levels, especially in relation to oxidative stress. For this article, research studies detailing the molecular mechanisms through which ginsenosides regulate oxidative stress and lower blood lipids in the treatment of hyperlipidemia and its related diseases (diabetes, nonalcoholic fatty liver disease, and atherosclerosis) were comprehensively reviewed. The relevant papers were search on seven literature databases. According to the studies reviewed, ginsenosides Rb1, Rb2, Rb3, Re, Rg1, Rg3, Rh2, Rh4, and F2 inhibit oxidative stress by increasing the activity of antioxidant enzymes, promoting fatty acid β-oxidation and autophagy, and regulating the intestinal flora to alleviate high blood pressure and improve the body's lipid status. These effects are related to the regulation of various signaling pathways, such as those of PPARα, Nrf2, mitogen-activated protein kinases, SIRT3/FOXO3/SOD, and AMPK/SIRT1. These findings suggest that ginseng is a natural medicine with lipid-lowering effects.

KEYWORDS

natural medicine, ginsenosides, oxidative stress, lipid metabolism, hyperlipidemia

1 Introduction

Changes in the modern diet of humans have led to a gradual increase in diseases related to abnormal lipid metabolism, which is represented by hyperlipidemia. This disorder is characterized by an increase in plasma total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) levels and a decrease in the serum high-density lipoprotein cholesterol (HDL-C) level. Hyperlipidemia is a risk factor for diseases such as nonalcoholic fatty liver disease (NAFLD), atherosclerosis, diabetes, cerebral infarction, acute myocardial infarction, and acute pancreatitis.

In the field of blood lipid control, new targets for lipid-lowering therapy have always been a hot topic of research. Methods for lowering blood lipid levels can be roughly classified into lifestyle adjustments, synthetic lipid-lowering drugs, and natural medicines. At present, statins, fibrates, niacin, and cholesterol absorption inhibitors are the most commonly used hypolipidemic drugs. However, despite their significant lipid-lowering effects, they may cause the development of liver damage, rhabdomyolysis, tumors, and diabetes. Moreover, withdrawal from these drugs may occur, limiting their clinical application to a certain extent (Ma, 2018). In recent years, it has been found that oxidative stress is closely related to elevated blood lipid levels. This has led to the clinical exploration of different antioxidative treatment strategies and the development of related drugs, such as superoxide dismutase (SOD), SOD–catalase (CAT) and glutathione peroxidase (GSH-Px) mimics, and nuclear factor erythroid-2-related factor 2 (Nrf2; encoded by gene *NFE2L2*) activators. However, researchers are yet to be able to achieve an effective concentration of these drugs in the body. Furthermore, how to suppress excessive reactive oxygen species (ROS) production from the source is the bottleneck of current research (Forman and Zhang, 2021). Traditional Chinese medicine, which includes the application of natural products as remedies, has a long history of use for the treatment of diseases related to abnormal lipid metabolism. Ginseng, the root of plants in the genus *Panax*, is a representative traditional Chinese medicine with lipid-lowering effects. Pharmacological studies have shown that this natural product has antitumor, anti-aging, and anti-fatigue effects and can regulate immunity as well as glucose and lipid metabolism (Kim et al., 2018). The main components of ginseng include saponins, polysaccharides, volatile oils, and amino acids. Ginsenosides, the major saponins of ginseng, are the key regulators of lipid metabolism. Not only do these compounds control appetite and reduce intestinal energy input by inhibiting pancreatic lipase activity, but they also inhibit lipid synthesis by activating the AMP-activated protein kinase (AMPK) pathway and stimulate energy consumption in skeletal muscle and liver tissues (Li and Ji, 2018). In animal models of hyperlipidemia, ginseng can reduce plasma levels of lipids and tissue cell concentrations of ROS and free radicals, indicating that its lipid-lowering effect is related to the inhibition of oxidative stress. Therefore, taking the inhibition of oxidative stress as a focal point, research findings on the role of ginsenosides in diseases related to abnormal lipid metabolism and the molecular mechanisms involved were systematically reviewed and summarized in this article.

2 Publication search strategy and selection criteria

Publications related to the review topic were searched on seven databases: PubMed, SciFinder, Scopus, The Web of Science, Embase, The Cochrane Library, and China National Knowledge Infrastructure (CNKI). Papers published from the time of establishment of these databases to December 2022 were retrieved. The keywords used were ginseng, ginsenoside, lipid metabolism disorder, hyperlipidemia, hypertriglyceridemia, hypercholesterolemia, oxidative stress, lipidemia, and substance peroxidation. There were no language restrictions on search. The following were the inclusion criteria: 1) studies using ginsenosides as intervention drugs; 2) studies with clear evaluation of lipid-lowering effects, where the blood lipid evaluation index was based on the levels of TC, TG, HDL-C, and LDL-C in serum or tissue in animal experiments and the number of lipid droplets in cell experiments; and 3) studies on oxidative stress regulation as a lipid-lowering mechanism, where the indicators of oxidative stress include the fluorescence intensity of ROS; the activities of SOD, CAT, GSH-Px, glutathione reductase (GST), heme oxygenase-1 (HO-1; encoded by gene *HMOX1*), and other antioxidant enzymes; and the content of malondialdehyde (MDA, a lipid peroxidation product). The following were the exclusion criteria: 1) literature on interventions using ginsenosides in combination with other drugs; 2) literature with unclear descriptions of research results; and 3) literature on repeated studies.

3 Summary of the blood lipid metabolic pathway

"Blood lipids" is the general name of the lipids in plasma. These include cholesterol esters, phospholipids, TGs, cholesterol, and free fatty acids (FFAs). Lipid metabolism is a complex process that involves the exogenous uptake and endogenous synthesis of lipids and the interactions of lipoproteins, receptors, and enzymes in the body. When the sources of lipids and the synthesis, metabolism, and transport of lipoproteins are disturbed, lipid metabolism disorders can develop (Zhu et al., 2016). Lipids are transported in blood as plasma lipoproteins, which can be in the form of chylomicrons (CMs), very-low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), LDLs, and HDLs (Zhang and Lu, 2003). These molecules are involved in the pathways of exogenous and endogenous lipid metabolism and reverse cholesterol transport in the human body (Figure 1). Ginseng participates in the regulation of lipid metabolism mainly by interfering with enzymes, signals, and receptors in each pathway.

3.1 Exogenous pathways of lipid metabolism

The exogenous metabolic pathways involve the synthesis of CMs from dietary cholesterol and TGs for the purpose of carrying FFAs to the peripheral tissues for metabolism. First, dietary TGs are hydrolyzed into glycerol, fatty acids, and monoglycerides in the small intestine. Once the long-chain fatty acids and glycerol monoesters enter the intestinal epithelial cells, most of them are re-synthesized into TGs in the endoplasmic reticulum. These TGs are combined with esterified cholesterol and cell-produced apolipoproteins (apoB48, apoA) to form primary CMs, which are then released from the cells via exocytosis and enter the bloodstream through lymphatic vessels. The primary CMs combine with apolipoproteins, such as apoC-Ⅱ from HDL, to form mature CMs. When these CMs are transported to adipose tissue, skeletal muscle, the myocardium, and other tissues by blood, their apoC-II component activates lipoprotein esterase (LPL) on the surface of capillary endothelial cells, resulting in the continuous hydrolysis of TG in the core to produce fatty acids and glycerol monoesters. Of

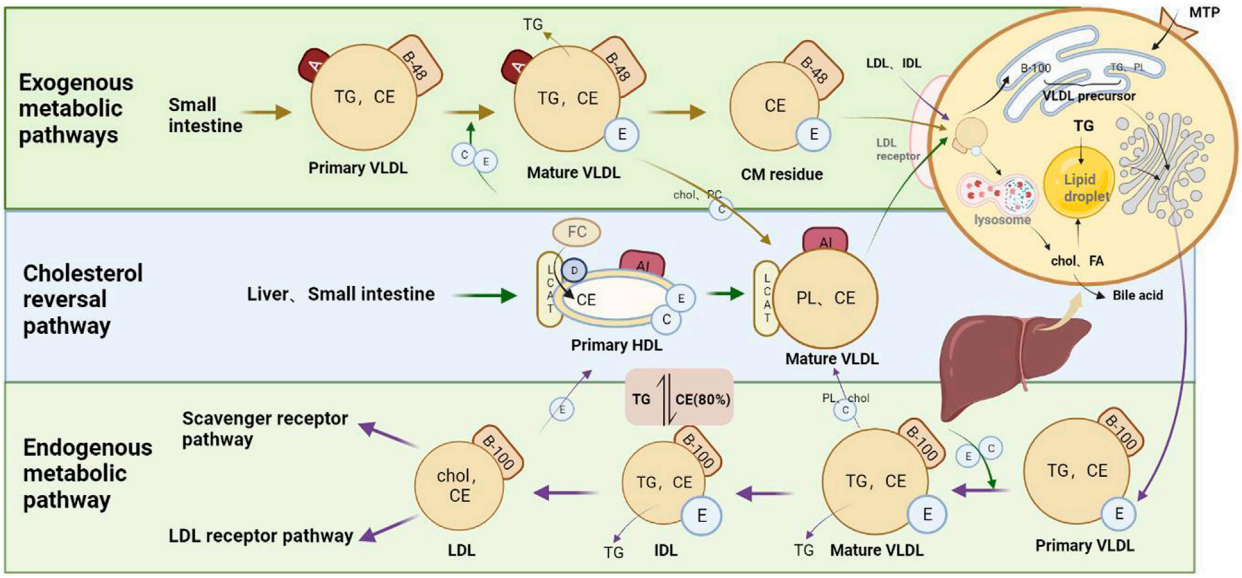


FIGURE 1
Blood lipid metabolism.

these TG hydrolysis products, 80% are absorbed by adipocytes and muscle cells and 20% are transported by albumin to the liver. Those in the liver are absorbed by hepatocytes, where they are re-esterified and used to form VLDLs, which are then secreted and transported to extrahepatic tissues. The CM remnants formed after hydrolysis of the TG in the mature CMs contain 6% apoE and 92% apoB-48. These CM remnants are ingested by hepatocytes and hydrolyzed in lysosomes, whereupon the released cholesteryl ester and fatty acids are re-esterified with hepatocyte apolipoproteins to form VLDLs.

It has been reported that ginseng may reduce the energy harvesting of rats by inhibiting pancreatic lipase activity in the exogenous pathways of lipid metabolism (Lee et al., 2013). Ginsenosides Rb1, Rb2, Rc, and Rd were found to significantly inhibit pancreatic lipase activity, which can prevent obesity by increasing fat excretion into feces (Liu et al., 2008). It has also been reported that 20(*S*)-protopanaxadiol-type ginsenoside can inhibit the expression of cholecystokinin in the hypothalamus of high-fat diet-fed mice (Kim et al., 2009). These effects indicate that supplementation with ginseng extracts can decrease obesity.

3.2 Endogenous pathways of lipid metabolism

The endogenous metabolic pathways involve the hepatic synthesis of VLDLs, which is transformed into the process in which IDL and LDL, LDL are metabolized by the liver or other organs. Glucose catabolism intermediates and fatty acids from both food sources and the body's own supply are the raw materials for the synthesis of TGs in liver cells. The TGs combine with apoB100, apoE, phospholipids, and cholesterol to form new VLDLs, which are then released into the blood. These plasma VLDLs combine with HDL-transferred apoC-Ⅱ and other molecules to form mature VLDLs and IDLs successively under the action of LPL. Approximately 30%–50% of IDLs are taken up by liver cells via LDL receptors, while 50%–70% continue to circulate. The core TGs are continuously hydrolyzed by LPL so that each endogenous TG molecule is transported to extrahepatic tissues and converted into rich LDLs containing cholesteryl esters, cholesterol, and a molecule of apoB-100. After most of the LDL molecule binds to the receptor, it is engulfed by the cell. Therein, the molecule is hydrolyzed and releases free cholesterol, which can penetrate the plasma membrane of the cell and be utilized by the cell membrane. In this way, endogenous cholesterol is transported from the liver to extrahepatic tissue. The other part of the LDL molecule is oxidized (ox-LDL), and its cellular uptake is then mediated by a group of macrophage transmembrane proteins known as scavenger receptors. Under normal circumstances, macrophages will transfer the cholesterol part of LDL-C to HDL via the cell membrane protein ATP-binding box transporter A1 (ABCA1). If the transfer is impaired, the macrophages will transform into foam cells and participate in the formation of atherosclerotic plaques. Lipid-lowering statins reduce the synthesis of cholesterol in the liver by competitively inhibiting the key rate-limiting enzyme HMG-CoA reductase in the cholesterol synthesis pathway.

In the hyperlipidemic state, the levels of fatty acid transporter (FATP2, FATP5) and translocase protein (CD36) expression in hepatocytes are upregulated, which promotes TG deposition in the liver. Fatty degeneration occurs when too many fat molecules accumulate in the liver, which affects the assembly of VLDLs and inhibits the endogenous metabolic pathways of blood lipids. Recent studies (Gao et al., 2020; Yoon et al., 2021) have found that ginsenosides can reduce the hepatocyte uptake of lipids by downregulating the hepatic expression of peroxisome proliferator-activated receptor gamma (PPARγ) and its downstream target genes *CD36*, *FATP2*, *FATP5*, and fatty acid

binding protein 1 (*FABP1*). Additionally, ginsenosides alleviate intracellular TG deposition by inhibiting the hepatocyte expression of sterol regulatory element binding protein 1c (*SREBP-1c*) and its target genes Fas cell surface death receptor (*FAS*) and acetyl-CoA carboxylase (*ACC*) as well as other adipogenic genes. These results show that ginsenosides can maintain normal endogenous metabolic pathways by reducing lipid accumulation in the hepatocytes and inhibiting hepatic steatosis.

3.3 Reverse cholesterol transport pathway

The excess cholesterol molecules that are not required for normal physiological needs have to be transported back to the liver for metabolism. This is accomplished through plasma HDLs, in a process known as reverse cholesterol transport (Zhang and Guo, 2012), which is essentially the metabolism of HDL-C. Cholesterol flows from the cells into the plasma and is converted into cholesterol ester, which in the final HDL core can eventually be transferred to TG-rich lipoproteins by the cholesterol ester transport protein (CETP), cleared in the liver by the low-density lipoprotein receptor (LDLR), or absorbed by the liver through the scavenger receptor, class B type 1 (SR-B1) pathway (Ouimet et al., 2019). In the liver, cholesterol ester is hydrolyzed, and the free cholesterol molecule is either converted into bile acid or transported to bile through ATP-binding cassette sub-family G member 5 (ABCG5) and ABCG8 for excretion in the feces. Through this mechanism, the body transfers cholesterol from the senescent cell membrane of peripheral tissues to the liver for metabolism and excretion and can prevent the accumulation of free cholesterol in the arterial wall and other tissues. Additionally, HDL also serves as the apoC reservoir, participating in the endogenous and exogenous pathways of blood lipid metabolism.

The cytochrome P450 (CYP) isoenzyme and ABCA and ABCG transporters that metabolize cholesterol are mainly regulated by PPARγ and liver X receptor-alpha/beta (LXR-α/β). In hepatocytes and macrophages, PPARγ activates LXR-α, upregulates CYP7A1 and CYP27A1, and promotes cholesterol metabolism. Studies have shown that ginseng has the effect of upregulating PPARγ expression and inducing CYP7A1, thereby regulating cholesterol metabolism.

CE: cholesterol ester, chol: cholesterol, FC: free cholesterol, MTP: microsomal triglyceride transfer protein, LCAT: cholesterol fat aminotransferase; DNL: *de novo* lipogenesis.

4 Hyperlipidemia and oxidative stress

4.1 The accumulation of free fatty acids in hyperlipidemia induces oxidative stress

Hyperlipidemia, which refers to an abnormal increase in lipid or lipoprotein levels in the blood as a result of dysregulated fat metabolism or function, is an important risk factor for cardiovascular disease (Yao et al., 2020). The clinical manifestations are high serum TC, TG, and LDL-C and low HDL-C levels. In the United States, although approximately 53% of adults have elevated LDL-C levels, less than 50% of such individuals receive treatment, and of those treated, less than 35% have adequately controlled lipid levels (Centers for Disease Control and Prevention, 2011). The deaths caused by hyperlipidemia account for nearly half of the global death toll every year (GBD, 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018). The active regulation of lipid metabolism and protection of target organs are the focus of hyperlipidemia treatment.

Blood lipid levels are related to many factors, such as heredity, eating habits, race, gender, and age. In recent years, research on the pathogenesis of hyperlipidemia has mainly been carried out in the subject areas of inflammatory response, oxidative stress, endoplasmic reticulum stress, intestinal flora, and gene polymorphism (Li et al., 2019). Among such studies, many have found that the oxidative stress response plays a key role in multiple organ lesions caused by hyperlipidemia.

Oxidative stress occurs when the levels of free radicals (e.g., ROS and reactive nitrogen species) increase as a result of imbalance between the oxidative and antioxidative capacities of the body, where the decrease in antioxidative components leads to structural and functional damage in tissues and organs. An increase in serum FFAs, an intermediate product of fat metabolism, is the main cause of mitochondrial dysfunction and oxidative stress induction. Under physiological conditions, FFAs are generated from the hydrolysis of TGs under the action of a hormone-sensitive TG enzyme. These FFAs are released from the liver into the blood, where they combine with plasma albumin to form a complex that is transported to various tissues and organs throughout the body for energy utilization. Under normal physiological conditions, 1 molecule of plasma albumin can be combined with 10 molecules of FFA. However, under the state of hypertriglyceridemia, the FFAs produced by lipid metabolism greatly exceed the binding capacity of plasma albumin, resulting in a large amount of these fat molecules remaining in the blood and becoming a risk factor for oxidative stress.

For a long time, researchers have considered the mitochondrial electron transport chain (ETC) to be one of the main sources of ROS in cells and surmised that the electron carrier at the flavin mononucleotide, Fe-S cluster, and Q-binding site in complexes I and III is in a highly reduced state, making electron "leakage" possible (Schönfeld and Wojtczak, 2008; Zorov et al., 2014). On the one hand, the mobilization and utilization of FFAs decrease when the body is in the state of hyperlipidemia, and the excess fat molecules can interact directly with other components for the generation of oxygen free radicals (Loskovich et al., 2005; Schönfeld and Reiser, 2006). On the other hand, the insufficient oxidation of β fatty acids and other saturated fatty acids can destroy the mitochondrial structure. Additionally, the amphiphilic property of FFAs facilitates their integration into the inner mitochondrial membrane, thereby increasing the fluidity of the membrane structure (Chen et al., 2020). All three aspects described above are important ways through which FFAs promote electron leakage and enhance ROS generation. Moreover, some ROS molecules are generated by peroxisome and endoplasmic reticulum and by NADPH oxidase (NOX) in the cytoplasm and plasma membrane.

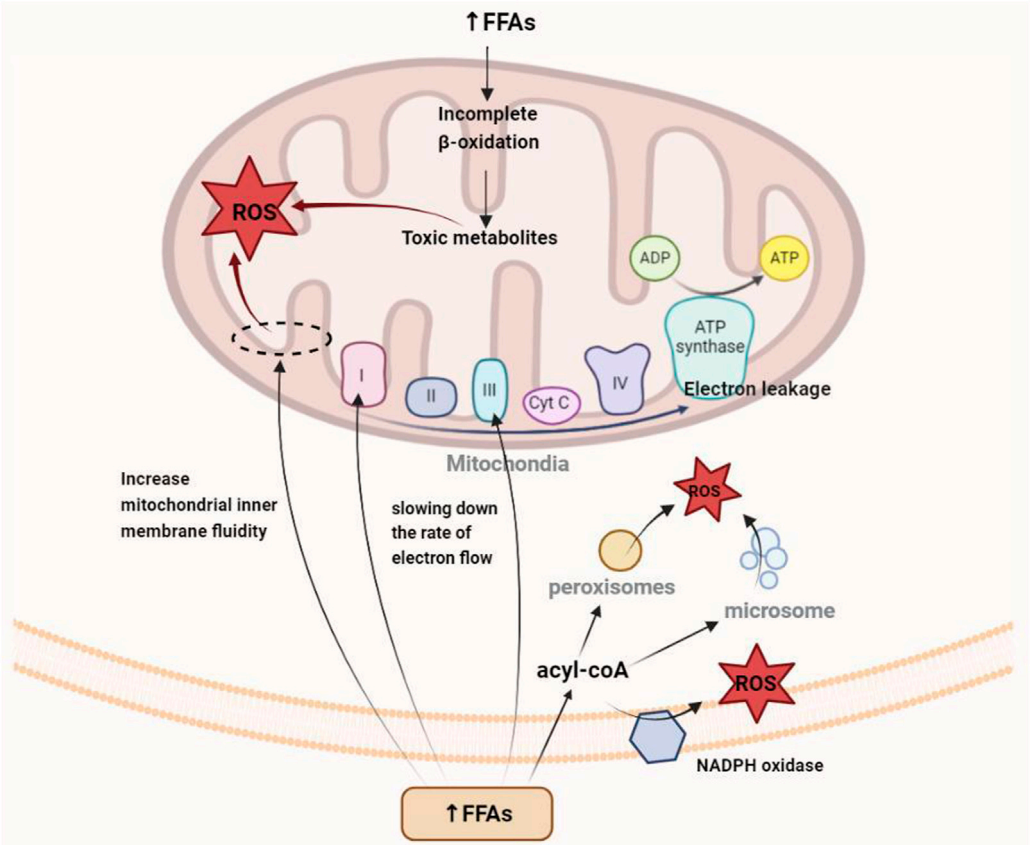


FIGURE 2
Mechanism of oxidative stress caused by the increase in free fatty acids in hyperlipidemia.

4.2 Oxidative stress is involved in hyperlipidemia-induced tissue damage

ROS is produced by enzymes (e.g., lipoxygenase) in the mitochondria, peroxisomes, and endoplasmic reticula as well as by NOX, xanthine oxidase, CYP2E1, and cyclooxygenase in the cytoplasm and plasma membrane. (Schieber and Chandel, 2014; Forrester et al., 2018). Physiological levels of ROS are involved in cellular metabolism, immune defense, proliferation, and differentiation. However, under oxidative stress conditions, the production of excess or cellular ROS shifts to that of more toxic oxidant species that can trigger pathological redox signaling and subsequent cell damage (Campbell and Colgan, 2019), thereby disrupting various signaling pathways and accelerating pathological processes by modifying proteins, promoting inflammation, inducing apoptosis, downregulating autophagy, and damaging mitochondrial function.

Numerous studies have shown that oxidative stress is an early event in the development of hyperlipidemia (Yang et al., 2008). The liver is the central organ of lipid metabolism (Figure 2), with the mitochondria being the key organelles responsible for the process. Oxidative stress can cause damage to mitochondrial DNA (mtDNA), phospholipids, and other molecular structures, resulting in mitochondrial dysfunction and liver damage. Additionally, apoptosis and fibrosis result in a further decline in lipid metabolism. Because of the short distance between mtDNA and the, ETC, the lack of protective histones, and the DNA repair mechanism being imperfect, mtDNA is prone to oxidative damage-induced breakage and mutation (Murphy, 2009). In addition to mtDNA and proteins, the phospholipid components of the mitochondrial membrane are particularly susceptible to ROS-induced oxidative attack, as high concentration of polyunsaturated fatty acids in mitochondrial phospholipids makes them a primary target for reaction with oxidants, resulting in the production of various byproducts of lipid peroxidation that interact with and inactivate, ETC components. Cardiolipin, a mitochondrial membrane phospholipid component that is rich in unsaturated fatty acids, carries a methylene bridge between the two double bonds of fatty acids, making it highly sensitive to ROS-induced oxidative damage (Paradies et al., 2009). Cardiolipin also exists in various mitochondrial inner membrane proteins and enzymes, including the, ETC complex and ADP/ATP transport proteins. Crystallographic studies have shown that a small number of tightly bound cardiolipin molecules exist in each crystal structure of complex III, complex IV, and ADP/ATP carriers (Maguire et al., 2017), suggesting that the phospholipid is a component of these proteins and its presence is critical for their folding, structure, and function. In mitochondria lacking cardiolipin, the respiratory supercomplex formed by complexes III and IV destabilizes (Zhang et al., 2005), and the dimer organization of the ADP/ATP vector and other supercomplexes

containing it is not stable (Claypool and Koehler, 2012). Damaged cardiolipin may produce more superoxide radicals, triggering a vicious cycle of ROS-induced mitochondrial membrane damage, leading to mitochondrial dysfunction, followed by increased mitochondrial permeability due to higher pore formation and the release of cytochrome *c* as well as the promotion of apoptosis and eventual hepatocyte damage (Petrosillo et al., 2006). At the same time, oxidative stress-induced Kupffer cell activation triggers innate and adaptive immune responses, resulting in the release of proinflammatory cytokines and chemokines, and the combined effect of oxidative stress and the inflammatory response further promotes the apoptosis of hepatocytes, with fibrosis ensuing.

Additionally, oxidative stress can induce insulin resistance by impairing insulin signal transduction and causing adipokine dysregulation, leading to systemic glucose and lipid metabolism disorders. Insulin receptor (InsR) and insulin receptor substrate (IRS) are important signaling elements in the insulin signaling pathway, with the former being the initial element of insulin signaling and the latter the connection between InsR and the downstream elements of the pathway bridge. Many studies have shown that oxidative stress can interfere with the phosphorylation of InsR and IRS through multiple pathways and hinder insulin signal transduction. For example, oxidative stress can activate mitogen-activated protein kinases (MAPK) family members, such as c-Jun N-terminal kinases (JNK), extracellular regulated protein kinases (ERK), and p38MAPK, aggravating the serine/threonine residues of InsR and IRS. The degree of phosphorylation reduces the protein binding ability between InsR and IRS and the ability of the latter to activate downstream signaling molecules that contain the SH2 domain (Henriksen et al., 2011). Phosphoinositide 3-kinase (PI3K), a signaling element located downstream of IRS in the insulin signaling pathway, consists of a catalytic p110 subunit and a regulatory p85 subunit. Upon insulin stimulation, PI3K translocates to the area of IRS-1 and IRS-2 accumulation and combines with these molecules, whereupon it is activated and catalyzes the formation of a series of downstream phosphoinositides to complete insulin signal transduction. Studies have found that oxidative stress can affect the combination of PI3K and protein kinase B (AKT) by reducing the activity of the PI3K-p110 subunit, thereby affecting insulin signal transmission (Shibata et al., 2010; Wang et al., 2010). Additionally, oxidative stress can affect insulin signaling by regulating the expression of the glucose transporter type 4 (*GLUT4*) gene (Henriksen et al., 2011).

Under the continuous cycle of oxidative stress and destruction of cellular structures and functions, the body finally manifests the coexistence of hyperlipidemia, hyperinflammation, and high oxidative stress levels, leading to other serious conditions such as atherosclerosis, progressive demyelination of peripheral nerves, and sarcopenia. If left untreated, these will eventually result in multiple organ and tissue damage throughout the body.

4.3 Alleviation of oxidative stress helps reduce tissue damage in hyperlipidemia

To defend against oxidative damage, organisms have evolved a defense system that can be roughly divided into three lines of defense. The first one is to remove ROS, such as hydrogen peroxide. However, because oxidants react too quickly with membrane lipids, proteins, and nucleic acids to be effectively removed by exogenous small molecules, preventing the production of ROS is the focus of the first line of defense. The second line of defense involves enhancing the activity of antioxidant enzymes, and the third line is to repair or remove oxidized macromolecules (Forman and Zhang, 2021).

Inhibiting excessive oxidative stress in patients with hyperlipidemia can help toward protecting their target organs. First, clearing the overloaded FFAs is beneficial in preventing ROS overproduction. Through facilitation by the cell membrane FATPs, the FFAs produced after fat mobilization are taken up and oxidized by various organ tissues, among which the liver, heart, and skeletal muscle are the most active in this regard. In different types of tissue, members of the PPAR family regulate the transcription of genes involved in fatty acid transport and metabolism, including *FABP1*, carnitine-palmitoyl transferase 1 (*CPT1*), and PPAR-gamma coactivator 1 alpha (*PGC-1α*). Among the PPARs, PPARα is mainly responsible for the uptake of fatty acids and regulation of oxidative stress and is highly expressed in the liver (Gross et al., 2017; Liss and Finck, 2017). Studies have shown that hepatic deficiency in PPARα can impair the ability of the liver to utilize fatty acids, resulting in lipid deposition (Benhamed et al., 2016), whereas its activation can enhance the expression of fatty acid oxidation genes and reduce steatosis (Zhu and Zhao, 2017). Additionally, AMPK activation increases the rate of fatty acid oxidation by enhancing glucose uptake and oxidation in skeletal muscle cells (O'Neill et al., 2013), and this may be related to the improvement of the insulin resistance state of the body through the regulation of PGC-1α to promote the activity of the mitochondrial oxidative respiratory chain (Kukidome et al., 2006).

With regard to the existing response to excessive oxidative stress, the main strategy is to improve the activity of antioxidants in the body, including SOD, CAT, and GSH-Px. Clinical studies have shown that the levels of MDA (a lipid peroxidation product) and advanced oxidation protein product (AOPP; an oxidative stress marker) are significantly increased in patients with hyperlipidemia, whereas those of SOD and GSH-Px (free radical scavengers) are significantly decreased (Zhang et al., 2016). Nrf2 is an important regulator of the expression of antioxidant enzymes and enhancement of the endogenous antioxidative capacity (Guariguata, 2013). Studies have shown that Nrf2-dependent antioxidant genes encode almost all antioxidant enzymes, including SOD, CAT, GST, glutathione peroxidase 1 (GPX1), and HO-1. The activation of Nrf2 has been shown to reduce bisphenol A-induced lipid accumulation in the liver of mice, and its efficacy in inhibiting lipid accumulation is considered to be related to the enhanced expression of genes coding for antioxidants and detoxification enzymes (Ludtmann et al., 2014; Shimpi et al., 2017). Promoting the expression of Nrf2 and downstream SOD, HO-1, and other signals can reduce oxidative stress and improve lipid metabolism. Another important pathway that protects cells from oxidative stress is that of sirtuin 3 (SIRT3), which achieves antioxidative effects by regulating the antioxidant enzyme SOD and upregulating the activities of manganese superoxide dismutase (MnSOD) and catalase (Sundaresan et al., 2009; Qiu et al., 2010; Cheung et al., 2015). Other studies have found that complex gut microbe–microbe and microbiota–host interactions may also interfere with ROS levels

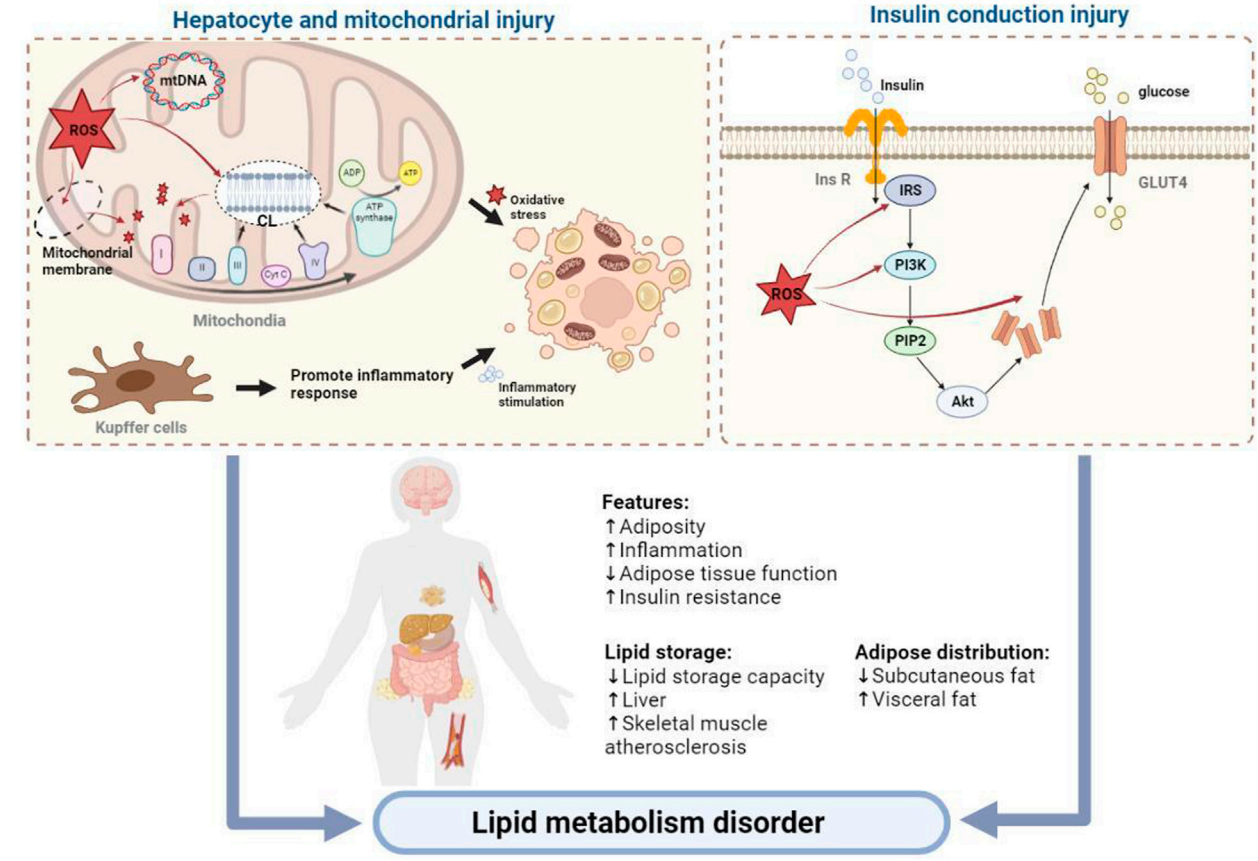


FIGURE 3
Related mechanisms of oxidative stress affecting body lipid metabolism.

and antioxidative systems at the organ level (Jones et al., 2012; Tse, 2017; Bonaz et al., 2018). However, the detailed relationship between oxidative stress and the gut microbiota remains to be elucidated.

Damaged tissue that has not been repaired or cleared in time can induce cascade reactions by releasing inflammatory factors and various cytokines. Therefore, repairing or removing oxidized macromolecules is also a key part of antioxidant therapy. Autophagy is one of the processes through which cellular components and damaged organelles are recycled. For example, damaged mitochondria are cleared through mitophagy to accommodate energy demands and support liver metabolic pathways and functions. Various mitochondrial adapter proteins, also known as mitophagy receptors, can be recruited to clear damaged mitochondria (Novak et al., 2010; Liu et al., 2012). Additionally, specific lipids such as cardiolipin are transferred from the inner to the outer membrane of damaged mitochondria where they interact with autophagosomes through lipidated microtubule-associated protein light chain 3 (LC3) for selective degradation (Kagan et al., 2016). Autophagy is related to the regulation of apoptosis. Taking the early stage of atherosclerosis as an example, autophagy can protect cells in atherosclerotic plaques from oxidative damage by degrading harmful substances, helping to inhibit apoptosis and delay aging of the vascular endothelium (Zhou et al., 2018).

In summary, hyperlipidemia can induce oxidative stress, which is the basis of tissue damage in this fat state (Figure 3). Therefore, the key to the treatment of hyperlipidemia and the prevention of secondary diseases is to prevent the production of oxidants that can cause direct damage to macromolecules, inhibit the downstream signals of oxidants that lead to inflammation or cell death signals, and increase the activity of antioxidant enzymes. Through our summarization of recent research studies, we found that ginsenosides used for the treatment of lipid metabolism disorders can exert antioxidative effects through multiple pathways and targets. These findings are expected to provide a direction for the research and development of antioxidants.

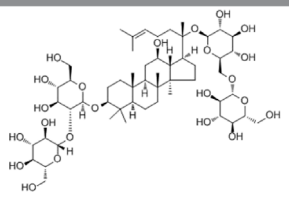
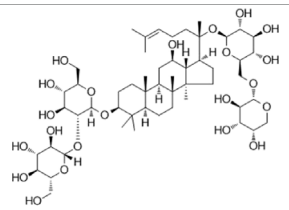
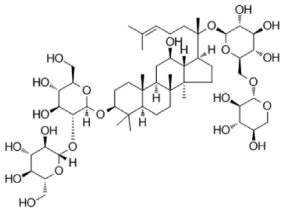
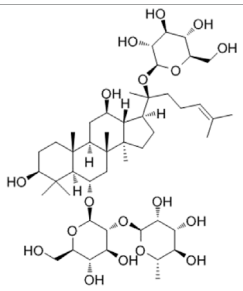
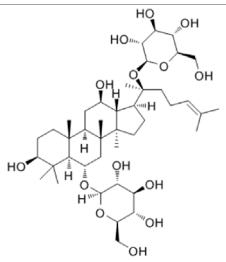
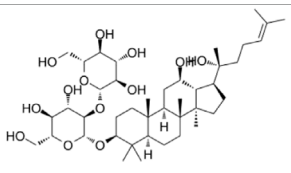
5 Ginsenosides regulate lipid metabolism by inhibiting oxidative stress

5.1 Ginseng and ginsenosides

Ginseng is one of the most widely used herbal product in the world, whether in its natural form or as a drug supplement. The plant root has a long history of use in Asian countries (especially China, South Korea, and Japan) as a dietary supplement for

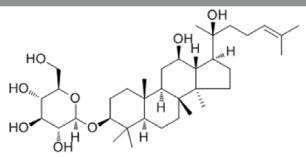
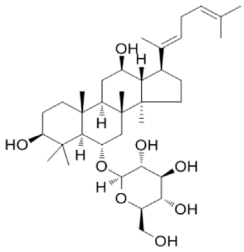
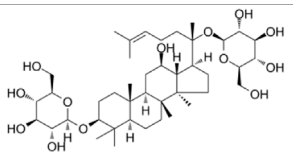
10.3389/fphar.2023.1166898

TABLE 1 Ginsenosides that cause antioxidative damage.

Name	Source	Chemical constitution	Molecular weight	Oral bioavailability (OB%)	Drug similarity (DL)
Rb1	Ginseng, Panax notoginseng, Panax quinquefolium, Panax ginseng		1109.295	6.24	0.04
Rb2	Ginseng, Panax notoginseng, American ginseng, gynostemma pentaphyllum		1,079.43	5.98	0.04
Rb3	Ginseng, Panax notoginseng, Panax quinquefolium, Panax ginseng		1079.269	6.02	0.04
Re	Red ginseng, gynostemma pentaphyllum, ginseng, ginseng leaf, Panax notoginseng, bamboo ginseng		947.30	4.27	0.12
Rg1	Red ginseng, ginseng, ginseng leaf, Panax notoginseng, bamboo ginseng		801.013	10.04	0.28
Rg3	Ginseng root, Panax notoginseng and American ginseng		785.14	12.43	0.22

(Continued on following page)

TABLE 1 (*Continued*) Ginsenosides that cause antioxidative damage.

Name	Source	Chemical constitution	Molecular weight	Oral bioavailability (OB%)	Drug similarity (DL)
Rh2	Red ginseng, ginseng leaf, Panax notoginseng, American ginseng		622.873	36.32	0.56
Rh4	ginseng		620.857	5.22	0.60
F2	Gynostemma pentaphyllum and Panax notoginseng		785.013	36.43	0.25

The above data is from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP).

relieving fatigue (Shi et al., 2019). Ginseng contains ginsenosides, ginseng polysaccharides, volatile oils (terpenoids, alcohols, fatty acids, etc.), and amino acids, which have multiple biological activities, such as anticancer, antioxidative, and anti-inflammatory effects (Metwaly et al., 2019). Among them, ginsenosides are the main contributors to the known pharmacological activities of ginseng from the genus *Panax* (Seo and Kim, 2011; Lee et al., 2012). These triterpine saponins are distributed in many parts of the ginseng plant, including the berries, seeds, roots, stems, leaves, and flowers, where they have different characteristics and pharmacological effects (Attele et al., 1999; Attele et al., 1999; Podolak et al., 2010). They exist not only in Asian ginseng (*Panax ginseng*) but also in American ginseng (*Panax quinquefolius*), Chinese ginseng (*Panax notoginseng*), and jiaogulan (*Gynostemma pentaphyllum*). So far, more than 180 types of ginsenosides have been isolated from *P. ginseng* (Yu et al., 2019).

Ginsenosides are divided into three categories according to the structure of their glycosides: 20(*S*)-protopanaxadiol, 20(*S*)-protopanaxatriol, and oleanolic acid saponins. The names of ginsenosides are often abbreviated as "Rx," where "R" refers to the root and "x" describes the chromatographic polarity in alphabetical order. Among them, Rb1 (20(*S*)-protopanaxadiol type) and Rg1 (20(*S*)-protopanaxatriol type) are the most valuable bioactive compounds. Additionally, there is a class of metabolically converted ginsenosides (derivatives), such as ginsenoside compound K (CK), which is an active metabolite of ginsenoside Rb1 and has stronger biological activity than the parent compound. Although the content of these saponins is very low, they show unique pharmacological activities.

Recent studies have found that ginsenosides play an important role in inhibiting oxidative stress, thereby preventing oxidative damage and protecting cells (Kang et al., 2013; Ong et al., 2015). Among them, ginsenosides Rb1, Rb2, Rb3, Re, Rg1, Rg3, Rh2, Rh4, F2 (Table 1), and CK have been proven to inhibit oxidative stress and alleviate hyperlipidemia by enhancing the expression of antioxidant enzymes, inhibiting the synthesis of triglycerides and cholesterol, and enhancing fatty acid oxidation.

5.2 Ginsenosides enhance the expression of antioxidant enzymes

It has been reported that ginsenosides such as Rb1 (Zhu et al., 2022), Re (Li et al., 2013), Rg3 (Li et al., 2021), Rg1 (Xu et al., 2019), Rb3 (Wu et al., 2022), and F2 (Zhou et al., 2021) and their metabolites can improve the level of lipid metabolism through antioxidation. Under physiological conditions, Nrf2 is anchored in the cytoplasm by Kelch-like epichlorohydrin-associated protein-1 (KEAP1) to prevent its translocation to the nucleus. During oxidative stress, Nrf2 dissociates from KEAP1 and is transported to the nucleus, where it binds to antioxidant response elements, regulates the production of downstream antioxidant enzymes such as SOD and CAT, and inhibits the increase in mitochondrial ROS production (Dinkova-Kostova and Abramov, 2015; Sabouny et al., 2017). Gao et al. (2020) demonstrated that ginsenoside Rg1 could protect mice with streptozotocin-induced diabetes from inflammation and oxidative stress by activating the KEAP1/Nrf2 pathway. Ginsenoside Rg1 also protected cardiomyocytes from hypoxia/reoxygenation injury by activating Nrf2/HO-

TABLE 2 Ginseng and its active components protect organs and tissues by increasing the activity of antioxidant enzymes to reduce oxidative stress.

Drugs	Animals/Cells	Intervention results	Mechanism	References
Rb1	*In vivo*, elderly rats with fatigue syndrome after major resection of the small intestine	MDA↓, SOD↑	Activate of the PI3K/Akt/Nrf 2 pathway	Zhuang et al. (2014)
Rg3	*In vivo*, high-fat diet feeding, STZ-induced diabetic nephropathy in C57BL/6 mice	TC, TG, LDL-C↓, HDL-C↑; MDA↓, SOD, CAT↑, HO-1↑	Regulate the MAPK/NF-κB pathway	Li et al. (2021)
Rb1	*In vivo*, immune liver injury induced by restraint stress (RS) combined with lipopolysaccharide (LPS) in mice	Liver fat vacuoles↓; MDA↓, SOD↑	SIRT3/FoxO3/SOD pathway↑	Wang et al. (2022)
Rb3	*In vitro*, the lipid hepatocyte model was prepared by mixed culture medium of oleic acid (OA) and palmitic acid (PA)	Lipid stock↓, TG↓; ROS↓	the expression of Klf16, Nrf2, and Sod2↑	Wu et al. (2022)
SGL 121	*In vivo*, mice fed a high-fat, high-carbohydrate diet (HFHC); *in vitro*, HepG2 cells	TG, TC, LDL↓, HDL↑, MDA↓	Activate of AMPK, the entry of Nrf 2 into the nucleus↑, HO-1 expression↑	Kim et al. (2020)
Rb1	*In vivo*, C57BL/6J mice	TC、TG、LDL-c, adipose cell↓; Expression of the oxidative stress genes SOD 1, CAT, and NFe212 in the colon↑	Regulate the structure of the intestinal flora and the intestinal fatty acid profile	Zou et al. (2022)

TABLE 3 Ginsenosides promote fatty acid oxidation, reduce oxidative stress, and protect organs and tissues.

Drugs	Model	Intervention results	Mechanism	References
Rb1	*In vitro*, fatty liver primary cells were prepared by mixed medium of oleic acid (OA) and palmitic acid (PA)	lipid droplet↓, TG↓; the fluorescence intensity of the ROS↓	the PPAR-α protein expression↑	Li et al. (2021)
Rb1	*In vivo*, HFD)-induced obese rats; *in vitro*, primary cultured rat hepatocytes	Liver weight, TG↓, lipid droplet↓	AMPK phosphorylation↑, CPT1 and ACC activities↑, PPAR-α and PGC-1α expression↑	Shen et al. (2013)
Rg1	*In vitro*, HepG2 cells were treated with 1 mmol L-1 free fatty acids for 24 h	TG, Fat droplet aggregation absorbance↓	PPARγ expression↓; the expression of PPAR-α, CPT-1, and ACOX1↑	Gao et al. (2020)
Rg1	*In vivo*, high-fat diet-induced NAFLD mice	Liver weight, TG, FFA↓; MDA↓, SOD↑	the PPAR-α expression↑	Xu et al. (2018)
Rh2	*In vivo*, high-fat diet-induced HCBP6 knockout (HCBP6-KO) in NAFLD mice	lipid deposition in the liver↓	HCBP 6 expression↑	Lu et al. (2020)
Rb2	*In vivo*, C57BL/6J mice induced by hyperfatty fatty liver fed 60% kcal for 8 weeks	Liver mass, fat vacuolar number and volume↓	the mRNA levels of CPT-1 and the PPAR-α gene and protein expression↑	Hong et al. (2018)
CK	*In vivo*, type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats fed on a high-fat diet	lipid droplets in hepatocytes ↓, hepatic steatosis↓	AMPK phosphorylation and the expression of PPAR-α and CPT-1↑	Hwang et al. (2018)

1 signaling, inhibiting JNK, and increasing the expression of SOD, GSH, and GSH-Px (Gao et al., 2020). It was found in another study that ginsenoside Rb1 could promote the nuclear translocation of Nrf2 and mediate its antioxidative effect, thereby preventing lipid peroxidation and protecting cells from oxidative stress (Li et al., 2017). *HMOX1*, one of the downstream genes of Nrf2, leads to the scavenging of free radicals accumulated by redox imbalance. SGL 121, a fabricated mixture in which the content of ginsenoside F2 has been enhanced through biotransformation, can increase the expression of HO-1 by promoting the entry of Nrf2 into the nucleus, thereby enhancing the cellular resistance to oxidative stress and helping to inhibit fat accumulation (Kim et al., 2020). Additionally, AKT and MAPK may be part of the central pathway related to Nrf2 translocation (Dai et al., 2007; Calabrese et al., 2010). Studies have found that islet dysfunction in mice with type 2 diabetes mellitus (T2DM; induced by a high-fat diet or streptozotocin) can be alleviated by ginsenoside Rh4, which it does by promoting Nrf2 nuclear translocation and upregulating the expression of HO-1, NAD(P)H dehydrogenase [quinone] 1 (NQO1), and glutamate-cysteine ligase catalytic subunit (GCLC). Conversely, an AKT signal inhibitor almost completely blocked Rh4-induced Nrf2 nuclear translocation, indicating that AKT is a key regulator of Rh4-induced Nrf2 activation. The MAPK family proteins JNK, ERK, and p38MAPK are key signal molecules in the response to mitogenic stimulation or environmental stress (Kong et al., 2001). Although it was shown that ginsenoside Rg3 could inhibit hyperlipidemia and streptozotocin-induced oxidative stress, increase the expression of SOD, CAT, and HO-1, and inhibit activation of the MAPK pathway, the exact relationship between the MAPK pathway and the antioxidant system was not further demonstrated (Li et al., 2021).

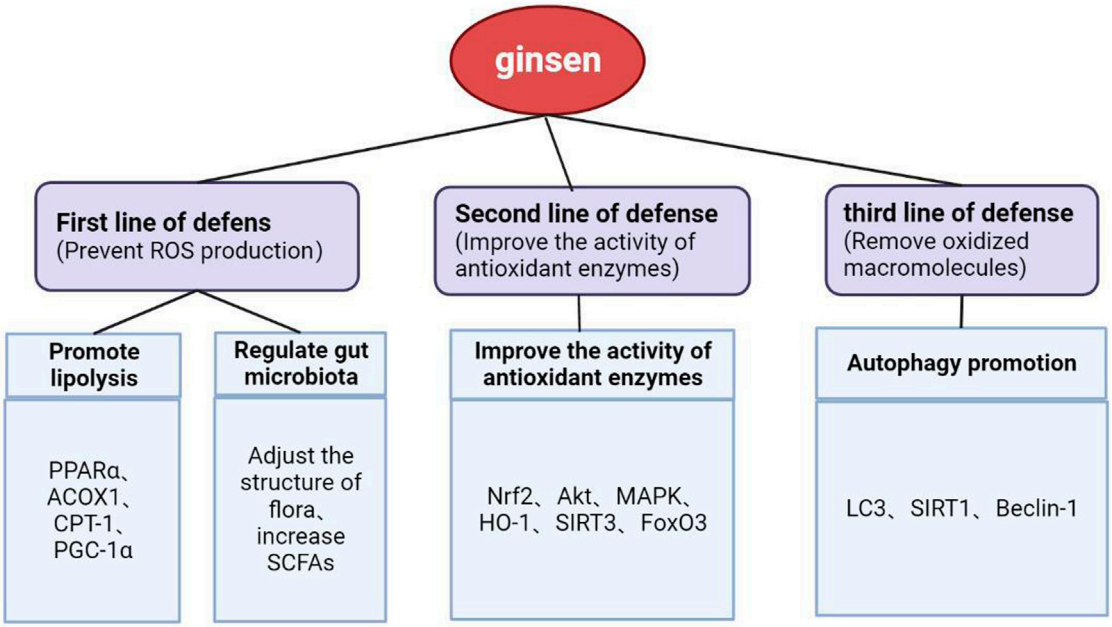


FIGURE 4
Ginseng improves lipid metabolism in hyperlipidemia by regulating oxidative stress.

The SIRT3 pathway is also one of the most important for protecting cells from oxidative stress. It has been reported that the liver contents of TG and cholesterol in *Sirt3*-knockout mice were significantly higher than those in the wild-type animals, resulting in accelerated obesity, insulin resistance, hyperlipidemia, and steatohepatitis (Hirschey et al., 2011). Forkhead box O3 (FOXO3) is one of the downstream target proteins of SIRT3. When SIRT3 is used as an oxidative stress-related deacetylase, FOXO3 is deacetylated and captured in the nucleus, where it enhances gene transcription of the FOXO-dependent antioxidants MnSOD and catalase, acts on the ROS/Ras signaling pathway, eliminates ROS, and reduces oxidative stress damage. Many studies have confirmed that SIRT3 can protect mitochondria from oxidative damage by reducing the acetylation of FOXO3 and promoting its expression, thus alleviating oxidative stress (Zhou et al., 2021). Wang et al. (2022) used ginsenoside Rb1 to ameliorate the immune-driven liver injury induced by restraint stress and lipopolysaccharide. After 7 days, the authors found that the number of fat vacuoles in the liver tissue and the expression of MDA had decreased, whereas the activity of SOD had increased. Those researchers verified that the mechanism through which Rb1 alleviates oxidative stress is related to the upregulation of *Fox3O* gene expression.

Additionally, a HFD can change the structure of the intestinal flora and thereby its symbiotic relationship with the host, and the products of microflora also play a regulatory role in the antioxidant system of the body. Zou et al. (2022) found that ginsenoside Rb1 upregulated the abundance of the genera *Akkermansia*, *Parasutterella*, and *Bacteroides* in the intestine of HFD-fed mice; decreased the abundance of species of *Intestinimonas*, *Oscillibacter*, and *Allobaculum*; and regulated the colonic expression of FFA receptors (FFAR4, PPARγ) and genes related to intestinal barrier function (claudin 4 (*Cldn4*) encoding dense protein 4, and *Cldn2* encoding dense protein 2), antimicrobial peptides (regenerating islet-derived 3 gamma (*Reg3g*)), and oxidative stress (*SOD1*, *CAT*, and *Nfe2l2*). Rb1 also protected the intestinal barrier and downregulated the oxidative stress response, thereby regulating the structure of the intestinal flora and the profile of intestinal fatty acids. At the same time, short-chain fatty acids (SCFAs) and other beneficial metabolites produced by intestinal bacteria help to reduce ROS by affecting mitochondrial activity. For example, total saponins from the stems and leaves of *Panax ginseng* can significantly increase the levels of acetic acid, propionic acid, butyric acid, and total SCFAs by regulating the proportion of intestinal microflora, which helps to improve the activity of antioxidants such as GSH-Px and SOD (Ding et al., 2023). However, more experiments are needed to verify how intestinal microorganisms participate in the lipid-lowering effect of ginsenosides (Table 2).

5.3 Ginsenosides enhance fatty acid beta-oxidation

Under physiological conditions, FFAs are regulated by PPARα and its target gene acyl-CoA oxidase 1 (*ACOX1*), and the fat molecules are transported to the mitochondria via the catalytic activity of CPT1 to produce energy (Papackova and Cahova, 2015). However, when the body is in a state of high fat, the mitochondria cannot completely oxidize the excess FFAs, resulting in the production of a large number of ROS and destruction of the mitochondrial structure.

At the transcriptional level, the initiation of fatty acid oxidation in different types of cells is regulated by PPARs, of which PPARα is

TABLE 4 Ginsenosides reduce oxidative stress by promoting autophagy.

Drugs	Model	Intervention results	Mechanism	References
Rb1	SD rats fed on a high-fat diet	Ox-LDL↓, vascular endothelial cell senescence↓	Regulate the SIRT1/Beclin-1/autophagy axis	Shi et al. (2020)
Rb3	EA.hy926 cells inoculated in DMEM/high glucose medium containing 10% fetal bovine serum and 1% double antibodies	endothelial cell damage↓	expression of Beclin1 mRNA, expression of LC3 and Beclin1 protein↑, the expression of P-mtor protein↓; autophagy↑	Cao et al. (2018)
Rb1	ApoE−/− male mice	Weight and intake↓, area of the atherosclerotic plaques↓, TC, TG, LDL-C↓, LDL-C↑	autophagy↑,apoptosis↓	Zhou et al.,2018bib_zhou_et_al_2018

TABLE 5 Ginsenosides inhibit oxidative stress in the treatment of nonalcoholic fatty liver disease.

Drugs	Model	Intervention results	Mechanism	References
Rg1	*In vitro*, HepG 2 cells were treated with 1 mmol L-1 free fatty acids for 24 h	TG, the lipid droplet aggregation absorbance↓	lipid uptake↓; lipid β-oxidation↑	Gao et al. (2020)
Rg2	*In vivo*, high-fat diet-induced NAFLD mice; *in vitro*, oleic acid and palmitic acid-induced primary hepatocytes	Accumulation of lipids↓, TG, TC↓	fat synthesis↓	Cheng et al. (2020)
Rg1	*In vivo*, high-fat diet (HFD)-induced NAFLD in rats	lipid droplet, AST, ALT↓; TC, TG , LDL-C↓, LDL-C↑	β-oxidation↑	Peng et al. (2015)
Rg1	*In vivo*, high-fat diet-induced NAFLD mice	Liver weight, TG, FFA↓; MDA↓, SOD↑	fatty acid β-oxidation↑; lipid peroxidation↓	Xu et al. (2018)
Rh2	*In vivo*, high-fat diet-induced HCBP6 knockout in NAFLD mice	lipid deposition in the liver↓	fat decomposition and fatty acid oxidation↑	Lu et al. (2020)
Rb1	*In vitro*, lipid liver primary cells were prepared with a mixed medium of oleic acid (OA) and palmitic acid (PA)	the lipid droplets gather↓, TG↓; ROS fluorescence intensity↓	the oxidation of fatty acids↑; fatty acid synthesis↓	Li et al. (2021)
CK	*In vivo*, type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats fed on a high-fat diet	lipid droplet ↓, Improvement of the hepatic steatosis	fatty acid synthesis↓; fatty acid oxidation↑	HWANG et al. (2018)
Rg1	*In vivo*, high-fat diet-induced C57/BL female mice	hepatic steatosis, FFA, TG, TC↓; MDA↓, SOD↑	antioxidant enzyme activity↑; ER stress↓	Xu et al. (2019)
Rg1	*In vivo*, SD rats, a nonalcoholic fatty liver model fed a high-glucose fatty fat diet	Liver fat vacuoles↓; GSH, SOD↑, MDA↓	oxidative stress↓; cell apoptosis in the liver↓	Xiao et al. (2019)
SGL 121	*In vivo*, mice fed a high-fat, high-carbohydrate diet (HFHC); *in vitro*, HepG2 cells	TG, TC, LDL↓, HDL↑; MDA↓	the antioxidant enzyme activity↑	Kim et al. (2020)

the key factor regulating this process and energy metabolism in the mitochondria, peroxisomes, and microsomes. As a fatty acid sensor, PPARα enhances lipid metabolism by promoting the β-oxidation of fatty acids in the peroxisomes and mitochondria, accelerating the decomposition of fatty acids, and regulating the expression of genes related to fatty acid and lipid metabolism (Wang et al., 2020). Some studies have shown that PPARα deficiency in the liver impairs the ability of the organ to utilize fatty acids, resulting in lipid deposition (Montagner et al., 2016), whereas PPARα activation enhances the expression of genes related to fatty acid oxidation and reduces steatosis (Zhu et al., 2017). It has been reported that fermented ginseng extract can increase the expression of PPARα in HepG2 cells (Chang, 2020). Rg1 has also been shown to upregulate the expression of PPARα to promote FFA oxidation and inhibit the upregulation of TG synthesis (Xu et al., 2018). Hong et al. (2018) found that the expression of PPARα was significantly decreased in mice with HFD-induced fatty liver, but Rb2 significantly improved the hepatic expression of this receptor at both the gene and protein levels, indicating that this ginsenoside could reverse the effect of HFD on PPARα expression. Similarly, using a mouse model of fatty hepatocytes, Li et al. (2021) found that the protein expression of PPARα was increased after Rb1 supplementation, indicating that this ginsenoside enhanced the oxidation of fatty acids. Furthermore, ginsenosides can upregulate *PGC-1α* gene expression (Zhao et al., 2019).

As a type of intracellular fuel receptor, AMPK also plays an important role in regulating lipid metabolism, where its activation can promote lipid catabolism by increasing the oxidation of fatty acids (Foretz et al., 2018; Fang et al., 2019). It was found that Rb1 can reduce the formation of malonyl-CoA and increase the oxidation of fatty acids by increasing the proportion of intracellular AMP/ATP, then activating AMPK phosphorylation, and subsequently inhibiting the activity of acetyl-CoA carboxylase (ACC). Rb1 can also significantly increase the expression of PGC-1α, PPAR-1α, and ACOX1, thus enhancing the oxidation of fatty acids in hepatocytes (Shen et al., 2013). In another study, ginsenoside CK was found to

increase fatty acid oxidation and alleviate liver steatosis by promoting AMPK phosphorylation and upregulating the expression of PPARα and CPT1 (Hwang et al., 2018).

It has been found that the lack of hepatitis C virus core-binding protein 6 (HCBP6) can reduce the expression of CPT1A, the rate-limiting enzyme of fatty acid β-oxidation, indicating that HCBP6 deficiency aggravates the accumulation of triglycerides by inhibiting fatty acid oxidation. Additionally, HCBP6 deficiency decreases the expression of p-AMPKα. In rats fed a HFD and CCl_4, the hepatic expression of HCBP6 decreased but was upregulated in rats treated with a mixture of water-soluble ginsenosides (Kim et al., 2011). Similarly, it was found that ginsenoside Rh2 could promote fatty acid oxidation and this effect depended on the expression of HCBP6 (Lu et al., 2020) (Table 3).

5.3.1 Ginsenosides promote autophagy and inhibit apoptosis

Autophagy is divided into three types: macroautophagy, microautophagy, and chaperone-mediated autophagy. Among them, macroautophagy is the process that has been the most studied. In autophagy, which is an important recycling process for maintaining cellular homeostasis, autophagosomes transfer aging or damaged organelles, misfolded or useless proteins, and intracellular pathogens to lysosomes for degradation (Zhang et al., 2021). For example, in pathological conditions, the mitochondria are not only the main organelles where ROS are produced but also the structures most vulnerable to ROS attack. The selective degradation of damaged mitochondria helps to restore the mitochondrial dysfunction caused by oxidative stress and reduces the accumulation of mtDNA mutations. The removal of oxidatively damaged macromolecules is beneficial to the protection of organs. Unfortunately, there are currently limited studies on the role that ginseng plays in the autophagic removal of oxidized macromolecules.

During autophagy, goblet-like structures (pro-autophagosomes) engulf cytosolic components and then close to form autophagosomes, which subsequently fuse with lysosomes, resulting in proteolytic degradation of the internalized components by lysosomal lyases. The modification of LC3 into two interconvertible forms (LC3-I and LC3-II) is required for the formation of mammalian autophagosomal membranes. First, newly synthesized LC3 in the cell is transformed into soluble LC3-I in the cytoplasm. Then, LC3-I is processed and modified by ubiquitination to membrane-bound LC3-II, which is localized in the pre-autophagosome and autophagosome. Both forms are biomarkers of autophagosomes. The LC3-II content or LC3-II/LC3-I ratio is positively correlated with the number of autophagosomes, reflecting the autophagic activity of cells to some extent. Additionally, many studies have suggested that autophagy is related to the regulation of apoptosis (Li et al., 2016), with B-cell leukemia/lymphoma 2 apoptosis regulator (BCL2) being the key protein linking and balancing the two processes to maintain homeostasis of the cellular environment. Proteins of the BCL2 family regulate the intrinsic apoptotic pathway by controlling mitochondrial outer membrane permeability. On the one hand, the phosphorylation of BCL2 at Ser70 is enhanced through interactions with BAX and BAD (BCL2-associated death promoter), inhibiting apoptosis, and on the other hand, BCL2 phosphorylation also leads to its dissociation from beclin 1 (BECN1), thereby activating autophagy (Liu et al., 2018).

Recent studies have found that the ox-LDL molecules produced during hyperlipidemia can induce vascular endothelial aging and inhibit autophagy, but ginsenosides can inhibit this process (Figure 4; Table 4). Ginsenoside Rb1 has been shown to play a protective role in mitochondrial apoptosis by regulating BCL2 family proteins (Wang et al., 2018; Zhao et al., 2018). In atherosclerosis, BCL2 accelerates autophagy by promoting the conversion of LC3-I to LC3-II to increase the autophagic flux and also inhibits apoptosis. Zhou et al. (2018) found that Rb1 feeding could reverse the levels of BCL2 and its binding protein BAX, protecting the vascular endothelium. SIRT1 was shown to prevent the progression of atherosclerosis by inhibiting foam cell formation (Kitada et al., 2016). On the basis of this study, Shi et al. (2020) further found that Rb1 could slow aging of the atherosclerotic vascular endothelium and reduce atherosclerotic plaque formation, results that were related to the increased expression of SIRT1 to reduce the autophagy induced by BECN1 acetylation. In another study, ginsenoside Rb3 was also confirmed to inhibit the ox-LDL-induced autophagic effect in endothelial cells by enhancing the expression of *BECN1* mRNA and LC3 and BECN1 proteins (Cao et al., 2018).

6 Ginsenosides inhibit oxidative stress in the treatment of diseases related to abnormal lipid metabolism

6.1 Nonalcoholic fatty liver disease

NAFLD, the most common form of chronic liver disease worldwide, is characterized by a range of histologic features—from simple steatosis featuring fat accumulation in the liver to hepatocyte swelling, inflammation, and/or fibrosis in nonalcoholic steatohepatitis, which can lead to cirrhosis and hepatocellular carcinoma (Sayiner et al., 2016). A variety of ginsenosides can slow progression of the disease by inhibiting lipid uptake by hepatocytes, reducing intrahepatic fat accumulation, and increasing the activity of antioxidant enzymes, thereby weakening oxidative stress and protecting the hepatocytes (Table 5).

In the hyperlipidemic state, the increased FFA content in blood triggers the upregulation of fatty acid transporters (FATP2 and FATP5) and translocase protein (CD36) in hepatocytes, leading to the promotion of FFA absorption into the cells and thus TG deposition in the liver. When excessive fat accumulation in the liver leads to steatosis, mitochondrial function is impaired, resulting in an increase in ROS release, which further promotes the development of NAFLD. It has been found that ginsenosides can reduce lipid uptake by downregulating the expression of PPARγ and *SREBP-1c* (Cheng et al., 2020; Gao et al., 2020). Additionally, for lipids that have been deposited in the liver, it has been found that ginsenosides Rg1, Rb1, Rh2, and CK can promote the expression of acyl CoA synthase 1 (CoASH1), carnitine acyltransferase 1 (CAT1), and ACOX1 in liver lipid metabolism to promote lipid oxidative decomposition and reduce liver lipid accumulation (Peng et al., 2015).

Under normal physiological conditions, oxidative stress plays an active role in “adaptive stress responses” (e.g., NAFLD detoxification, molecular damage repair, anti-inflammation, and tissue regeneration), such as by promoting cell autophagy

TABLE 6 Ginsenosides inhibit oxidative stress in the treatment of atherosclerosis.

Drugs	Model	Intervention results	Mechanism	References
CK	*In vivo*, a rat model of atherosclerosis prepared by high-fat diet + vitamin D3 by gavage	TC, TG, LDL-C↓, HDL-C; atherosclerotic index ↓	antioxidant enzyme activity↑	Gao et al. (2020)
Rh2	*In vivo*, a rat model of atherosclerosis prepared by high-fat diet + vitamin D3 by gavage	TC, LDL-c, AI↓; SOD↑, MDA↓	antilipid peroxidation function ↑; scavenge of oxygen free radicals	Kong et al. (2010)
Rg1	*In vivo*, a coronary atherosclerotic heart disease model rat	TG, TC, LDL-c↓, HDL-c↑; SOD, NO↑, MDA, ET↓	Balance the vasomotor function; the body's antioxidant enzyme activity↑	Chen et al. (2020)
Rb1	*In vitro*, human aortic endothelial cells, HAECs	Cell viability, SOD, Bcl-2, HRD1↑, ROS, MDA, the rate of apoptosis, Caspase-3, Bax↓	oxidative stress↓; vascular endothelial cell apoptosis↑	Ren et al. (2022)

TABLE 7 Ginsenosides inhibit oxidative stress in the treatment of type 2 diabetes mellitus and its complications.

Drugs	Model	Intervention results	Mechanism	References
Rb1	*In vivo*, a high-glucose and high-fat diet was induced by intraperitoneal injection of streptozotocin (STZ) to construct type 2 diabetic rats	TC, TG, LDL-C↓; MDA↓, CAT, SOD, GSH-Px ↑	Improve insulin resistance and enhance the body	Zhu et al. (2022)
Re	*In vivo*,C57BL/6 mice fed on a high-fat diet	TC, TG, LDL-C↓,HDL-c↑, SOD↑, MDA↓	Protecte the antioxidant system in brain tissue and improve insulin resistance by regulating the JNK pathway	Kim et al. (2017)
Rb1	*In vivo*,High-fat feeding for 12 weeks induced obese C57BL/6J mice	Body quality↓, Blood lipid level↓, skeletal muscle endurance, and insulin sensitivity↑	Activate of proteins involve in the AMPK signaling pathway in skeletal muscle	Zhao et al. (2019)
Rg3	*In vivo*, in rats with diabetic retinopathy	Proportion of apoptotic cells, MDA and LDH expression in retinal tissues↓, SOD↑	the oxidative stress response in diabetic retinal tissue↓; activating PI3K/Akt/PKB signaling pathway; the expression of apoptosis and VE GF, ICAM-1 protein ↓	Wang et al. (2020)
Rg3	*In vivo*, C57BL/6 mice with diabetic nephropathy fed a high fat diet with a single injection of 100 mg/kg streptozotocin (STZ)	TC, TG, LDL-C↓, HDL-C↑; MDA↓、SOD, CAT↑, HO-1↑	Restrain antioxidant enzyme activity by regulating the MAPK/NF- κB pathway	Li et al. (2021)
Rb1	*In vivo*, male db/m and db/db mice	Body quality↓, CHO, LDL, Adiponectin levels↓, Lipid droplets in the liver as well as in the myocardial tissue↓; ROS, MDA↓, SOD↑	Nrf 2, GSH-Px, Sod 1, and Keap 1↑; insulin resistance and leptin resistance↓	Zhang et al. (2022)

and mitochondrial autophagy, which can effectively deal with acute exposure to cellular stressors. However, under the pathological condition, the production of excessive ROS will also promote steatosis, produce lipotoxic substances, and lead to organelle dysfunction and cell damage and death. It has been shown that oxidative stress is involved in the occurrence of NAFLD at an early stage (Mann et al., 2017). The increased ROS molecules in NAFLD can directly consume antioxidant molecules and inhibit antioxidant enzymes (Chen et al., 2020). The antioxidative capacity of hepatocytes in patients with NAFLD was shown to be significantly decreased (Reccia et al., 2017), owing to a decrease in CAT, SOD, GPX, GSH, thioredoxin, α-tocopherol, and ubiquinone levels (Koruk et al., 2004; Kumar et al., 2013; Leghi et al., 2015). Xu et al. (2019) measured the antioxidants and oxidizing compounds in a mouse model of NAFLD following intervention with different doses of ginsenoside Rg1 and metformin. The results showed that high-dose Rg1 was better than metformin in significantly reducing the MDA and FFA levels and increasing the SOD level. This indicates that Rg1 can improve antioxidation and reduce the formation of ROS and free radicals, thus ameliorating NAFLD. Xiao et al. (2019) also found that Rg1 could increase the concentrations of SOD and GSH, reduce the concentration of serum MDA, decrease oxidative stress, inhibit hepatocyte apoptosis, and alleviate the progression of the disease. These effects were related to the ginsenoside-mediated activation of AMPK and ACC phosphorylation, inhibition of the synthesis of transcription factor SREBP-1c and its target genes in adipocytes, and reduction of lipid deposition in cells (Xiao et al., 2019; Zhang et al., 2019). Additionally, it was found in liver biopsies of patients with nonalcoholic steatohepatitis that the expression of Nrf2 increased when ROS was produced at the pathological level, and drug activation of this gene in mice could reverse insulin resistance, hepatic steatosis, and liver fibrosis (Sharma et al., 2017). Moreover, the ginsenoside F2-enhanced mixture SGL121 increased the expression of HO-1 by promoting the entry of Nrf2 into the nucleus, enhanced resistance to oxidative stress, inhibited fat accumulation in the liver, and effectively eliminated liver inflammation (Kim et al., 2020). Furthermore, ginsenosides can increase the antioxidative activity of hepatocytes by upregulating the expression of antioxidant genes such as *SOD2*, *HMOX1*, and *NFE2L2* (Cheng et al., 2020).

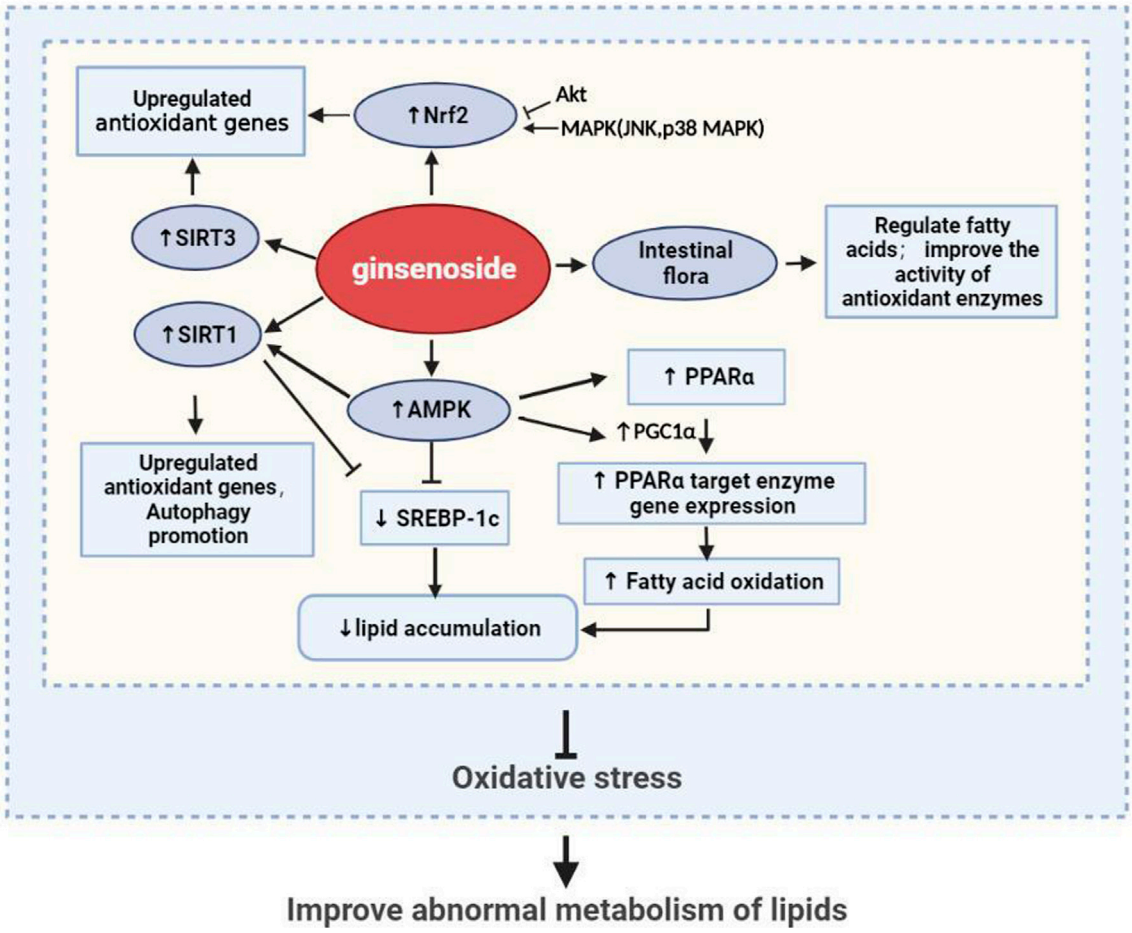


FIGURE 5
Ginseng and its active ingredients reduce lipid accumulation and promote antioxidation through multiple pathways.

6.2 Atherosclerosis

Atherosclerosis is a chronic inflammatory disease that is characterized by the accumulation of lipids and inflammatory cells in the walls of medium and large arteries. The severity of the disease is aggravated with the continuous increase in plasma cholesterol levels, especially those of LDL and VLDL, and the decrease in HDL, which are positively correlated with the incidence of the disease. Ever since lipid hydroperoxides were first found in human atherosclerotic aorta, many studies have shown an increase in lipid oxidation and other markers of oxidative stress in atherosclerotic lesions. Oxidative stress is responsible for converting LDL-C into ox-LDL, its atherogenic form. Ox-LDL plays an important role in initiating and promoting the inflammatory response and leukocyte recruitment, which inhibits or damages the defense systems (e.g., antioxidant enzymes) of the cell, thereby decreasing its ability to scavenge oxygen free radicals and finally leading to vascular endothelial cell apoptosis (Yang et al., 2017). Consequently, plaques (fatty deposits) develop on the smooth blood vessel wall, causing it to harden and damage easily. (Araujo et al., 2008). Additionally, excessive ROS can damage endothelium-dependent relaxation and trigger endothelium-dependent contraction, which are related to atherosclerotic vascular dysfunction and the subsequent development of hypertension. Studies that have tested total ginsenosides of ginseng in rats with NAFLD and ginsenoside Rg1 in mice with HFD-induced liver weight gain have shown positive effects of these saponins in decreasing the TG and FFA levels in the animals. Moreover, it was shown that ginsenoside Rh2 stimulated SOD activity and fatty acid β-oxidation (and hence increased the MDA level) *in vivo* and inhibited the formation of lipid hydroperoxides. (Nie et al., 2019) (Table 6). Ginsenoside Rg1 was shown to improve cardiac function and alleviate pathological injury of the myocardium and coronary artery in rat models of coronary heart disease. The mechanism may be related to the balance of vasomotor function and increase in the activity of antioxidant enzymes.

Ginsenosides can also protect vascular endothelial cells and stabilize atherosclerotic plaques. Rb1 may inhibit the ox-LDL-induced apoptosis of atherosclerotic endothelial cells by activating the hydroxymethyl glutaryl-coenzyme A reductase degradation protein 1 (HRD1) pathway to inhibit oxidative stress (Ren et al., 2022). Intercellular adhesion molecule 1 (ICAM-1) is a single-chain glycoprotein on the surface of

vascular endothelial cells. Studies have shown that ox-LDL can upregulate ICAM-1, thus promoting leukocyte adhesion to and migration through vascular endothelial cells, whereas panaxadiol saponins can inhibit monocyte infiltration into the intima by downregulating the protein expression of aortic endothelial ICAM-1 (Li et al., 2005; Li et al., 2006; Li et al., 2009). Monocytes migrate to the intima and differentiate into macrophages, engulfing lipids to form foam cells that express scavenger receptors, which participate in the formation of atherosclerotic plaques. These plaques are extremely unstable and rupture easily, causing thrombosis. Ginsenosides Rg1, Rg3, and panaxadiol saponins have been found to inhibit the ROS-induced release of matrix metalloproteinases and degradation of the fibrous wall of atherosclerotic plaques and the basement membrane of endothelial cells, thus weakening the physical destruction caused by atherosclerotic plaques (Bao et al., 2009; Han et al., 2013).

6.3 Type 2 diabetes mellitus and its complications

Non-insulin-dependent T2DM, which is characterized by hyperglycemia, insulin resistance, and low-grade inflammation, is a chronic metabolic disease that affects glucose, lipid, and protein metabolism. At present, the main drugs for the treatment of T2DM are insulin, insulin secretion enhancers, insulin sensitizers, α-glucosidase inhibitors, biguanides, and glucagon-like peptide-1 receptor agonists, but these can generate adverse side effects such as hypoglycemia and gastrointestinal reactions (Jendle et al., 2016). The main pathological features of T2DM are impaired islet function and insulin resistance. Ginsenosides have been shown to improve insulin sensitivity, protect islet β cells, and promote insulin secretion, and their mechanism of action is related to antioxidation (Table 7). Therefore, ginseng has great significance as a natural medicine with low side effects and multiple targets and pathways to interfere with T2DM.

The overproduction of ROS and elevated FFA content mediated by hyperglycemia can lead to insulin resistance by damaging insulin signaling and activating proinflammatory signaling proteins, which are related to the activation of p38 MAPK, JNK, and inhibitor of nuclear factor kappa B kinase beta (IKKβ)/nuclear factor kappa B (NF-κB) signals. Ginseng improves insulin resistance through multiple components, targets, and pathways. For example, ginsenoside Re was shown to ameliorate hyperglycemia by protecting the cholinergic and antioxidative systems in the brain of mice, thus alleviating HFD-induced insulin resistance (Kim et al., 2017). Chen et al. (2016) pointed out that ginsenosides Rb1 and CK could improve the IRS-1/PI3K/AKT insulin signaling pathway in adipose tissue by inhibiting the activation of NOD-like receptor protein 3 (NLRP3) inflammasomes and thereby improve insulin resistance. IRS-1 is the main substrate of insulin resistance. It was found that ginsenoside Re could inhibit JNK, MAPK, and serine phosphorylation of IRS-1 in 3T3-L1 adipocytes and HFD-fed rats and reduce insulin resistance (Zhang et al., 2008). Skeletal muscle cells, in which metabolism is mainly regulated by AMPK, are the main targets of insulin. Ginsenoside Rb1 can significantly reduce serum FFA levels and increase insulin sensitivity by activating the AMPK signaling pathway (Zhao et al., 2019).

Additionally, the antioxidative effect of ginsenosides can protect islet cells. Previous studies have shown that ginsenoside Rb1 can protect pancreatic β cells by inhibiting the apoptosis and oxidative stress induced by hyperglycemia and selectively reduce hydroxyl radicals and hypochlorite, the two strongest ROS (Chen et al., 2012; Lü et al., 2012). Ginseng berry extract (mainly ginsenoside Re) administered intragastrically (100 and 200 mg/kg) to mice with streptozotocin-induced T2DM for 10 weeks could promote β-cell proliferation and insulin secretion and decrease the blood glucose level in the animals (Park et al., 2012).

As mentioned above, oxidative stress is related to the pathogenesis and progression of diabetic vascular complications, such as retinopathy, nephropathy, and cardiovascular disease. Hyperglycemia can increase the level of ROS in endothelial cells through polyols and the advanced glycation end products and their receptors (AGEs/RAGEs), then activate NF-κB, and subsequently induce vascular endothelial inflammation and thrombosis by enhancing the expression of various related genes, including those coding for vascular endothelial growth factor (VEGF), vascular cell adhesion molecule-1 (VCAM-1), and endothelin-1 (ET-1). ICAM-1 and VCAM-1 are the main biomarkers of endothelial cell injury. One study found that ginsenoside Rg3 could inhibit apoptosis and VEGF and ICAM-1 protein expression by reducing oxidative stress in the diabetic retina and activating the PI3K/AKT signaling pathway (Wang et al., 2020). Ginsenosides can also upregulate the expression of vascular endothelial antioxidant enzymes (e.g., Nrf2 and NQO1), thereby playing a therapeutic role in diabetic nephropathy (Wu and Wang, 2021). Similarly, they upregulate the expression of genes encoding Nrf2, GSH-Px, SOD1, and KEAP1 to reduce oxidative stress and thereby improve the tissue pathological changes of diabetic cardiomyopathy (Zhang et al., 2022).

7 Discussion

According to current research on the pathogenesis of hyperlipidemia, the pathogenic factors are highly complex. This not only hampers the research and development of therapeutic drugs but also encourages the further study of the role that natural products can play in the regulation of lipid metabolism. Ginseng is widely used to treat various diseases in Asian countries, and the hypolipidemic and antioxidative effects of the plant roots have been verified. Ginseng contains saponins, sugars, volatile components, organic acids and their esters, proteins, enzymes, sterols and their glycosides, peptides, nitrogen compounds, lignins, flavonoids, vitamins, inorganic elements, and other components. The main active components are ginseng polysaccharides and ginsenosides, with the latter being the most abundant (Gao and Lu, 2021). At present, more than 180 ginsenosides have been isolated from *Panax ginseng*, among which ginsenosides Rb1, Rb2, Rb3, Rg3, Rh2, Re, Rg1, Rh4, and F2 have demonstrated effects in the treatment of hyperlipidemia.

In general, ginsenosides have been proven to treat hyperlipidemia and its secondary diseases by inhibiting oxidative stress *in vitro* and *in vivo* through their prevention of ROS production, enhancement of antioxidant enzyme activity, and removal of oxidative macromolecules, using processes that

involve PPARα, Nrf2, MAPK, SIRT3/FOXO3/SOD, AMPK/SIRT1, and other signaling pathways (Figure 5). Nrf2 and AMPK are the most-studied signaling factors at present. Among the various ginsenosides, Rg1 and Rb1 are the most promising adjuvant drugs for the treatment of hyperlipidemia.

Although the effect of ginsenosides in improving lipid metabolism in hyperlipidemia by inhibiting oxidative stress has been investigated in many preclinical studies, there are still not many clinical trials of these compounds. Therefore, further high-quality studies are needed to determine the clinical efficacy of ginsenosides. Moreover, some studies have only shown that ginseng affects the relevant markers of oxidative stress and the indexes of lipid metabolism in serum and tissue cells. Although the data obtained from the measurement of these markers clearly show that oxidative stress is closely related to lipid metabolic disorders such as fatty liver and atherosclerotic diabetes, the biomarkers provide limited information about the type, number, and location of ROS as well as their participation in specific pathophysiological processes. The causal relationship between oxidative stress and lipid metabolism disorder in hyperlipidemia needs to be demonstrated using a more rigorous experimental design. The findings summarized in this review suggest that hyperlipidemia causes excessive FFA flow into the liver, resulting in electron leakage of the liver mitochondrial, ETC. Subsequently, oxidative stress is caused by ROS accumulation, resulting in oxidative stress damage to the mitochondria and other cell structures, which further inhibits lipid metabolism, forming a vicious cycle of hyperlipidemia and oxidative stress. However, mitochondria are not the only source of ROS. The other sources of excess ROS in lipid metabolism disorders remain to be identified and the related pathogenesis needs to be further explored.

8 Conclusion

At present, the treatment of hyperlipidemia and prevention of its secondary lesions remain a challenge to healthcare providers. Ginseng, as a famous natural Chinese herbal medicine with effective bioactive components (especially ginsenosides), has proven to be capable of lowering lipid levels through various mechanisms. In recent years, there has been vast progress made in research on the positive effects of ginseng in alleviating antioxidative stress and improving lipid metabolism. In this review, we have comprehensively summarized recent studies on the effects of ginsenosides on lipid metabolism disorders in animal models of hyperlipidemia and secondary diseases, such as diabetes, NAFLD, and atherosclerosis. The current findings show that ginseng can inhibit oxidative stress and improve the level of lipid metabolism in hyperlipidemia by strengthening the expression of antioxidant enzymes, promoting fatty acid β-oxidation, and promoting autophagy. Its molecular mechanism is also related to the PPARα, Nrf2, MAPK, SIRT3/FOXO3/SOD, AMPK/SIRT1, and other signaling pathways. In conclusion, current pharmacological research appears to support the hypothesis that ginsenosides regulate lipid metabolism by regulating oxidative stress. However, the definitive relationship between oxidative stress and hyperlipidemia still needs to be verified through further studies, the results of which would have great significance for the research and development of new drugs for the treatment of hyperlipidemia, especially the use of ginseng and its ginsenosides.

Author contributions

WJ and YS contributed the central idea and wrote the initial draft of the paper. QD contributed to refining the ideas. SY, WH carried out additional comment. CL and SS finalized this paper. WJ and CL contributed equally to the research.

Funding

This work was supported by Science and Technology Development Foundation of the Hospital of Chengdu University of Traditional Chinese Medicine (NO.21HL05; NO. 20YY11); Sichuan Financial Society (2022) No.79 TCM Talent Training Program (CJJ2022119); and Sichuan Natural Science Foundation (NO. 2022NSFSC1277).

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

Attele, A. S., Wu, J. A., and Yuan, C. S. (1999). Ginseng pharmacology: Multiple constituents and multiple actions. *Biochem. Pharmacol.* 58 (11), 1685–1693. doi:10.1016/s0006-2952(99)00212-9

Bao, C. F., Liu, X., Wei, J., Liang, J., and Qin, S. J. (2009). Effects of ginsenoside Rg1 on expression of matrix metalloproteinases 2 and 9 in cerebral ischemia-reperfusion rats. *Chin. J. Cerebrovasc. Dis.* (02), 88–92.

Bonaz, B., Bazin, T., and Pellissier, S. (2018). The vagus nerve at the interface of the microbiota-gut-brain Axis. *Front. Neurosci.* 12, 49. doi:10.3389/fnins.2018.00049

Calabrese, V., Cornelius, C., Dinkova-Kostova, A. T., Calabrese, E. J., and Mattson, M. P. (2010). Cellular stress responses, the hormesis paradigm, and vitagenes: Novel targets for therapeutic intervention in neurodegenerative disorders. *Antioxidants redox Signal.* 13 (11), 1763–1811. doi:10.1089/ars.2009.3074

Campbell, E. L., and Colgan, S. P. (2019). Control and dysregulation of redox signalling in the gastrointestinal tract. *Nat. Rev. Gastroenterology hepatology* 16 (2), 106–120. doi:10.1038/s41575-018-0079-5

Cao, H. M., Song, N., Jia, L. Q., Chen, W. N., Yang, G. L., Yang, F., et al. (2018). Effects of different active ingredients of Gynostemma pentaphyllum on the autophagy effect of endothelial cells induced by ox-LDL. *Chin. J. Traditional Chin. Med.* (05), 1166–1168. doi:10.13193/j.issn.1673-7717.2018.05.036

Carrier, A. (2017). Metabolic syndrome and oxidative stress: A complex relationship. *Antioxidants redox Signal.* 26 (9), 429–431. doi:10.1089/ars.2016.6929

Centers for Disease Control and Prevention (Cdc) (2011). Vital signs: Prevalence, treatment, and control of high levels of low-density lipoprotein cholesterol—United States, 1999-2002 and 2005-2008. *MMWR Morb. Mortal. Wkly. Rep.* 60 (4), 109–114.

Chang, R. X. (2020). *Study on the preventive effect and mechanism of modified sijunzi decoction on non-alcoholic fatty liver in mice*. Daqing: Heilongjiang Bayi Agricultural University.[dissertation/master's thesis][China(Heilongjiang)].

Chen, F., Chen, Y., Kang, X., Zhou, Z., Zhang, Z., and Liu, D. (2012). Anti-apoptotic function and mechanism of ginseng saponins in Rattus pancreatic β-cells. *Biol. Pharm. Bull.* 35 (9), 1568–1573. doi:10.1248/bpb.b12-00461

Chen, W., Wang, J., Luo, Y., Wang, T., Li, X., Li, A., et al. (2016). Ginsenoside Rb1 and compound K improve insulin signaling and inhibit ER stress-associated NLRP3 inflammasome activation in adipose tissue. *J. ginseng Res.* 40 (4), 351–358. doi:10.1016/j.jgr.2015.11.002

Chen, Y. X., Li, S. S., and Zhang, H. F. (2020). Effects of ginsenoside Rg1 on cardiac function and vasomotor function in rats with coronary atherosclerotic heart disease. *Chin. Med. J.* (07), 1491–1496. doi:10.16368/j.issn.1674-8999.2020.07.333

Chen, Z., Tian, R., She, Z., Cai, J., and Li, H. (2020). Role of oxidative stress in the pathogenesis of nonalcoholic fatty liver disease. *Free Radic. Biol. Med.* 152, 116–141. doi:10.1016/j.freeradbiomed.2020.02.025

Cheng, B., Gao, W., Wu, X., Zheng, M., Yu, Y., Song, C., et al. (2020). Ginsenoside Rg2 ameliorates high-fat diet-induced metabolic disease through SIRT1. *J. Agric. food Chem.* 68 (14), 4215–4226. doi:10.1021/acs.jafc.0c00833

Cheung, K. G., Cole, L. K., Xiang, B., Chen, K., Ma, X., Myal, Y., et al. (2015). Sirtuin-3 (SIRT3) protein attenuates doxorubicin-induced oxidative stress and improves mitochondrial respiration in H9c2 cardiomyocytes. *J. Biol. Chem.* 290 (17), 10981–10993. doi:10.1074/jbc.M114.607960

Claypool, S. M., and Koehler, C. M. (2012). The complexity of cardiolipin in health and disease. *Trends Biochem. Sci.* 37 (1), 32–41. doi:10.1016/j.tibs.2011.09.003

Dai, G., Vaughn, S., Zhang, Y., Wang, E. T., Garcia-Cardena, G., and Gimbrone, M. A. (2007). Biomechanical forces in atherosclerosis-resistant vascular regions regulate endothelial redox balance via phosphoinositol 3-kinase/Akt-dependent activation of Nrf2. *Circulation Res.* 101 (7), 723–733. doi:10.1161/CIRCRESAHA.107.152942

Ding, Q., Feng, S. W., Xu, G. H., Chen, Y. Y., and Shi, Y. Y. (2023). Effects of total saponins from ginseng stems and leaves on intestinal flora and short-chain fatty acid metabolism in mice with acute lung injury. *Chin. J. Traditional Chin. Med.* doi:10.19540/j.cnki.cjcmm.20221111.702

Dinkova-Kostova, A. T., and Abramov, A. Y. (2015). The emerging role of Nrf2 in mitochondrial function. *Free Radic. Biol. Med.* 88 (1), 179–188. doi:10.1016/j.freeradbiomed.2015.04.036

Fang, K., Wu, F., Chen, G., Dong, H., Li, J., Zhao, Y., et al. (2019). Diosgenin ameliorates palmitic acid-induced lipid accumulation via AMPK/ACC/CPT-1A and SREBP-1c/FAS signaling pathways in LO2 cells. *BMC complementary Altern. Med.* 19 (1), 255. doi:10.1186/s12906-019-2671-9

Filomeni, G., De Zio, D., and Cecconi, F. (2015). Oxidative stress and autophagy: The clash between damage and metabolic needs. *Cell. death Differ.* 22 (3), 377–388. doi:10.1038/cdd.2014.150

Foretz, M., Even, P. C., and Viollet, B. (2018). AMPK activation reduces hepatic lipid content by increasing fat oxidation *in vivo*. *Int. J. Mol. Sci.* 19 (9), 2826. doi:10.3390/ijms19092826

Forman, H. J., and Zhang, H. (2021). Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. *Nat. Rev. Drug Discov.* 20 (9), 689–709. doi:10.1038/s41573-021-00233-1

Forrester, S. J., Kikuchi, D. S., Hernandes, M. S., Xu, Q., and Griendling, K. K. (2018). Reactive oxygen species in metabolic and inflammatory signaling. *Circulation Res.* 122 (6), 877–902. doi:10.1161/CIRCRESAHA.117.311401

Frühbeck, G., Catalán, V., Rodríguez, A., Ramírez, B., Becerril, S., Salvador, J., et al. (2017). Involvement of the leptin-adiponectin axis in inflammation and oxidative stress in the metabolic syndrome. *Sci. Rep.* 7 (1), 6619. doi:10.1038/s41598-017-06997-0

Gao, J., and Lu, S. W. (2021). Research progress on chemical constituents and pharmacological effects of ginseng. *Her. Traditional Chin. Med.* (01), 127–130+137. doi:10.13862/j.cnki.cn43-1446/r.2021.01.030

Gao, Y., Huang, W. X., Zhang, S. J., Li, J. J., Yang, C., Zhao, J. Q., et al. (2020). Effects of ginsenoside Rg_1 on lipid uptake and oxidation in nonalcoholic fatty liver HepG2 cells. *Chin. J. Exp. Formulas* (12), 100–106. doi:10.13422/j.cnki.syfjx.20201101

Gao, Y., Li, J., Chu, S., Zhang, Z., Chen, N., Li, L., et al. (2020). Ginsenoside Rg1 protects mice against streptozotocin-induced type 1 diabetic by modulating the NLRP3 and Keap1/Nrf2/HO-1 pathways. *Eur. J. Pharmacol.* 866, 172801. doi:10.1016/j.ejphar.2019.172801

Gao, Y., Zhang, S., Li, J., Zhao, J., Xiao, Q., Zhu, Y., et al. (2020). Effect and mechanism of ginsenoside Rg1-regulating hepatic steatosis in HepG2 cells induced by free fatty acid. *Biosci. Biotechnol. Biochem.* 84 (11), 2228–2240. doi:10.1080/09168451.2020.1793293

GBD 2017 Disease and Injury Incidence and Prevalence Collaborators (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: A systematic analysis for the global burden of disease study 2017. *Lancet (London, Engl.* 392 (10159), 1789–1858. doi:10.1016/S0140-6736(18)32279-7

Gross, B., Pawlak, M., Lefebvre, P., and Staels, B. (2017). PPARs in obesity-induced T2DM, dyslipidaemia and NAFLD. *Nat. Rev. Endocrinol.* 13 (1), 36–49.

Guariguata, L. (2013). Contribute data to the 6th edition of the IDF diabetes atlas. *Diabetes Res. Clin. Pract.* 100 (2), 280–281. doi:10.1016/j.diabres.2013.02.006

Han, D., Zhang, Y. W., Liu, M., Dai, D. Q., Zhang, Y. J., Wang, C. Y., et al. (2013). Effects of panaxadiol group saponins on matrix metalloproteinase-2, -9 and ventricular remodeling rats after myocardial infarction. Effect of tissue inhibitor-1 levels. *Chin. J. Gerontology* (24), 6225–6227.

Helgason, G. V., Holyoake, T. L., and Ryan, K. M. (2013). Role of autophagy in cancer prevention, development and therapy. *Essays Biochem.* 55, 133–151. doi:10.1042/bse0550133

Henriksen, E. J., Diamond-Stanic, M. K., and Marchionne, E. M. (2011). Oxidative stress and the etiology of insulin resistance and type 2 diabetes. *Free Radic. Biol. Med.* 51 (5), 993–999. doi:10.1016/j.freeradbiomed.2010.12.005

Hirschey, M. D., Shimazu, T., Jing, E., Grueter, C. A., Collins, A. M., Aouizerat, B., et al. (2011). SIRT3 deficiency and mitochondrial protein hyperacetylation accelerate the development of the metabolic syndrome. *Mol. Cell.* 44 (2), 177–190. doi:10.1016/j.molcel.2011.07.019

Hong, Y. L., Gu, X. J., Xu, J., Lin, Y., and Si, Q. Y. (2018). *The effect and mechanism of ginsenoside Rb2 on liver lipid metabolism in mice with hyperlipidemia fatty liver*. Wenzhou: Journal of Wenzhou Medical University, 338–341+349.05

Hu, C. F., Lu, D. X., Sun, L. P., and Qin, L. (2009). The effect and mechanism of ginseng stem and leaf saponins on fatty liver in mice. *Chin. Pharmacol. Bull.* (05), 663–667.

Hwang, Y. C., Oh, D. H., Choi, M. C., Lee, S. Y., Ahn, K. J., Chung, H. Y., et al. (2018). Compound K attenuates glucose intolerance and hepatic steatosis through AMPK-dependent pathways in type 2 diabetic OLETF rats. *Korean J. Intern. Med.* 33 (2), 347–355. doi:10.3904/kjim.2015.208

Jendle, J., Grunberger, G., Blevins, T., Giorgino, F., Hietpas, R. T., and Botros, F. T. (2016). Efficacy and safety of dulaglutide in the treatment of type 2 diabetes: A comprehensive review of the dulaglutide clinical data focusing on the AWARD phase 3 clinical trial program. *Diabetes/metabolism Res. Rev.* 32 (8), 776–790. doi:10.1002/dmrr.2810

Jones, R. M., Mercante, J. W., and Neish, A. S. (2012). Reactive oxygen production induced by the gut microbiota: Pharmacotherapeutic implications. *Curr. Med. Chem.* 19 (10), 1519–1529. doi:10.2174/092986712799828283

Kagan, V. E., Jiang, J., Huang, Z., Tyurina, Y. Y., Desbourdes, C., Cottet-Rousselle, C., et al. (2016). NDPK-D (NM23-H4)-mediated externalization of cardiolipin enables elimination of depolarized mitochondria by mitophagy. *Cell. death Differ.* 23 (7), 1140–1151.

Kang, K. S., Ham, J., Kim, Y. J., Park, J. H., Cho, E. J., and Yamabe, N. (2013). Heat-processed Panax ginseng and diabetic renal damage: Active components and action mechanism. *J. ginseng Res.* 37 (4), 379–388. doi:10.5142/jgr.2013.37.379

Kawahara, H., Fukura, M., Tsuchishima, M., and Takase, S. (2007). Mutation of mitochondrial DNA in livers from patients with alcoholic hepatitis and nonalcoholic steatohepatitis. *Alcohol. Clin. Exp. Res.* 31 (1), S54–S60. doi:10.1111/j.1530-0277.2006.00287.x

Kim, D. E., Chang, B. Y., Jeon, B. M., Baek, J. I., Kim, S. C., and Kim, S. Y. (2020). SGL 121 attenuates nonalcoholic fatty liver disease through adjusting lipid metabolism through AMPK signaling pathway. *Int. J. Mol. Sci.* 21 (12), 4534. doi:10.3390/ijms21124534

Kim, H. J., Jung, S. W., Kim, S. Y., Cho, I. H., Kim, H. C., Rhim, H., et al. (2018). *Panax* ginseng as an adjuvant treatment for Alzheimer's disease. *J. ginseng Res.* 42 (4), 401–411. doi:10.1016/j.jgr.2017.12.008

Kim, I. H., Kim, S. K., Kim, E. H., Kim, S. W., Sohn, S. H., Lee, S. C., et al. (2011). Korean red ginseng up-regulates C21-steroid hormone metabolism via Cyp11a1 gene in senescent rat testes. *J. ginseng Res.* 35 (3), 272–282. doi:10.5142/jgr.2011.35.3.272

Kim, J. H., Kang, S. A., Han, S. M., and Shim, I. (2009). Comparison of the antiobesity effects of the protopanaxadiol- and protopanaxatriol-type saponins of red ginseng. *Phytotherapy Res. PTR* 23 (1), 78–85. doi:10.1002/ptr.2561

Kim, J. M., Park, C. H., Park, S. K., Seung, T. W., Kang, J. Y., Ha, J. S., et al. (2017). Ginsenoside Re ameliorates brain insulin resistance and cognitive dysfunction in high fat diet-induced C57bl/6 mice. *J. Agric. food Chem.* 65 (13), 2719–2729. doi:10.1021/acs.jafc.7b00297

Kim, Y. K., Yoo, D. S., Xu, H., Park, N. I., Kim, H. H., Choi, J. E., et al. (2009). Ginsenoside content of berries and roots of three typical Korean ginseng (Panax ginseng) cultivars. *Nat. Product. Commun.* 4 (7), 903–906.

Kitada, M., Ogura, Y., and Koya, D. (2016). The protective role of Sirt1 in vascular tissue: Its relationship to vascular aging and atherosclerosis. *Aging* 8 (10), 2290–2307. doi:10.18632/aging.101068

Kong, A. N., Owuor, E., Yu, R., Hebbar, V., Chen, C., Hu, R., et al. (2001). Induction of xenobiotic enzymes by the MAP kinase pathway and the antioxidant or electrophile response element (ARE/EpRE). *Drug metab. Rev.* 33 (3-4), 255–271. doi:10.1081/dmr-120000652

Kong, F. L., Sun, X., Zhao, X. J., and Li, Y. L. (2010). Experimental study on anti-atherosclerotic effect of ginsenoside (Rh_2). *J. Beihua Univ. Nat. Sci. Ed.* (06), 520–523.

Koruk, M., Taysi, S., Savas, M. C., Yilmaz, O., Akcay, F., and Karakok, M. (2004). Oxidative stress and enzymatic antioxidant status in patients with nonalcoholic steatohepatitis. *Ann. Clin. laboratory Sci.* 34 (1), 57–62.

Kukidome, D., Nishikawa, T., Sonoda, K., Imoto, K., Fujisawa, K., Yano, M., et al. (2006). Activation of AMP-activated protein kinase reduces hyperglycemia-induced mitochondrial reactive oxygen species production and promotes mitochondrial biogenesis in human umbilical vein endothelial cells. *Diabetes* 55 (1), 120–127.

Kumar, A., Sharma, A., Duseja, A., Das, A., Dhiman, R. K., Chawla, Y. K., et al. (2013). Patients with nonalcoholic fatty liver disease (NAFLD) have higher oxidative stress in comparison to chronic viral hepatitis. *J. Clin. Exp. hepatology* 3 (1), 12–18. doi:10.1016/j.jceh.2012.10.009

Lee, D. H., Cho, H. J., Kang, H. Y., Rhee, M. H., and Park, H. J. (2012). Total saponin from Korean red ginseng inhibits thromboxane A2 production associated microsomal enzyme activity in platelets. *J. ginseng Res.* 36 (1), 40–46. doi:10.5142/jgr.2012.36.1.40

Lee, M. R., Kim, B. C., Kim, R., Oh, H. I., Kim, H. K., Choi, K. J., et al. (2013). Anti-obesity effects of black ginseng extract in high fat diet-fed mice. *J. ginseng Res.* 37 (3), 308–349. doi:10.5142/jgr.2013.37.308

Leghi, G. E., Domenici, F. A., and Vannucchi, H. (2015). Influence OF oxidative stress and obesity IN patients with nonalcoholic steatohepatitis. *Arq. Gastroenterol.* 52 (3), 228–233. doi:10.1590/S0004-28032015000300014

Li, D. C., Zhang, H., Zhang, F. H., and Wang, D. Q. (2005). Effect of compound qishen jiangzhi decoction on the expression of intercellular adhesion molecule-1 in the aortic wall of rats with hypercholesterolemia. *Chin. J. Clin. Pharm.* (05), 282–284. doi:10.19577/j.cnki.issn10074406.2005.05.006

Li, F. G., Kong, F. L., Sun, X., Wang, D. M., Zhao, X. J., Li, Y. L., et al. (2009). The anti-atherosclerotic effect and mechanism of panaxadiol saponins. *Chin. J. Gerontology* (24), 3234–3236.

Li, J., Song, J. Y., Zhang, L. Y., and Wu, S. S. (2013). Myocardial protective effect and mechanism of ginsenoside Re on diabetic rats. *Shandong Med.* (42), 9–11.

Li, Q., Xiang, Y., Chen, Y., Tang, Y., and Zhang, Y. (2017). Ginsenoside Rg1 protects cardiomyocytes against hypoxia/reoxygenation injury via activation of Nrf2/HO-1 signaling and inhibition of JNK. *Cell. physiology Biochem. Int. J. Exp. Cell. physiology, Biochem. Pharmacol.* 44 (1), 21–37. doi:10.1159/000484578

Li, Q. X., Zhong, H. J., Zhou, M., He, J. L., Li, W., Wu, Q., et al. (2006). Puerarin regulates blood lipids and aortic intercellular adhesion molecule-1 expression in diabetic rats. *Chin. Behav. Med. Sci.* (11), 981–983.

Li, X., Zhu, F., Jiang, J., Sun, C., Zhong, Q., Shen, M., et al. (2016). Simultaneous inhibition of the ubiquitin-proteasome system and autophagy enhances apoptosis induced by ER stress aggravators in human pancreatic cancer cells. *Autophagy* 12 (9), 1521–1537. doi:10.1080/15548627.2016.1191722

Li, Y., Hou, J. G., Liu, Z., Gong, X. J., Hu, J. N., Wang, Y. P., et al. (2021). Alleviative effects of 20(R)-Rg3 on HFD/STZ-induced diabetic nephropathy via MAPK/NF-κB signaling pathways in C57BL/6 mice. *J. Ethnopharmacol.* 267, 113500. doi:10.1016/j.jep.2020.113500

Li, Y. P., Miao, W., Gao, W. H., and Hu, L. (2021). Effects of ginsenoside Rb1 on lipid deposition and oxidative stress in mouse fatty liver cell model. *New Chin. Med. Clin. Pharmacol.* (07), 939–944. doi:10.19378/j.issn.1003-9783.2021.07.006

Li, Y., Sun, K. H., Bai, F., Luo, H. M., and Wu, Z. Z. (2019). Research progress in the pathogenesis of hyperlipidemia-related diseases. *J. Liaoning Univ. Traditional Chin. Med.* (04), 84–87. doi:10.13194/j.issn.1673-842x.2019.04.023

Li, Z., and Ji, G. E. (2018). Ginseng and obesity. *J. ginseng Res.* 42 (1), 1–8. doi:10.1016/j.jgr.2016.12.005

Liss, K. H., and Finck, B. N. (2017). PPARs and nonalcoholic fatty liver disease. *Biochimie* 136, 65–74.

Liu, J., Liu, W., Lu, Y., Tian, H., Duan, C., Lu, L., et al. (2018). Piperlongumine restores the balance of autophagy and apoptosis by increasing BCL2 phosphorylation in rotenone-induced Parkinson disease models. *Autophagy* 14 (5), 845–861. doi:10.1080/15548627.2017.1390636

Liu, L., Feng, D., Chen, G., Chen, M., Zheng, Q., Song, P., et al. (2012). Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. *Nat. Cell. Biol.* 14 (2), 177–185. doi:10.1038/ncb2422

Liu, W. (2010). Experimental study on anti-atherosclerosis effect of total ginseng saponins. *Aerosp. Med.* (11), 1968–1970.

Liu, W., Zheng, Y., Han, L., Wang, H., Saito, M., Ling, M., et al. (2008). Saponins (Ginsenosides) from stems and leaves of Panax quinquefolium prevented high-fat diet-induced obesity in mice. *Phytomedicine Int. J. phytotherapy Phytopharm.* 15 (12), 1140–1145. doi:10.1016/j.phymed.2008.07.002

Liu, Y., Deng, J., and Fan, D. (2021). G-Rh4 improves pancreatic β-cells dysfunction *in vivo* and *in vitro* by increased expression of Nrf2 and its target genes. *Food Chem. Toxicol. Int. J. Publ. Br. Industrial Biol. Res. Assoc.* 148, 111925. doi:10.1016/j.fct.2020.111925

Loskovich, M. V., Grivennikova, V. G., Cecchini, G., and Vinogradov, A. D. (2005). Inhibitory effect of palmitate on the mitochondrial NADH:ubiquinone oxidoreductase (complex I) as related to the active-de-active enzyme transition. *Biochem. J.* 387 (3), 677–683. doi:10.1042/BJ20041703

Lu, H., Yuan, X., Zhang, Y., Han, M., Liu, S., Han, K., et al. (2020). HCBP6 deficiency exacerbates glucose and lipid metabolism disorders in non-alcoholic fatty liver mice. *Biomed. Pharmacother. = Biomedecine Pharmacother.* 129, 110347. doi:10.1016/j.biopha.2020.110347

Lü, J. M., Weakley, S. M., Yang, Z., Hu, M., Yao, Q., and Chen, C. (2012). Ginsenoside Rb1 directly scavenges hydroxyl radical and hypochlorous acid. *Curr. Pharm. Des.* 18 (38), 6339–6347. doi:10.2174/138161212803832254

Ludtmann, M. H., Angelova, P. R., Zhang, Y., Abramov, A. Y., and Dinkova-Kostova, A. T. (2014). Nrf2 affects the efficiency of mitochondrial fatty acid oxidation. *Biochem. J.* 457 (3), 415–424. doi:10.1042/BJ20130863

Ma, L. Y. (2018). Research progress on adverse reactions and non-cardiovascular effects of statins. *Chin. Med. Guide* (04), 8–9. doi:10.15912/j.cnki.gocm.2018.04.006

Maguire, J. J., Tyurina, Y. Y., Mohammadyani, D., Kapralov, A. A., Anthonymuthu, T. S., Qu, F., et al. (2017). Known unknowns of cardiolipin signaling: The best is yet to come. *Biochimica biophysica acta. Mol. Cell. Biol. lipids* 1862 (1), 8–24. doi:10.1016/j.bbalip.2016.08.001

Mann, J. P., Raponi, M., and Nobili, V. (2017). Clinical implications of understanding the association between oxidative stress and pediatric NAFLD. *Expert Rev. gastroenterology hepatology* 11 (4), 371–382. doi:10.1080/17474124.2017.1291340

Metwaly, A. M., Lianlian, Z., Luqi, H., and Deqiang, D. (2019). Black ginseng and its saponins: Preparation, phytochemistry and pharmacological effects. *Mol. (Basel, Switz.* 24 (10), 1856. doi:10.3390/molecules24101856

Mohs, A., Otto, T., Schneider, K. M., Peltzer, M., Boekschoten, M., Holland, C. H., et al. (2021). Hepatocyte-specific NRF2 activation controls fibrogenesis and carcinogenesis in steatohepatitis. *J. hepatology* 74 (3), 638–648. do:. doi:10.1016/j.jhep.2020.09.037

Montagner, A., Polizzi, A., Fouché, E., Ducheix, S., Lippi, Y., Lasserre, F., et al. (2016). Liver PPARα is crucial for whole-body fatty acid homeostasis and is protective against NAFLD. *Gut* 65 (7), 1202–1214. doi:10.1136/gutjnl-2015-310798

Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. *Biochem. J.* 417 (1), 1–13. doi:10.1042/BJ20081386

Nie, Z. F., Sun, X. Y., Zhang, T. P., and Peng, T. (2019). Effects of ginsenoside Compound K on oxidative stress, inflammatory factors and vasoactive substances in rats with atherosclerosis. *Hebei Tradit. Chin. Med.* (07), 1042–1047.

Nomoto, K., Tsuneyama, K., Takahashi, H., Murai, Y., and Takano, Y. (2008). Cytoplasmic fine granular expression of 8-hydroxydeoxyguanosine reflects early mitochondrial oxidative DNA damage in nonalcoholic fatty liver disease. *Appl. Immunohistochem. Mol. Morphol. AIMM* 16 (1), 71–75. doi:10.1097/PAI.0b013e31803156d5

Novak, I., Kirkin, V., McEwan, D. G., Zhang, J., Wild, P., Rozenknop, A., et al. (2010). Nix is a selective autophagy receptor for mitochondrial clearance. *EMBO Rep.* 11 (1), 45–51.

O'Neill, H. M., Holloway, G. P., and Steinberg, G. R. (2013). AMPK regulation of fatty acid metabolism and mitochondrial biogenesis: Implications for obesity. *Mol. Cell. Endocrinol.* 366 (2), 135–151.

Ong, W. Y., Farooqui, T., Koh, H. L., Farooqui, A. A., and Ling, E. A. (2015). Protective effects of ginseng on neurological disorders. *Front. aging Neurosci.* 7, 129. doi:10.3389/fnagi.2015.00129

Ouimet, M., Barrett, T. J., and Fisher, E. A. (2019). HDL and reverse cholesterol transport. *Circulation Res.* 124 (10), 1505–1518.

Papackova, Z., and Cahova, M. (2015). Fatty acid signaling: The new function of intracellular lipases. *Int. J. Mol. Sci.* 16 (2), 3831–3855. doi:10.3390/ijms16023831

Paradies, G., Petrosillo, G., Paradies, V., and Ruggiero, F. M. (2009). Role of cardiolipin peroxidation and Ca^{2+} in mitochondrial dysfunction and disease. *Cell. calcium* 45 (6), 643–650. doi:10.1016/j.ceca.2009.03.012

Park, E. Y., Kim, H. J., Kim, Y. K., Park, S. U., Choi, J. E., Cha, J. Y., et al. (2012). Increase in insulin secretion induced by Panax ginseng berry extracts contributes to the amelioration of hyperglycemia in streptozotocininduced diabetic mice. *J. ginseng Res.* 36 (2), 153–160. doi:10.5142/jgr.2012.36.2.153

Peng, X., Huang, D. B., Yan, M., and Peng, S. J. (2015). Ginsenoside Rg1 improves liver function in rats with nonalcoholic fatty liver disease by regulating fat metabolism. *Chin. J. Pathophysiol.* (05), 864–870.

Petrosillo, G., Casanova, G., Matera, M., Ruggiero, F. M., and Paradies, G. (2006). Interaction of peroxidized cardiolipin with rat-heart mitochondrial membranes: Induction of permeability transition and cytochrome c release. *FEBS Lett.* 580 (27), 6311–6316.

Podolak, I., Galanty, A., and Sobolewska, D. (2010). Saponins as cytotoxic agents: A review. *Phytochemistry Rev. Proc. Phytochemical Soc. Eur.* 9 (3), 425–474. doi:10.1007/s11101-010-9183-z

Qiu, X., Brown, K., Hirschey, M. D., Verdin, E., and Chen, D. (2010). Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. *Cell. metab.* 12 (6), 662–667. doi:10.1016/j.cmet.2010.11.015

Ren, L., Wu, X. H., Liu, T., Mei, Y. Z., and Su, S. Y. (2022). *The effect of ginsenoside Rb1 on the apoptosis of atherosclerotic endothelial cells by activating the HRD1 signaling pathway*. Guiyang: Journal of Guizhou Medical University, 779–784+795. doi:10.19367/j.cnki.2096-8388.2022.07.00607

Sabouny, R., Fraunberger, E., Geoffrion, M., Ng, A. C., Baird, S. D., Screaton, R. A., et al. (2017). The keap1-nrf2 stress response pathway promotes mitochondrial hyperfusion through degradation of the mitochondrial fission protein Drp1. *Antioxidants redox Signal.* 27 (18), 1447–1459. doi:10.1089/ars.2016.6855

Sayiner, M., Koenig, A., Henry, L., and Younossi, Z. M. (2016). Epidemiology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in the United States and the rest of the world. *Clin. liver Dis.* 20 (2), 205–214. doi:10.1016/j.cld.2015.10.001

Schieber, M., and Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Curr. Biol. CB* 24 (10), R453–R462. doi:10.1016/j.cub.2014.03.034

Schönfeld, P., and Reiser, G. (2006). Rotenone-like action of the branched-chain phytanic acid induces oxidative stress in mitochondria. *J. Biol. Chem.* 281 (11), 7136–7142. doi:10.1074/jbc.M513198200

Schönfeld, P., and Wojtczak, L. (2008). Fatty acids as modulators of the cellular production of reactive oxygen species. *Free Radic. Biol. Med.* 45 (3), 231–241. doi:10.1016/j.freeradbiomed.2008.04.029

Seo, E. Y., and Kim, W. K. (2011). Red ginseng extract reduced metastasis of colon cancer cells *in vitro* and *in vivo*. *J. ginseng Res.* 35 (3), 315–324. doi:10.5142/jgr.2011.35.3.315

Sharma, R. S., Harrison, D. J., Kisielewski, D., Cassidy, D. M., McNeilly, A. D., Gallagher, J. R., et al. (2017). Experimental nonalcoholic steatohepatitis and liver fibrosis are ameliorated by pharmacologic activation of Nrf2 (NF-E2 p45-related factor 2). *Cell. Mol. gastroenterology hepatology* 5 (3), 367–398. doi:10.1016/j.jcmgh.2017.11.016

Shen, L., Xiong, Y., Wang, D. Q., Howles, P., Basford, J. E., Wang, J., et al. (2013). Ginsenoside Rb1 reduces fatty liver by activating AMP-activated protein kinase in obese rats. *J. lipid Res.* 54 (5), 1430–1438. doi:10.1194/jlr.M035907

Shi, G., Liu, D., Zhou, B., Liu, Y., Hao, B., Yu, S., et al. (2020). Ginsenoside Rb1 alleviates oxidative low-density lipoprotein-induced vascular endothelium senescence via the SIRT1/beclin-1/autophagy Axis. *J. Cardiovasc. Pharmacol.* 75 (2), 155–167. doi:10.1097/FJC.0000000000000775

Shi, Z. Y., Zeng, J. Z., and Wong, A. S. T. (2019). Chemical structures and pharmacological profiles of ginseng saponins. *Mol. (Basel, Switz.* 24 (13), 2443. doi:10.3390/molecules24132443

Shibata, M., Hakuno, F., Yamanaka, D., Okajima, H., Fukushima, T., Hasegawa, T., et al. (2010). Paraquat-induced oxidative stress represses phosphatidylinositol 3-kinase activities leading to impaired glucose uptake in 3T3-L1 adipocytes. *J. Biol. Chem.* 285 (27), 20915–20925.

Shimpi, P. C., More, V. R., Paranjpe, M., Donepudi, A. C., Goodrich, J. M., Dolinoy, D. C., et al. (2017). Hepatic lipid accumulation and Nrf2 expression following perinatal and peripubertal exposure to bisphenol A in a mouse model of nonalcoholic liver disease. *Environ. health Perspect.* 125 (8), 087005. doi:10.1289/EHP664

Sundaresan, N. R., Gupta, M., Kim, G., Rajamohan, S. B., Isbatan, A., and Gupta, M. P. (2009). Sirt3 blocks the cardiac hypertrophic response by augmenting Foxo3a-dependent antioxidant defense mechanisms in mice. *J. Clin. investigation* 119 (9), 2758–2771. doi:10.1172/JCI39162

Teng, S. N., and Yang, J. (2017). The relationship between the cholesterol and the incidence of coronary heart disease. *Electron. J. Cardiovasc. Dis. Integr. Traditional Chin. West. Med.* (23), 20–22. doi:10.16282/j.cnki.cn11-9336/r.2017.23.013

Tse, J. K. Y. (2017). Gut microbiota, nitric oxide, and microglia as prerequisites for neurodegenerative disorders. *ACS Chem. Neurosci.* 8 (7), 1438–1447. doi:10.1021/acschemneuro.7b00176

Varghese, J. F., Patel, R., and Yadav, U. C. S. (2018). Novel insights in the metabolic syndrome-induced oxidative stress and inflammation-mediated atherosclerosis. *Curr. Cardiol. Rev.* 14 (1), 4–14. doi:10.2174/1573403X13666171009112250

Wang, G. E., Yang, F., Liu, X. Y., Ye, Y. J., Wang, R. H., Lu, X. T., et al. (2022). Mechanism of ginsenoside Rb_1 in improving restraint stress combined with lipopolysaccharide-induced immune liver injury in mice. *Chin. Herb. Med.* (13), 4028–4034.

Wang, J. J., Hu, R. R., Zhang, D., and Qiao, Y. B. (2020). Effects of ginsenoside Rg3 on PI3K-Akt/PKB pathway and expression of vascular endothelial growth factor and intercellular adhesion molecule-1 in rats with diabetic retinopathy. *Ophthalmol. new Prog.* (01), 20–25. doi:10.13389/j.cnki.rao.2020.0005

Wang, L., Zhu, L. H., Jiang, H., Tang, Q. Z., Yan, L., Wang, D., et al. (2010). Grape seed proanthocyanidins attenuate vascular smooth muscle cell proliferation via blocking phosphatidylinositol 3-kinase-dependent signaling pathways. *J. Cell. physiology* 223 (3), 713–726.

Wang, Y., Li, Y., Yang, W., Gao, S., Lin, J., Wang, T., et al. (2018). Ginsenoside Rb1 inhibit apoptosis in rat model of Alzheimer's disease induced by Aβ1-40. *Am. J. Transl. Res.* 10 (3), 796–805.

Wang, Y., Nakajima, T., Gonzalez, F. J., and Tanaka, N. (2020). PPARs as metabolic regulators in the liver: Lessons from liver-specific PPAR-null mice. *Int. J. Mol. Sci.* 21 (6), 2061. doi:10.3390/ijms21062061

Wu, S. B., and Wang, Y. D. (2021). Effects of ginsenosides on the expression of ColⅣ, Nrf2 and NQO1 in rats with diabetic nephropathy. *Hebei Med.* (15), 2245–2249.

Wu, X. T., Wu, X. J., Cui, X. L., Li, X., and Zhang, D. (2022). Ginsenoside Rb3 improves lipid deposition and oxidative stress in a mouse fatty liver cell model by up-regulating the expression of Klf16/Nrf2. *New Drugs Clin. Pharmacol. Traditional Chin. Med.* (04), 477–483. doi:10.19378/j.issn.1003-9783.2022.04.009

Xiao, Q., Huang, W. X., Zhang, S. J., Yang, C., Li, J. J., Zhao, J. Q., et al. (2019). *Ginsenoside Rg1 activates adenylate-activated protein kinase to inhibit lipid deposition in a nonalcoholic fatty liver cell model induced* in vitro. Chongqing: No. 3 Journal of Military Medical University, 1343–1349. doi:10.16016/j.1000-5404.20190107414

Xiao, Y., Hou, Y. H., Yin, X., Kang, F., Li, S. D., Yang, S. K., et al. (2019). Ginsenoside Rg1 interferes with the apoptosis of liver cells in non-alcoholic fatty liver model rats. *China Tissue Eng. Res.* (03), 384–390.

Xu, Y. S., Huang, W. X., Yang, C., Zhang, S. J., Zhao, L., and Qin, Y. Y. (2019). Improvement effect and mechanism of ginsenoside Rg1 on nonalcoholic fatty liver disease in mice. *J. Chongqing Med. Univ.* (11), 1434–1438.

Xu, Y., Yang, C., Zhang, S., Li, J., Xiao, Q., and Huang, W. (2018). Ginsenoside Rg1 protects against non-alcoholic fatty liver disease by ameliorating lipid peroxidation, endoplasmic reticulum stress, and inflammasome activation. *Biol. Pharm. Bull.* 41 (11), 1638–1644. doi:10.1248/bpb.b18-00132

Yang, R. L., Shi, Y. H., Hao, G., Li, W., and Le, G. W. (2008). Increasing oxidative stress with progressive hyperlipidemia in human: Relation between malondialdehyde and atherogenic index. *J. Clin. Biochem. Nutr.* 43 (3), 154–158. doi:10.3164/jcbn.2008044

Yang, X., Li, Y., Li, Y., Ren, X., Zhang, X., Hu, D., et al. (2017). Oxidative stress-mediated atherosclerosis: Mechanisms and therapies. *Front. physiology* 8, 600. doi:10.3389/fphys.2017.00600

Yao, Y. S., Li, T. D., and Zeng, Z. H. (2020). Mechanisms underlying direct actions of hyperlipidemia on myocardium: An updated review. *Lipids health Dis.* 19 (1), 23. doi:10.1186/s12944-019-1171-8

Yazıcı, D., and Sezer, H. (2017). Insulin resistance, obesity and lipotoxicity. *Adv. Exp. Med. Biol.* 960, 277–304. doi:10.1007/978-3-319-48382-512

Yoon, S. J., Kim, S. K., Lee, N. Y., Choi, Y. R., Kim, H. S., Gupta, H., et al. (2021). Effect of Korean red ginseng on metabolic syndrome. *J. ginseng Res.* 45 (3), 380–389. doi:10.1016/j.jgr.2020.11.002

Yu, S. E., Mwesige, B., Yi, Y. S., and Yoo, B. C. (2019). Ginsenosides: The need to move forward from bench to clinical trials. *J. ginseng Res.* 43 (3), 361–367. doi:10.1016/j.jgr.2018.09.001

Zhang, C., Han, M., Zhang, X., Tong, H., Sun, X., and Sun, G. (2022). Ginsenoside Rb1 protects against diabetic cardiomyopathy by regulating the adipocytokine pathway. *J. Inflamm. Res.* 15, 71–83. doi:10.2147/JIR.S348866

Zhang, L. C., Liu, X., Liang, W. W., and Zhong, C. (2016). Study of oxidative stress levels in patients with hyperlipidemia. *Trace Elem. Health Res.* (01), 9–10.

Zhang, L., Wang, X., Cueto, R., Effi, C., Zhang, Y., Tan, H., et al. (2019). Biochemical basis and metabolic interplay of redox regulation. *Redox Biol.* 26, 101284. doi:10.1016/j.redox.2019.101284

Zhang, M., and Lu, G. P. (2003). Dyslipidemia and metabolic syndrome. *Chin. J. Cardiovasc. Dis.* (12).

Zhang, M., Mileykovskaya, E., and Dowhan, W. (2005). Cardiolipin is essential for organization of complexes III and IV into a supercomplex in intact yeast mitochondria. *J. Biol. Chem.* 280 (33), 29403–29408.

Zhang, P. R., and Guo, G. A. (2012). Pathogenesis and classification of hyperlipidemia. *Chin. Clin.* (03), 18–20.

Zhang, Q., Jin, X. Y., and Quan, H. Y. (2019). Mechanism of ginsenoside CompoundK improving non-alcoholic fatty liver through AMPK and SREBP1 pathways. *Chin. J. Hosp. Pharm.* (08), 791–795. doi:10.13286/j.cnki.chinhosppharmacyj.2019.08.05

Zhang, Y., Marcillat, O., Giulivi, C., Ernster, L., and Davies, K. J. (1990). The oxidative inactivation of mitochondrial electron transport chain components and ATPase. *J. Biol. Chem.* 265 (27), 16330–16336.

Zhang, Z., Li, X., Lv, W., Yang, Y., Gao, H., Yang, J., et al. (2008). Ginsenoside Re reduces insulin resistance through inhibition of c-Jun NH2-terminal kinase and nuclear factor-kappaB. *Mol. Endocrinol. Baltim. Md* 22 (1), 186–195. doi:10.1210/me.2007-0119

Zhang, Z., Yang, X., Song, Y. Q., and Tu, J. (2021). Autophagy in Alzheimer's disease pathogenesis: Therapeutic potential and future perspectives. *Ageing Res. Rev.* 72, 101464. doi:10.1016/j.arr.2021.101464

Zhao, D. D., Bai, Y., Wu, R., Mo, F. F., Fang, X., Tian, T., et al. (2019). Effects of ginsenoside Rb1 on insulin resistance and AMPK signaling pathway in skeletal muscle of obese mice. *World Tradit. Chin. Med.* (04), 852–858.

Zhao, D., Zhang, M., Yuan, H., Meng, C., Zhang, B., and Wu, H. (2018). Ginsenoside Rb1 protects against spinal cord ischemia-reperfusion injury in rats by downregulating the Bax/Bcl-2 ratio and caspase-3 and p-Ask-1 levels. *Exp. Mol. pathology* 105 (3), 229–235. doi:10.1016/j.yexmp.2018.09.001

Zhou, C., Zhang, Y., Jiao, X., Wang, G., Wang, R., and Wu, Y. (2021). SIRT3 alleviates neuropathic pain by deacetylating FoxO3a in the spinal

dorsal horn of diabetic model rats. *Regional Anesth. pain Med.* 46 (1), 49–56. doi:10.1136/rapm-2020-101918

Zhou, J., Zhang, J., Li, J., Guan, Y., Shen, T., Li, F., et al. (2021). Ginsenoside F2 suppresses adipogenesis in 3T3-L1 cells and obesity in mice *via* the AMPK pathway. *J. Agric. food Chem.* 69 (32), 9299–9312. doi:10.1021/acs.jafc.1c03420

Zhou, P., Xie, W., Luo, Y., Lu, S., Dai, Z., Wang, R., et al. (2018). Inhibitory effects of ginsenoside Rb1 on early atherosclerosis in ApoE-/- mice via inhibition of apoptosis and enhancing autophagy. *Mol. (Basel, Switz.* 23 (11), 2912. doi:10.3390/molecules23112912

Zhu, J. R., Gao, R. L., Zhao, S. P., Lu, G. P., Zhao, D., and Li, J. J. (2016). Guidelines for the prevention and treatment of dyslipidemia in Chinese adults (2016 revised edition). Chin. J. Circulation (10), 937–953.

Zhu, M., Gong, X. C., Liu, D. Y., Wang, Y. W. Q., Li, L., Wang, X. L., et al. (2022). The effect of ginsenoside Rb1 on improving glucose and lipid metabolism disorders in type 2 diabetic rats. *Food Industry Sci. Technol.* (03), 367–373. doi:10.13386/j.issn1002-0306.2021050272

Zhu, S. T., and Zhao, Y. (2017). Research progress on the role of PPARα in liver-related diseases. *Life Sci.* (05), 479–484. doi:10.13376/j.cbls/2017063

Zhuang, C. L., Mao, X. Y., Liu, S., Chen, W. Z., Huang, D. D., Zhang, C. J., et al. (2014). Ginsenoside Rb1 improves postoperative fatigue syndrome by reducing skeletal muscle oxidative stress through activation of the PI3K/Akt/Nrf2 pathway in aged rats. *Eur. J. Pharmacol.* 740, 480–487. doi:10.1016/j.ejphar.2014.06.040

Zorov, D. B., Juhaszova, M., and Sollott, S. J. (2014). Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol. Rev.* 94 (3), 909–950. doi:10.1152/physrev.00026.2013

Zou, H., Zhang, M., Zhu, X., Zhu, L., Chen, S., Luo, M., et al. (2022). Ginsenoside Rb1 improves metabolic disorder in high-fat diet-induced obese mice associated with modulation of gut microbiota. *Front. Microbiol.* 13, 826487. doi:10.3389/fmicb.2022.826487

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 12 May 2023
DOI 10.3389/fphar.2023.1166178

OPEN ACCESS

EDITED BY
Cailu Lin,
Monell Chemical Senses Center,
United States

REVIEWED BY
Mengmeng Zhang,
Shaanxi University of Chinese Medicine,
China
Yunbin Jiang,
Southwest University, China

*CORRESPONDENCE
Peiyang Gao,
gaopy930@126.com
Chuantao Zhang,
zhangchuantao@cdutcm.edu.cn

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

RECEIVED 14 February 2023
ACCEPTED 21 April 2023
PUBLISHED 12 May 2023

CITATION
Luo M, Zheng Y, Tang S, Gu L, Zhu Y, Ying R, Liu Y, Ma J, Guo R, Gao P and Zhang C (2023), Radical oxygen species: an important breakthrough point for botanical drugs to regulate oxidative stress and treat the disorder of glycolipid metabolism.
Front. Pharmacol. 14:1166178.
doi: 10.3389/fphar.2023.1166178

Radical oxygen species: an important breakthrough point for botanical drugs to regulate oxidative stress and treat the disorder of glycolipid metabolism

Maocai Luo[1†], Yuhong Zheng[1†], Shiyun Tang[2†], Linsen Gu[1], Yi Zhu[1], Rongtao Ying[1], Yufei Liu[1], Jianli Ma[1], Ruixin Guo[1], Peiyang Gao[3*] and Chuantao Zhang[1*]

[1]Department of Respiratory Medicine, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]GCP Center, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China, [3]Department of Critical Care Medicine, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China

Background: The incidence of glycolipid metabolic diseases is extremely high worldwide, which greatly hinders people's life expectancy and patients' quality of life. Oxidative stress (OS) aggravates the development of diseases in glycolipid metabolism. Radical oxygen species (ROS) is a key factor in the signal transduction of OS, which can regulate cell apoptosis and contribute to inflammation. Currently, chemotherapies are the main method to treat disorders of glycolipid metabolism, but this can lead to drug resistance and damage to normal organs. Botanical drugs are an important source of new drugs. They are widely found in nature with availability, high practicality, and low cost. There is increasing evidence that herbal medicine has definite therapeutic effects on glycolipid metabolic diseases.

Objective: This study aims to provide a valuable method for the treatment of glycolipid metabolic diseases with botanical drugs from the perspective of ROS regulation by botanical drugs and to further promote the development of effective drugs for the clinical treatment of glycolipid metabolic diseases.

Methods: Using herb*, plant medicine, Chinese herbal medicine, phytochemicals, natural medicine, phytomedicine, plant extract, botanical drug, ROS, oxygen free radicals, oxygen radical, oxidizing agent, glucose and lipid metabolism, saccharometabolism, glycometabolism, lipid metabolism, blood glucose, lipoprotein, triglyceride, fatty liver, atherosclerosis, obesity, diabetes, dysglycemia, NAFLD, and DM as keywords or subject terms, relevant literature was retrieved from Web of Science and PubMed databases from 2013 to 2022 and was summarized.

Results: Botanical drugs can regulate ROS by regulating mitochondrial function, endoplasmic reticulum, phosphatidylinositol 3 kinase (PI3K)/protein kinase B (AKT), erythroid 2-related factor 2 (Nrf-2), nuclear factor κB (NF-κB), and other signaling pathways to improve OS and treat glucolipid metabolic diseases.

Conclusion: The regulation of ROS by botanical drugs is multi-mechanism and multifaceted. Both cell studies and animal experiments have demonstrated the effectiveness of botanical drugs in the treatment of glycolipid metabolic diseases

by regulating ROS. However, studies on safety need to be further improved, and more studies are needed to support the clinical application of botanical drugs.

KEYWORDS

botanical drugs, glycolipid metabolic diseases, oxidative stress, radical oxygen species, mitochondria function, nicotinamide adenine dinucleotide phosphate hydrogen oxidase, signaling pathways

1 Introduction

Glycolipid metabolic diseases are a large group of diseases characterized by disorders of glycolipid metabolism, including many diseases, such as diabetes mellitus (DM), non-alcoholic fatty liver disease (NAFLD), obesity, and atherosclerosis (AS). This kind of disease tends to be chronic, which affects the extension of human life to a certain extent and brings a heavy burden to human health cause. According to the statistics of "The International DM Federation," there will be 536.6 million DM patients worldwide in 2021 (Sun et al., 2022). Studies have found that about 1/4 of the global population suffers from NAFLD (Huang et al., 2021). In addition, the incidence of obesity, AS, hyperlipidemia, and other diseases presents a gradually increasing trend (Barquera et al., 2015; Bluher, 2019; Pirillo et al., 2021). Oxidative stress (OS) plays an important role in the pathogenesis of glycolipid metabolic diseases.

OS is the role of REDOX signal transduction widely existing in organisms (Sies, 2015), which is the imbalance between oxidizing free radicals and antioxidants. It is as broad and important as body PH regulation (Sies et al., 2017). Radical oxygen species (ROS) is a general term for a large group of oxidants extended from molecular oxygen, including superoxide (O^{2-}) and hydrogen peroxide (H_2O_2) (Sies et al., 2022). There are two main sources of ROS in the body: one is from enzymes and the other is from mitochondria. The enzymes that generate ROS include NADPH oxidase (NOX), lipoxygenase (LOX), and nitric synthase (NOS), among which the most important is NADPH oxidase (Brown and Griendling, 2015). Mitochondrial ROS, as a by-product of the ATP production process, increases during anoxia or mitochondrial dysfunction (Shadel and Horvath, 2015). As a key factor of signal transduction in OS, ROS plays a role in regulating cell proliferation, inflammation, and body aging (Schieber and Chandel, 2014) and progressing diseases related to glucose and lipid metabolism.

2 Association of ROS with common glycolipid metabolic diseases

2.1 ROS and DM

DM is a group of complex metabolic diseases, often manifested by the abnormal metabolism of carbohydrates, fats, and proteins due to the insufficient action of insulin and pancreatic islet B cells (Farmer and Fox, 2011). In the OS mode, ROS and DM are closely related. DM promotes ROS production by reducing intracellular antioxidant levels (Vezza et al., 2021), and ROS accelerates the dysregulation of glucose metabolism and tissue damage through a series of signal transduction.

In DM, ROS can be produced by NADPH oxidase, ER stress (Leenders et al., 2021), mitochondrial stress, and abnormal fatty acid metabolism (Drose and Brandt, 2012; Qiu and Zhang, 2019; Zhou L. et al., 2021; Zhou Y. et al., 2021). Under pathological stimulation, glucose glycolysis produces a large number of ROS, resulting in elevated blood sugar and forming a vicious cycle of hyperglycemia-OS (Qiu and Zhang, 2019).

The accumulation of ROS will damage islet B cells and weaken their function (Blesia et al., 2021). ROS inhibited the expression of insulin promoter factor 1 (Pdx-1) by activating the JNK pathway (Kajimoto and Kaneto, 2004), thus reducing insulin production. In addition, ROS can induce apoptosis of islet B cells by, for example, regulating intracellular C_a^{2+} concentration (Gier et al., 2009) and consuming heparan sulfate proteoglycan (Dhounchak et al., 2021). In addition, ROS can further cause mitochondrial dysfunction, resulting in reduced proliferation and differentiation of islet B cells (Nahdi et al., 2017). ROS can cause not only islet B-cell dysfunction, but also insulin resistance (IR). On the one hand, ROS can induce insufficient glucose uptake and trigger IR (Bhattacharya et al., 2017) by inhibiting GLUT-4 expression (Yaribeygi et al., 2020). On the other hand, ROS can also impair insulin sensitivity and promote the occurrence of IR by interfering with signaling pathways or downregulating the concentration of signaling molecules (Cheng et al., 2021). In addition, ROS influence on mitochondrial function can lead to IR production (Yaribeygi et al., 2020). Therefore, ROS and DM have a mutually reinforcing relationship.

2.2 ROS and NAFLD

NAFLD covers a wide spectrum of liver injury pathologic spectrum, from general steatosis and steatohepatitis to liver fibrosis and cirrhosis, and it is a typical disorder of lipid metabolism. Currently, there is a "multiple shocks" hypothesis related to the pathogenesis of NAFLD, and OS is a major factor in liver injury and this disease progression (Takaki et al., 2013; Friedman et al., 2018). Abnormal lipid metabolism further promotes ROS production, whereas ROS accumulation aggravates OS, leading to the further development of NAFLD (Mansouri et al., 2018), forming a vicious cycle.

ROS in NAFLD can be produced by mitochondria, and mitochondrial fatty acid oxidative overload leads to increased mitochondrial ROS production. However, the mitochondrial electron transport chain (ETC) complex cannot be upregulated in a coordinated manner, which leads to ROS overproduction (Begriche et al., 2013). In addition, mitochondrial flavoenzymes (including pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, glycerol phosphate, and electron transfer flavoprotein) in mitochondria are considered a major source of ROS (Chen et al.,

2020). ER stress is also an important source of ROS in NAFLD. The increase in ROS in the ER is mainly due to the excessive utilization of reduced GSH to reduce the oxidized unfolded protein response (UPR) (Zeeshan et al., 2016) and the increase in UPR-mediated ROS production by the upregulation of CHOP activity (Fuchs et al., 2017).

On the one hand, excessive production of ROS can affect mitochondrial function and lead to abnormal fatty acid oxidation. On the other hand, ROS can damage macromolecules and lead to the production of toxic substances. In addition, ROS and gut microbiota interact to promote liver inflammation. All these contribute to the development of NAFLD (Arroyave-Ospina et al., 2021).

2.3 ROS and AS

AS is a chronic multifactorial inflammatory disease of arterial blood vessels and is the main cause of cardiovascular disease morbidity and mortality. ROS accompanies every step in the development of AS, including the expression of adhesion molecules, stimulation of vascular smooth muscle proliferation and migration, endothelial cell apoptosis, lipid oxidation, and activation of matrix metalloproteinases (Park and Oh, 2011). Matrix metalloproteinases degraded the fibrous wall of AS plaques and the basement membrane of endothelial cells. This leads to the physical destruction and shedding of plaques, ultimately leading to microvascular destruction, microbleeds, and thrombosis (Kattoor et al., 2017).

The generation of ROS in AS mainly depends on various oxidases (Forstermann et al., 2017), among which NADPH oxidases are the main ROS generators in the cardiovascular system, and the deletion of its two subtypes, NOX1 and NOX2, has been confirmed to be related to the reduction of AS in mice (Kattoor et al., 2017). In macrophages, NOX activity plays a role in endothelial adhesion molecule expression, monocyte infiltration, and vascular smooth muscle cell VSMC proliferation (Vendrov et al., 2007). In addition, elevated levels of other enzymes, such AS superoxide anions, were associated with the acceleration of AS (Madamanchi and Runge, 2007). 5/12/15-lipoxygenases are correlated with the occurrence of AS (Cervantes Gracia et al., 2017), which induce NOX activation in vascular endothelial cells and lead to OS. The end product, leukotriene, also has a pro-inflammatory effect.

ROS has multiple effects on the development of AS, including oxidative modification of lipids and DNA, endothelial dysfunction, and the stability of plaque fiber caps (Batty et al., 2022). ROS accumulation increased the content of ox-LDL and the lesion area of AS (Shih et al., 2000). ROS can damage nuclear and mitochondrial DNA (mtDNA) damage, accelerate the development of AS, and increase susceptibility to AS (Yu et al., 2013; Shah et al., 2018). It can also cause endothelial cell inflammation and damage endothelial function (Li and Mehta, 2000). In addition, ROS can promote the production of interleukin to mediate the recruitment of macrophages (Libby, 2017). All these functions play an important role in the development of AS.

2.4 ROS and other glycolipid metabolic diseases

2.4.1 Obesity

Obesity, which is usually defined as being severely over recommended weight levels, is associated with excess accumulation of fat. In obesity, excessive fat accumulation and IR promote ROS production, whereas moderate caloric restriction increases antioxidant activity (Kanikowska et al., 2021). The accumulation of ROS further promotes the occurrence of obesity and its development (Martinez-Martinez and Cachofeiro, 2022).

2.4.2 Hyperlipidemia

Hyperlipidemia is characterized by increased blood lipid levels, including increased LDL and VLDL, and decreased high-density lipoprotein particles. Hypercholesterolemia leads to ROS production; decreases the antioxidant activities of GSH, SOD, and catalase enzymes leading to homocysteine autoxidation; and weakens the intracellular PI3K/AKT pathway due to the decrease in PTP enzymes, JAK/STAT, and PARP. It accelerates endothelial dysfunction (Jamwal and Sharma, 2018). However, the high level of ROS further increases the level of ox-LDL and the severity of hyperlipidemia.

In addition, hypoglycemia (Ceriello et al., 2013) and metabolic syndrome (Ando and Fujita, 2009) are also related to ROS. The overall performance is mutual promotion and mutual influence.

In conclusion, ROS is produced in glycolipid metabolic diseases and plays an important role in the occurrence, development, and deterioration of such diseases (Figure 1). In order to improve the development process of glucose and lipid metabolism diseases, special treatment for ROS in OS is still an emerging direction.

Some previous drugs for the treatment of glycolipid metabolic diseases by inhibiting OS are metformin (Wang et al., 2017; Apostolova et al., 2020), sodium-glucose co-transporter-2 inhibitors (Trnovska et al., 2021), and statins for the treatment of hypercholesterolemia (Nagila et al., 2009; Carnevale et al., 2010). However, ROS as an entry point for the treatment of glycolipid metabolic diseases is still a new direction. In recent years, nano-targeted drugs targeting ROS have been developed to antagonize or eliminate ROS (Yoshitomi and Nagasaki, 2014; Saifi et al., 2021). However, nanomedicine has not been fully transformed into clinical medication due to the differences in production quality, insufficient large-scale production plans, and the need to evaluate drug safety (Zhang Z. et al., 2022). These reasons make us find safer drugs with excellent anti-OS effects, and botanical drugs give us a new choice.

To determine how botanical drugs modulate ROS in diseases of glycolipid metabolic, herb, plant medicine, Chinese herbal medicine, phytochemicals, natural medicine, phytomedicine, plant extract, botanical drug, ROS, oxygen free radicals, oxygen radical, oxidizing agent, glucose and lipid metabolism, saccharometabolism, glycometabolism, lipid metabolism, blood glucose, lipoprotein, and triglyceride, fatty "liver", "atherosclerosis", "obesity OR diabetes", "dysglycemia", "NAFLD", and "DM" were used as keywords or subject headings to search for relevant articles in the Web of Science and PubMed databases from 2013 to 2022. A total of 680 articles were retrieved, of which 126 review articles were excluded and 554 articles were selected. Subsequently, we screened the articles and excluded those with the following conditions: 1) non-medical articles; 2) articles belonging to the scope of medicine but not to the diseases of glucose

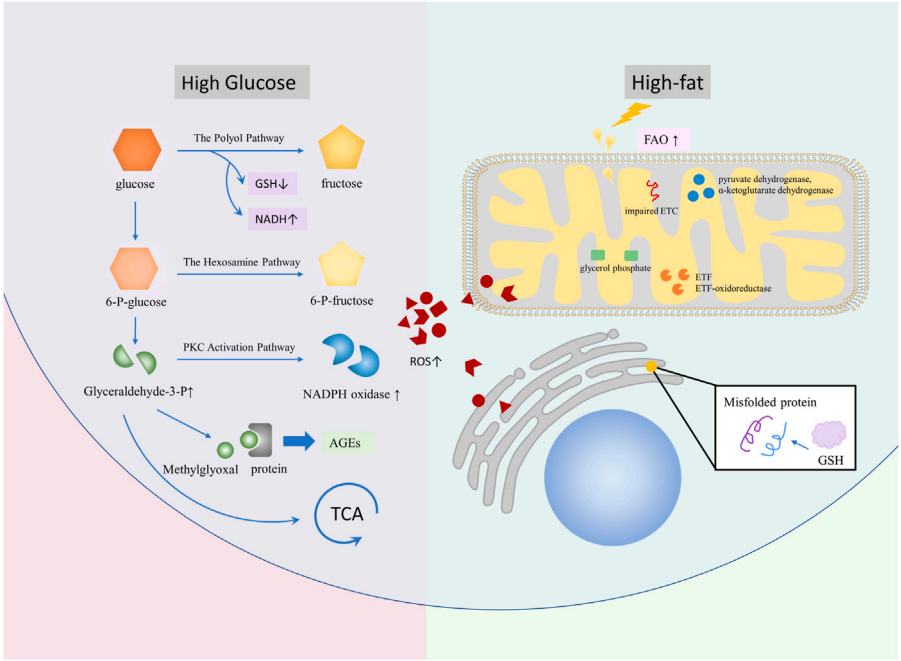


FIGURE 1
Diseases, such as DM, NAFLD, and AS, lead to glycolipid metabolism disorder and promote ROS production. In a high-sugar environment, ROS can generate more ROS via branching off pathways, such as the polyol pathway, the hexosamine pathway, the PKC activation pathway, and AGE, on the basis of glycolysis. In the high-fat environment, ROS is mainly caused by excessive oxidative stress of mitochondria and ER caused by increased FAO. A large number of ROS are involved in glycolipid metabolism, which causes complications.

and lipid metabolism; 3) articles without animal or cell experiments; 4) articles with serious missing experimental data; 5) articles referring only to extracts but not to plant sources; and 6) articles that study combinations composed of various botanical drugs (e.g., decoctions). Finally, 89 articles were included, involving 81 kinds of botanical drugs, which summarized the species and genera of plants and the extraction methods involved in plant extraction. Unfortunately, due to the incomplete information provided by the author, five species of plants have not been found (Table 1) (the screening process is shown in Figure 2).

(Botanical drugs through the following URL and the relationship between species: https://www.plantplus.cn/cn/sp/Homalium%20zeylanicum; https://www.catalogueoflife.org/; http://mpns.kew.org/mpns-portal/; http://www.plantsoftheworldonline.org).

In the following, we will explain the regulatory effects of botanical drugs on ROS regulation from the direct generation and scavenging of ROS, the regulation of related pathways, and the tolerance to reduce the adverse consequences caused by excessive ROS.

3 Multi-mechanism regulation of ROS by botanical drugs

3.1 Direct modulation of ROS production and scavenging by botanical drugs

Mitochondria, endoplasmic reticulum, and related oxidases are important sources of ROS. Botanical drugs can directly regulate the production of ROS by regulating mitochondria, NADPH oxidase, and endoplasmic reticulum stress. Botanical drugs can also play a role by regulating the activity and expression of anti-OS enzymes (Table 2) (Figure 3).

3.1.1 Botanical drugs reduce mitochondrial ROS production

Mitochondria are an important source of ROS in mammals. Molecular oxygen undergoes single-electron reduction through the ETC to form ROS, which is then converted to H_2O_2 by SOD in the mitochondrial matrix (Murphy, 2009). This effect is enhanced when cells are under hypoxia, and the ROS produced can cause damage to mitochondrial proteins and DNA (Hamanaka and Chandel, 2010; Juan et al., 2021). Botanical drugs can improve mitochondrial dysfunction and reduce ROS production by maintaining mitochondrial membrane potential (MMP), promoting the expression of mitochondrial respiratory chain complex, and reducing mitochondrial autophagy *in vitro* and *in vivo*.

3.1.1.1 Mitochondrial membrane potential

MMP abnormalities lead to ROS production. Previous studies have shown that maintaining a physiological MMP below 140 mv can reduce ROS production, whereas membrane potential hyperpolarization leads to increased ROS production in mitochondrial respiratory chain complexes I and III (Kaim and Dimroth, 1999; Starkov and Fiskum, 2003; Liu, 2010). Experiments have shown that botanical drugs can restore MMP and reduce ROS production. For example, plants *Salvia plebeia* and *Pueraria lobata* (Willd.) Ohwi effectively reversed MMP

TABLE 1 Botanical drugs information table.

Plant source	Family	Used part	Extract	Extraction method	Qualitative phytochemical analysis	Ref.
Alisma plantago-aquatica subsp. *orientale*	Alismataceae	Rhizome	Alisol A 24-acetate		Triterpenoids	Wu et al. (2018)
Alnus firma	Betulaceae	Leaves	Ethanolic extract	Ethanol extraction	Phenolic, flavonoids, proanthocyanidin	Choi et al. (2018)
Alpinia officinarum Hance	Zingiberaceae	Rhizome	DPH5	Extract by petroleum ether and ethyl acetate	Diarylheptanoid component	Zhang et al. (2022a)
Amaranthus viridis	Amaranthaceae	Leaves		Moringa methanol extracts	Kaempferol, quercetin, catechin, gallic acid, caffeic acid, p-coumaric acid, vanillin, ferulic acid, protocatechuic acid, cinnamic acid, and epicatechin	Omodanisi et al. (2017)
Angelica gigas Nakai	Umbelliferae	Root	Ligustilide		Phthalide derivative	Choi et al. (2018)
Annickia polycarpa	Annickia	Stem bark		Water extraction	Saponins, reducing sugars, phenolic compounds, alkaloids, flavonoids	Lartey et al. (2021)
Anoectochilus roxburghii	Orchidaceae		ARP		Six monosaccharides	Liu et al. (2017)
Anoectochilus roxburghii Wall. Lindl.	Orchidaceae		Kinsenoside			Liu et al. (2016)
Antrodia Cinnamomea	Polyporales			Concentration		Yen et al. (2020)
Artemisia capillaris	Asteraceae		Capillin		Polyacetylene	Li et al. (2021a)
Artemisia caruifolia	Asteraceae	Leaves		Water extraction	Polyphenols, flavonoids, condensed tannins	Sekiou et al. (2021)
Astragalus mongholicus	Fabaceae		Astragaloside IV			Zhu et al. (2019)
Calotropis procera	Apocynaceae	Latex	Laticifer proteins	Centrifugal method	Abundant proteins	De Oliveira et al. (2021)
Cannabis sativa	Cannabaceae		Cannabidiol			Jiang et al. (2021)
Cassia auriculata Linn	Leguminosae	Flower		Ethanolic extract of *C. auriculata* flowers		Vijayaraj et al. (2013)
Catharanthus roseus	Apocynaceae		Vindoline		Indole alkaloid	Goboza et al. (2019)
Cistanche tubulosa	Orobanchaceae		Echinacoside			Kong et al. (2018)
Cnidium monnieri	Apiaceae	Rhizome	Ligustilide		Phthalide derivative	Choi et al. (2018)
Crassocephalum crepidioides	Asteraceae	Aerial parts		Methanol extraction	Phenolic and flavonoids	Bahar et al. (2017)
Crataegus aronia	Rosaceae	Aerial parts		Water extraction		Mani et al. (2022)
Curcuma	Zingiberaceae	rhizome	Curcumin		Polyphenolic compound	Cao et al. (2022)
Dendrobium huoshanense	Orchidaceae	Stems	DHP	Water extraction	Polysaccharide	Fan et al. (2020)
Dendrobium officinale	Orchidaceae					Han et al. (2021)
Dillenia indica	Dilleniaceae	Leaves	DI-HET	Extract by n-hexane, ethyl acetate, hydroethanolic		Poornima et al. (2022)

(Continued on following page)

TABLE 1 (*Continued*) Botanical drugs information table.

Plant source	Family	Used part	Extract	Extraction method	Qualitative phytochemical analysis	Ref.
Echinodorus grandiflorus	Alismataceae	Leaves				Gasparotto et al. (2019)
Enicostemma littorale Blume	Gentianaceae	Whole plant	Betulin, swertiamarin, enicoflavine, swertisin	EL MeOH ext	Triterpenoid sapogenin, secoiridoid glycoside, flavonoid, and gentiocrucine	Srivastava et al. (2016)
Epimedium	Berberidaceae		Icariside II		Flavonoid	Zhang et al. (2020)
Eugenia jambolana	Myrtaceae	Seeds		Extract by petroleum ether, aqueous acetone, EtOAc, and n-BuOH	Hyperglycemia	Liu et al. (2018)
Galega officinalis	Fabaceae	Aboveground part		Ethanol extraction	Non-alkaloid fraction	Hachkova et al. (2021)
Ganoderma lucidum	Ganodermataceae	Fruiting bodies	FYGL	Extract by ethanol and acetic acid	Proteoglycan	Liang et al. (2020)
Ginkgo biloba	Ginkgoaceae		Bilobalide			Su et al. (2022)
Ginkgo biloba	Ginkgoaceae		*Ginkgo biloba* extract			Chang et al. (2021)
Gynura bicolor	Ginkgoaceae	Leaves	GBEE	Ethanol extraction		Hsieh et al. (2020)
Herba Epimedii	Berberidaceae		Icariside II			Li et al. (2022)
Herba Erigerontis	Aristolochiaceae		Scutellarin			Fu et al. (2019)
Hibiscus sabdariffa	Malvaceae					Herranz-Lopez et al. (2020)
Homalium zeylanicum		Calyx	Quercetin		Polyphenol	Herranz-Lopez et al. (2020)
Hydrangea paniculata	Hydrangeaceae					Sen et al., 2019
Hypoxis hemerocallidea	Amaryllidaceae	Corm		Hypoxis hemerocallidea aqueous extract		Oguntibeju et al. (2016)
Ilex chinensis Sims	Aquifoliaceae		Coumarin glycosides			Deng et al. (2021)
Inonotus obliquus	Hymenochaetaceae	Sclerotium	Phelligridin D	Extract by petroleum ether and ethyl acetate	Phenolic compound	Li et al. (2021b)
Juglans regia	Juglandaceae	Husk		Ethanol extraction		Fang et al. (2022)
Lannea coromandelica	Anacardiaceae	Bark	LCBE		Polyphenolic compounds	Alam et al. (2017)
Laurus nobilis Linn		Leaves		Ethanol extraction	Methyl eugenol, kaempferol rutinoside/isomer, gallic acid	Bourebaba et al. (2021)
Laurus nobilis Linn.	Lauraceae		*Laurus nobilis*	*Laurus nobilis* ethanolic extract		Bourebaba et al. (2021)
Ligusticum chuanxiong Hort.	Umbelliferae	Rhizome	*Ligusticum chuanxiong* 19	LC ethanolic extract	17b-Estradiol, phenylephrine hydrochloride, acetylcholine hydrochloride, sodium nitroprusside, pentobarbital sodium, ferulic acid, and tetramethylpyrazine	Li et al. (2014)
Lindera obtusiloba	Lauraceae	Branch	LOE	Ethanol extraction	Hyperin, isoquercitrin, guaijaverin, avicularin, and quercitrin	Ihm et al. (2021)
Lithospermum erythrorhizon Sieb. et Zucc	Boraginaceae		Shikonin			Huang et al. (2015)

(Continued on following page)

TABLE 1 (*Continued*) Botanical drugs information table.

Plant source	Family	Used part	Extract	Extraction method	Qualitative phytochemical analysis	Ref.
Momordica charantia	Cucurbitaceae	Leaves		Water extraction	Polyphenol and flavonoids	Hsu et al. (2021)
Morinda citrifolia	Rubiaceae	Pulp, seeds		Extract by n-butanol		Ishibashi et al. (2017)
Moringa oleifera	Moringaceae	Leaves	Ascorbic acid, rutin, quercetin, and catechin	Concentrated methanol extract from crape myrtle leaves	Flavonoids, phenols, saponins, tannins, alkaloids, terpenoids, and steroid	Salvamani et al. (2016)
Nepeta angustifolia C. Y. Wu	Lamiaceae	Areal parts		Ethanol extraction	Oleanolic acid, betulinic acid, and ursolic acid	Huang et al. (2020)
Ophiocordyceps sinensis	Ophiocordycipitaceae		Cordycepin			Ku et al. (2021)
Padina pavonia				Extract by dichloromethane	Terpenoids	Germoush et al. (2020)
Paeonia lactiflora Pall.	Paeoniaceae		Paeoniflorin			Yang et al. (2016)
Paeonia suffruticosa Andr.	Paeoniaceae	Root	Moutan	Moutan ethanolic extract		Zhang et al. (2014)
Panax ginseng C. A. Meyer	Araliaceae		Panax notoginseng saponins	PNS	Ginsenoside Rb1	Fan et al. (2016)
Parkia biglobosa	Fabaceae	Seeds		N-Hexane, butanol extraction	Protein	Ogunyinka et al. (2019)
Picrorhiza kurroa	Plantaginaceae		Apocynin			Gimenes et al. (2018)
Premna herbacea	Lamiaceae	Leaves	Isoverbascoside	Methanol extraction		Kashyap et al. (2021)
Prosopis Strombulifera	Fabaceae					Quesada et al. (2021)
Prunella vulgaris Linn.	Labiatae		*P. vulgaris*	*P. vulgaris* ethanol extract		Park et al. (2013)
Pueraria lobata Willd. Ohwi	Leguminosae	Root	Puerarin, daidzin, and daidzein	An ethanol extract from kudzu		Gao et al. (2016)
Romina strawberry		Pulp		Methanol extraction	Polyphenol and flavonoids	Forbes-Hernández et al. (2017)
Rubia cordifolia, *Rubia tinctorum* L.	Rubiaceae	Root	Purpurin		Anthraquinones	Nam et al. (2019)
Rubus amabilis	Rosaceae	Stems		Acetone extraction	Procyanidins	Sun et al. (2020)
Rubus coreanus Miq.	Rosaceae		Rubus coreanus	URFE		Kim et al. (2013)
Rumex dentatus L.	Polygonaceae	Acrial part	RDE	Extract by methylene chloride, ethyl acetate, and n-butanol	Phenolic compounds	Elsayed et al. (2020)
Salvia miltiorrhiza	Lamiaceae			Water extraction	Dihydrotanshinone I, cryptotanshinone, tanshinone I, dihydrotanshinone I, and tanshinonellA	Qin et al. (2016)
Salvia miltiorrhiza Bunge	Lamiaceae		Danshenol A		Abietane-type diterpenoid	Zhao et al. (2017a)
Salvia plebeia	Lamiaceae		Hispidulin	DMSO solution extraction	Flavone	Qin et al. (2016)

(Continued on following page)

TABLE 1 (*Continued*) Botanical drugs information table.

Plant source	Family	Used part	Extract	Extraction method	Qualitative phytochemical analysis	Ref.
Scutellaria baicalensis	Lamiaceae	Root	Baicalin		Flavonoids	Chen et al. (2019)
Scutellaria baicalensis Georgi	Labiatae		Baicalin, wogonin		Baicalin, baicalein, and wogonin	Ku and Bae (2015)
Silybum marianum Linn. Gaertn	Compositae		Silymarin	Diluted with DMSO	DMSO, propylene glycol, and normal saline	Khazim et al. (2013)
Smallanthus sonchifolius	Asteraceae	Leaves		Acetone extraction	Non-alkaloid fraction	Hachkova et al. (2021)
Sophora flavescens	Fabaceae		Oxymatrine		Quinolizidine alkaloid	Jin et al. (2021a)
Syzygium aqueum	Myrtaceae	Leaves		Methanol extraction	Glucosides, flavonols myricetin, quercetin, and proanthocyanidins	Mahmoud et al. (2021a)
Syzygium jambos	Myrtaceae	Bark		Water extraction	Flavonoids, tannins, chalcones, phloroglucinol, and triterpenoids	Mahmoud et al. (2021b)
Tessaria Absinthioides	Asteraceae			Water extraction		Quesada et al. (2021)
Tinospora sinensis	Menispermaceae			Water extraction	Organic acids, phenolic acids, procyanidins, flavonoids, and oxylipins	Banerjee et al. (2020)
Toxicodendron vernicifluum	Anacardiaceae		IBF-R	Water extraction	Fisetin	Hoang et al. (2021)
Tribulus terrester Linn.	Zygophyllaceae		*Tribulus terrestris*	Aqueous extracts of *Tribulus terrestris*		Jiang et al. (2016)
Trigonella foenum-graecum	Fabaceae	Seeds	Orientin, isoorientin, vitexin, and isovitexin		Flavonoid glycosides	Luan et al. (2018)
Trigonella foenum-graecum	Fabaceae	Seeds	Polyphenol stilbenes		Polyphenol	Li et al. (2018)
Tripterygium wilfordii Hook. F	Celastraceae		Celastrol	Diluted with DMSO		Jiang et al. (2016)
Zingiber officinale	Zingiberaceae			Steam extraction, ethanol extraction	6-Gingerol	Lee et al. (2021)
	Ginkgoaceae			*Ginkgo biloba* extract		Tsai et al. (2013)

NOTE: ARP: *Anoectochilus roxburghii* polysaccharide, DPH5: 1,7-diphenyl-4E-en-3-heptanone, DHP: *Dendrobium huoshanense* C. Z. Tang et S. J. Cheng polysaccharide, DI-HET: *D. indica* hydroethanolic extract, FYGL: Fudan-Yueyang *G. lucidum*, Fisetin: a flavanol compound majorly found in IBF-R, GBEE: *G. bicolor* ether extract, IBF-R: lyophilized to obtain dried *R. verniciflua* extract, LCBE: *Lannea coromandelica* (Houtt.) Merr. Bark extract, LOE: *Lindera obtusiloba* extract, RDE: *R. dentatus* extract, Six monosaccharides: L-rhamnose, L-arabinose, D-xylose, D-mannose, D-glucose, and D-galactose.

loss, reduced ROS production, and protected vascular endothelial cells (Gao et al., 2016; Qin et al., 2016). *In vitro* studies have shown that *Laurus nobilis* Linn. not only maintains the MMP but also increases insulin sensitivity (Bourebaba et al., 2021). *Tinospora sinensis* maintains MMP while reducing islet B-cell apoptosis (Banerjee et al., 2020).

3.1.1.2 Mitochondrial ETC complex

Mitochondrial ETC complexes exist in the mitochondrial and membrane space (Muller et al., 2004) and play an important role in the formation of ROS. For mitochondrial complex Ⅰ in the mitochondrial matrix, when the NADH/NAD ratio increases, the FMN binding site on complex Ⅰ decreases and superoxide is formed (Murphy, 2009). Mitochondrial complex Ⅲ is located in the inner mitochondrial membrane, which produces superoxide through the Q cycle (Hamanaka and Chandel, 2010). *In vitro* and *in vivo* studies have shown that botanical drugs can improve ROS production by regulating the expression of mitochondrial respiratory chain complexes. For example, latex protein from *Calotropis procera* increased the expression of mitochondrial complexes I, III, and V; improved glucose tolerance; and inhibited hepatic glucose production in mice. This makes it a potential agent for the treatment of type 2 DM (De Oliveira et al., 2021).

3.1.1.3 Mitochondrial DNA

MtDNA damage is closely related to mutation and ROS production (Passos et al., 2007). *In vitro* and *in vivo* studies have

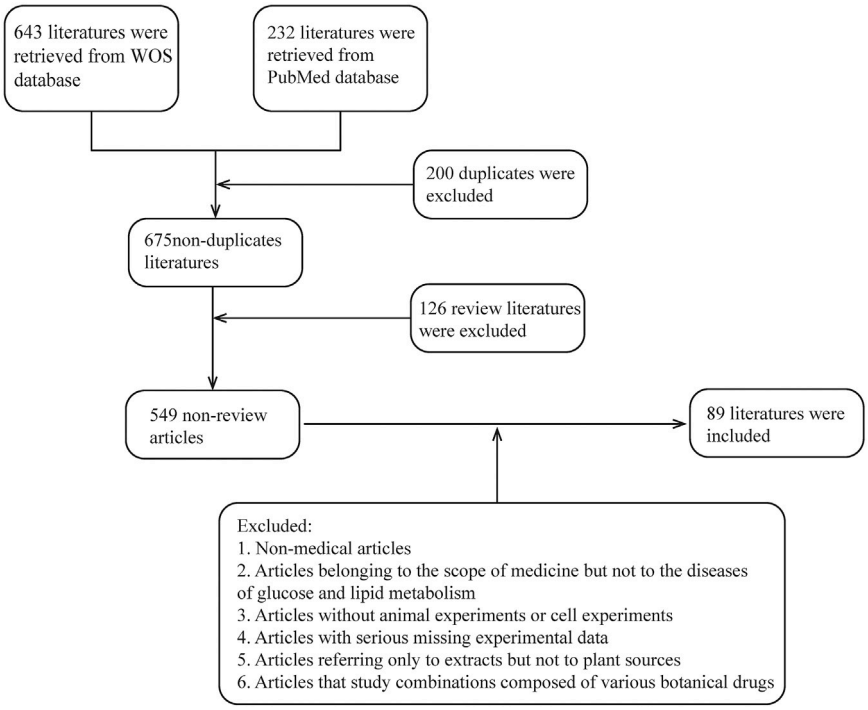


FIGURE 2
Literature screening process; review literature is automatically identified by the database.

found that *Trigonella foenum-graecum* seeds can protect the mtDNA of 3T3-L1 adipocytes from the damage caused by ROS induced by high glucose *in vitro* (Luan et al., 2018). *Zingiber officinale Roscoe*'s steamed ginger extract can enhance the mtDNA content and increase thermogenesis to reduce the body weight of HFD-fed mice, which has a certain effect on improving obesity (Lee et al., 2021).

3.1.1.4 Mitochondrial autophagy

Excessive accumulation of ROS leads to mitochondrial autophagy (Schofield and Schafer, 2021). Studies have shown that botanical drugs can maintain mitochondrial function by reducing mitophagy. For example, *Epimedium* and *Cordyceps sinensis* reduce mitophagy *in vivo* (Zhang et al., 2020; Ku et al., 2021), and *Hibiscus hibiscus* increased mitochondrial mass and content of various cell types, activated AMPK pathway, reduced mitochondrial autophagy and alleviated OS induced by glucose lipid toxicity (Herranz-Lopez et al., 2020).

In addition, mitochondrial phosphorylation is an important mechanism for mitochondrial energy production, and electron transport and ATP formation in mitochondrial ETC are related to mitochondrial phosphorylation (Foo et al., 2022). Some botanical drugs have the effect of regulating mitochondrial phosphorylation. As mentioned earlier, the plant *L. nobilis* Linn. plays a role in regulating mitochondrial phosphorylation and MMP (Bourebaba et al., 2021). However, there is a close relationship between the regulation of mitochondrial dysfunction and mitochondrial phosphorylation in plants. However, few studies have focused on the effect of botanical drugs on mitochondrial phosphorylation separately.

3.1.2 Botanical drugs downregulated NADPH expression to reduce ROS production

NADPH oxidase is another major source of ROS. NOX enzymes are membrane-associated proteins that transfer electrons across biofilms, allowing molecular oxygen to accept electrons to form ROS (Bedard and Krause, 2007). The effect of botanical drugs on NOX enzymes is more common in AS. As demonstrated *in vitro*, treatment of HUVECs with *Astragalus mongholicus* can reduce the activity of the NOX enzyme and the level of ROS under oxLDL conditions (Zhu et al., 2019). *In vivo* studies have found that *Lindera obtusiloba* extract can reduce the expression of NOX oxidase subunits p22phox and p47phox and inhibit NOX activity (Ihm et al., 2021). *Prosopis strombulifera*, *Tessaria absinthioides* (Quesada et al., 2021), and *Salvia miltiorrhiza* significantly inhibited the expression of NOX2 and NOX4 (Zhao W. W. et al., 2017; Zhao et al., 2019), while reducing the activation of NF-κB and the expression of vascular adhesion factors and protecting vascular endothelial cells to play an anti-AS role. Regarding DN, extracts from *Silybum marianum* downregulated the activity of the NOX enzyme and reduced ROS production, which played a protective role in podocytes (Khazim et al., 2013).

3.1.3 Botanical drugs alleviate ER stress to reduce ROS production

The accumulation of unfolded proteins in the ER has been termed ER stress (Gardner and Walter, 2011). On the one hand, ER stress can produce ROS through NOX4 and microsomal monooxygenase systems (Zeeshan et al., 2016). On the other

TABLE 2 Research on ROS production directly regulated by botanical drugs.

Disease	Source	Animals/cell lines	Dose	Duration	Detail	Ref.
AS	*Salvia plebeia*	HUVECs	0.1, 1, 10 μM	24 h	Maintain the MMP	Qin et al. (2016)
AS	*Astragalus mongholicus*	HUVECs	10, 20, 50 μM	1 h	Inhibit NADPH oxidase activity	Zhu et al. (2019)
AS	*Lindera obtusiloba*	ApoE$^{-/-}$ mice	100 mg/kg	20 weeks	Inhibit NADPH oxidase activity	Ihm et al. (2021)
AS	*Prosopis Strombulifera Tessaria Absinthioides*	VSMCs	2.5–40 μg/ml	24–48 h	Inhibit NADPH oxidase activity	Quesada et al. (2021)
AS	*Salvia miltiorrhiza* Bunge	HUVECs	10 nM	1 h	Inhibit TNF-α-induced NOX4 expression Inhibit TNF-α-induced NF-κB activation Inhibit ICAM-1 expression	Zhao et al. (2017a)
AS	*Pueraria lobata* Willd. Ohwi	HUVECs	1, 5, 10, 25 g/ml	48 h	Maintain the MMP	Gao et al. (2016)
AS	*Tripterygium wilfordii* Hook. F.	Macrophages	25–200 nmol/L	24 h	Scavenger receptor LOX-1	Gu et al. (2013)
		C57BL/6J mice	1, 2 mg/kg		Suppression of NF-κB pathway	
DM	*Laurus nobilis* Linn.	HepG2 cells	1 μg/ml	24 h	Regulate mitochondrial phosphorylation	Bourebaba et al. (2021)
					Maintain the MMP	
					Increase cell sensitivity to insulin	
DM	*Tinospora sinensis*	Wister rats	100–400 mg/kg	4 weeks	Maintain the MMP	Banerjee et al. (2020)
					Reduce apoptosis	
DM	*Calotropis procera*	Swiss mice	5 mg/kg		Improve IR	De Oliveira et al. (2021)
		HepG2	100 μg/ml	3 h	Increase ETC complex proteins	
DM	*Trigonella foenum-graecum*	3T3-L1 preadipocytes	0–100 μM	48 h	Enhance mitochondrial function	Luan et al. (2018)
					Protect mtDNA	
					Activate the AKT/AMPK pathway	
DM	*Epimedium*	Wistar rats	10 mg/kg	12 weeks	Reduce mitochondrial autophagy	Zhang et al. (2020)
DM	*Ophiocordyceps sinensis*	HUVECs	200 mM	24 h	Reduce mitophagy	Ku et al. (2021)
DM	*Salvia miltiorrhiza*	SD rats	50, 200 mg/kg	7 weeks	Inhibit the NOX4 expression	Zhao et al. (2019)
DM	*Curcuma*	MIN6 cells	20 μM	1 h	Inhibit ER stress	Cao et al. (2022)
					Reduce apoptosis	
DM	*Silybum marianum* Linn. Gaertn	Mouse podocytes	10 μM	24 h	Inhibit NADPH oxidase activity	Khazim et al. (2013)
		OVE26 mouse	100 mg/kg	6 weeks	Inhibit the NOX4 expression	
Obesity	*Zingiber officinale*	3T3-L1 adipocytes			Protect mtDNA	Lee et al. (2021)
		C57BL mice	40, 80 mg/kg	8 weeks	Activate the AMPK/SIRT pathway	
					Enhance mitochondrial function	
					Inhibit ER stress	
Obesity	*Hibiscus sabdariffa*	3T3-L1 adipocytes		48 h	Activate the AMPK pathway	Herranz-Lopez et al. (2020)
					Reduce mitochondrial autophagy	
Obesity	*Toxicodendron vernicifluum*	ob/ob mice, ob/+ mice	20, 40, 80 mg/kg	8 weeks	Activate the AMPK signaling pathway	Hoang et al. (2021)
					Inhibit ER stress	
NAFLD	*Momordica charantia*	HepG2 cells	5 μg/ml	24 h	Inhibit ER stress	Hsu et al. (2021)

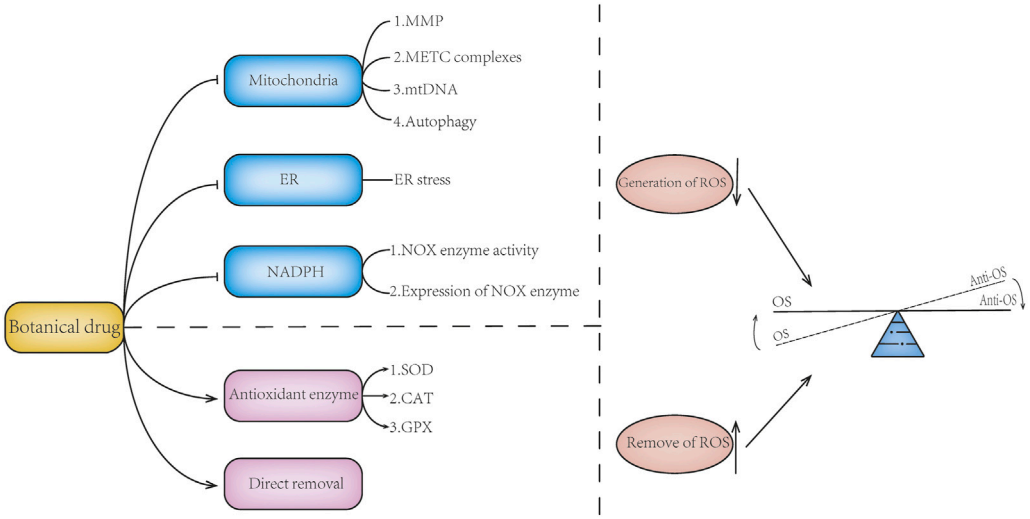


FIGURE 3
Botanical drugs directly modulate ROS generation or scavenging. Botanical drugs can regulate the balance of oxidative stress by reducing the generation of ROS and promoting the removal of ROS. Botanical drugs can reduce ROS production by regulating mitochondrial function, maintaining mitochondrial membrane potential, regulating the expression of mitochondrial ETC complex, protecting mitochondrial DNA, regulating mitophagy, improving ER stress, and reducing NOX enzymes. There are also botanical drugs that can reduce the accumulation of ROS and regulate the balance of oxidative stress by regulating the expression and activity of antioxidant enzymes and scavenging ROS. At the same time, some of the mechanisms of botanicals are not reflected in the aforementioned; for example, botanical drugs can inhibit the expression of LOX enzyme and reduce ROS production.

hand, ER stress can aggravate mitochondrial dysfunction and produce ROS by regulating Ca ions (Bhandary et al., 2012).

In vitro and *in vivo* experiments showed that *Z. officinale* and *Toxicodendron vernicifluum* could reduce ROS production through mTOR/SREBP1/ER stress and then improve fat metabolism to reduce body weight in high-fat diet mice (Hoang et al., 2021; Lee et al., 2021). *Curcuma* can alleviate ER stress and reduce MIN6 cell apoptosis by interfering with the PERK/CHOP pathway (Cao et al., 2022). In addition, *Momordica charantia* also has anti-ER stress and reduces ROS generation (Hsu et al., 2021).

In addition to the aforementioned mechanisms, some botanical drugs can play a role in alleviating AS by regulating the LOX enzyme, such as *S. miltiorrhiza* (Liu et al., 2015) and *Tripterygium wilfordii* Hook F. (Gu et al., 2013).

3.2 Scavenging effect of botanical drugs on ROS

Antioxidant enzymes are an important mechanism for the body to cope with the excessive accumulation of ROS. Botanical drugs can play an anti-ROS effect by activating antioxidant enzymes. At the same time, they can play an anti-ROS effect via their reaction with ROS to contribute to alleviating glycolipid metabolic diseases (Table 3).

3.2.1 Botanical drugs activate antioxidant enzymes to reduce ROS accumulation

Antioxidant enzymes are located in the middle of one of the three layers of antioxidant defense (Lei et al., 2016). These enzymes include SOD, CAT, and GPX. The SOD is a group of metal-containing enzymes that play a crucial antioxidant role in human health. ROS can be converted into H_2O_2 under the action of the SOD (Buettner, 2011), which is then decomposed into water by CAT and GPX (Kirkman and Gaetani, 2007; Bhabak and Mugesh, 2010).

Studies have shown that it is effective and feasible to intervene in OS-related diseases with antioxidant enzymes as the key point. For example, the use of oral antioxidant enzymes can enhance ROS clearance and reduce inflammatory responses (Zeng et al., 2021). Treating T2DM rats with *Homalium zeylanicum* and *Padina pavonia* can enhance the activity of antioxidant enzymes, reduce ROS production, and protect islet B cells (Germoush et al., 2020; Rout et al., 2020). *In vivo* experiments have found that *Enicostemma littorale* Blume can enhance the activity of antioxidant enzymes, reduce ROS production, and treat DM (Srivastava et al., 2016). Moreover, some botanical drugs can enhance the activity and expression of antioxidant enzymes and treat DM-related complications (Mihailovic et al., 2021). For example, *Inonotus obliquus* and *Artemisia caruifolia* can improve diabetic kidney damage (Li Y. et al., 2021; Sekiou et al., 2021); *Parkia biglobosa* seeds and *Picrorhiza kurroa* could improve heart damage in DM mice; and the activities of GPX, SOD, and CAT were higher after their intervention (Gimenes et al., 2018; Ogunyinka et al., 2019). The CAT was significantly increased in *Hypoxis hemerocallidea* diabetic rats, which showed anti-hyperglycemic and antioxidant effects (Oguntibeju et al., 2016). All these indicate that botanical drugs play a protective role against DM nephropathy (DN) or heart disease by interfering with antioxidant enzymes.

In AS, *in vitro* experiments found that the *Romina strawberry* variety (AN99.78.51), which is commonly consumed, can activate antioxidant enzymes and damage HepG2 cells (Forbes-Hernandez et al., 2017). Plants such as *Amaranthus viridis* and *Moringa oleifera* also showed excellent enhancement of antioxidant enzyme activity *in vivo* (Salvamani et al., 2016; Omodanisi et al., 2017). *In vivo* experiments found that the flower extract of *Cassia auriculata* could

TABLE 3 Research on the removal of ROS by botanical drugs.

Disease	Source	Animals/cell lines	Dose	Duration	Detail	Ref.
DM	*Homalium zeylanicum*	Wistar rats	300, 400 mg/kg	4 weeks	Enhance the activity of antioxidant enzymes	Rout et al. (2020)
DM	*Padina pavonia*	Wistar Rats	50, 100, 200 mg/kg	4 weeks	Enhance the activity of antioxidant enzymes	Germoush et al. (2020)
					Inhibit PPAR-γ pathway	
DN	*Inonotus obliquus*	HGMCs		24 h	Enhance the activity of SOD and CAT	Li et al. (2021b)
DM	*Artemisia caruifolia*	Wistar rats	400 mg/kg	30 days	Enhance the activity of antioxidant enzymes	Sekiou et al. (2021)
DM	*Parkia biglobosa*	Sprague–Dawley rats	200, 400 mg/kg	4 weeks	Enhance the activity of SOD, CAT, and GPX	Ogunyinka et al. (2019)
DM	*Picrorhiza kurroa*	Wistar rats	16 mg/kg	8 weeks	Enhance the activity of SOD, CAT, and GPX	Gimenes et al. (2018)
DM	*Annickia polycarpa*	Wistar rats	20, 100, 500 mg/kg	4 weeks	Clear DPPH free radicals	Lartey et al. (2021)
DM	*Eugenia jambolana*	RAW264.7 cells	10 μM	1 h	Clear ROS	Liu et al. (2018)
DM	*Galega officinalis Smallanthus sonchifolius*	Wistar rats	600, 1,200 mg/kg	14 days	Clear ROS	Hachkova et al. (2021)
DM	*Rumex dentatus* L.	Wistar rats	50, 100, 200 mg/kg	4 weeks	Increase cell sensitivity to insulin	Elsayed et al. (2020)
					Clear ROS	
					Reduce hyperglycemia	
					Enhance the activity of antioxidant enzymes	
DM	*Enicostemma littorale* Blume	Mice	2 mg/ml	2 h	Counteract inflammatory cytokines Oxidative stress-mediated cytotoxicity	Srivastava et al. (2016)
DM	*Hypoxis hemerocallidea*	Wistar rat	200, 800 mg/kg	6 weeks	Antihyperglycemic and antioxidant	Oguntibeju et al. (2016)
DM	*Amaranthus viridis*	Wistar rat	250 mg/kg	6 weeks	Antidiabetic and antioxidant properties	Omodanisi et al. (2017)
AS	*Strawberry*	HepG2	0–1 mg/ml	24, 48, 72 h	Enhance the activity of antioxidant enzymes	Forbes-Hernández et al. (2017)
	Alnus firma	3T3-L1 preadipocytes	25, 50, 100 μg/ml		Remove ROS produced during adipose differentiation	Choi et al. (2018)
	Lannea coromandelica	RAW264.7 cells Skin Fibroblast cells		1 h	Enhance antioxidant enzyme activity	Alam et al. (2017)
					Activate the Nrf-2 pathway	
					Enhance the expression of antioxidant enzymes	
AS	*Moringa oleifera*	Rabbits	100, 200 mg/kg	4 weeks	Increases antioxidants in plasma	Salvamani et al. (2016)
AS	*Cassia auriculata Linn*	Rats	150, 300, 450 mg/kg	14 days	Investigate antihyperlipidemic	Vijayaraj et al. (2013)
					Antioxidative effect	

improve hyperlipidemia and the activity of various antioxidant enzymes without obvious adverse reactions (Vijayaraj et al., 2013).

3.2.2 Direct scavenging effect of botanical drugs on ROS

Antioxidants can directly or indirectly inhibit cell damage caused by OS. Antioxidants scour ROS by providing hydrogen or electron antioxidants, and ROS and reactive nitrogen are thought to be a direct pathway (Dinkova-Kostova and Talalay, 2008). Polyphenol extracts from plants have obvious advantages in the removal of ROS and play a role by removing chelating metal ions of ROS (Yahfoufi et al., 2018). For example, *Alnus firma* can eliminate ROS produced during fat accumulation and adipose differentiation in 3T3-L1 cells (Choi et al., 2020), and *Rumex dentatus* L. can

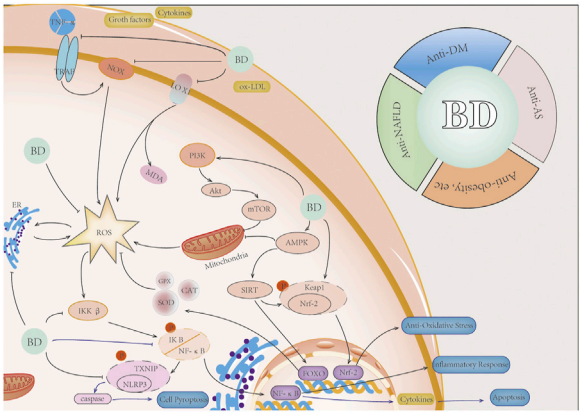


FIGURE 4
Botanical drugs regulate ROS-related pathways and corresponding anti-inflammatory effects. Botanical drugs can regulate mitochondrial function and reduce ROS production by regulating P13K/AKT/mTOR pathway. Botanical drugs can regulate TNF-α, cytokines, growth factors, and other signaling molecules and reduce the generation of ROS. Botanical drugs can regulate the AMPK pathway, improve mitochondrial energy metabolism, and reduce ROS production. Botanical drugs can regulate the Nrf-2 pathway, activate FOXO and other genes, and increase the expression of antioxidant enzyme genes. Botanical drugs can reduce ROS-induced apoptosis and caspase activation-induced pyroptosis by inhibiting the NF-κB/NLRP3 pathway. Botanical drugs eventually played a role in anti-oxidation, anti-inflammation, anti-apoptosis, and anti-pyroptosis. It has a therapeutic effect on NAFLD, DM, AS, and obesity, among others.

improve OS in DM rats by scavenging ROS (Elsayed et al., 2020). In addition, other extracts have a significant free radical scavenging ability; for example, *in vitro* experiments found that *Lannea coromandelica* bark extract (Alam et al., 2017), *Annickia polycarpa* (Lartey et al., 2021), *Eugenia jambolana*, *Galega officinalis*, and *Smallanthus sonchifolius* also had significant ROS clearance ability (Liu et al., 2018; Hachkova et al., 2021).

ROS is a key factor in the OS. Botanical drugs can regulate OS by regulating ROS generation and clearance so that ROS levels tend to be balanced. At the same time, this effect can also play a role by indirectly regulating the up–down pathways related to ROS, which will be described as follows.

3.3 Upstream and downstream regulation related to ROS by botanical drugs

Botanical drugs can regulate ROS accumulation and improve the damage caused by ROS accumulation by regulating upstream signals of ROS, such as PI3K signaling pathway and inflammatory factor (tumor necrosis factor) TNF-α, and downstream signals, such as AMPK, NF-κB, and Nrf-2 (Figure 4).

3.3.1 Botanical drugs regulate the upstream signal of ROS and reduce the accumulation of ROS

Botanical drugs can play a regulatory role in regulating ROS by regulating PI3K/AKT pathway and inflammatory factors (Table 4).

3.3.1.1 The PI3K/AKT pathway

Studies have shown that the PI3K/AKT pathway is correlated with ROS, and activated AKT can reduce the production of ROS caused by ischemia (Chatterjee et al., 2012). The mechanism is related to the regulation of mitochondrial function and NOX enzyme by the PI3K/AKT (Stiles, 2009; Nakanishi et al., 2014).

In recent studies, treatment targeting the PI3K/AKT pathway has a certain effect on glycolipid metabolic diseases, such as obesity and DM (Huang et al., 2018). Moderate pharmacological inhibition of the PI3K could be a therapeutic strategy for obesity and metabolic syndrome (Vanhaesebroeck et al., 2021). Moreover, PI3K/AKT is the main pathway of botanical drugs regulating OS-related glycolipid metabolic disorders. For example, botanical drugs can improve DN by regulating the PI3K pathway (Tang et al., 2021). *In vitro* experiments have shown that *Alpinia officinarum* Hance can improve the IR of HepG2 cells through the PI3K/AKT pathway (Zhang X. G. et al., 2022). *Trigonella foenum-graecum* seeds restored dexamethasone-induced glucose uptake in IR3T3-L1 cells by activating the AKT and AMPK (Luan et al., 2018). *Rubus amabilis* active ingredients treat MINI6*β* cells to regulate the activation of the AKT/FoxO1 pathway and play an anti-apoptotic role (Sun et al., 2020). *Epimedium* can reduce mitochondrial autophagy of cavernous smooth muscle cells through PI3K/AKT/mTOR pathway and improve glucose metabolism and ROS production in T2DM rats with erectile dysfunction (Zhang et al., 2020).

For AS, *in vivo* experiments showed that *Herba Erigerontis* could upregulate the expression of Hipo/FoxO3a and PI3K/AKT and reduce the occurrence of OS in vascular endothelial cells in high-fat diet rats (Fu et al., 2019). *In vitro*, *S. miltiorrhiza* Bunge has been found to reduce the production of intracellular reactive oxygen species through the PI3K/Akt/MEK1/Nrf-2 pathway and has therapeutic effects on vascular diseases (Lee et al., 2012). *Lithospermum erythrorhizon* and *Tribulus terrestris* can inhibit OS and protect vascular endothelial cells by activating the PI3K/AKT pathway and inhibiting the NF-κB pathway, and *L. erythrorhizon* Sieb. et Zucc. can also inhibit the NF-κB pathway activation and improve inflammation (Huang et al., 2015; Jiang et al., 2016). *Ginkgo biloba* Linn. extract can reduce ROS production by activating the AKT/eNOS and play a protective role in endothelial cells *in vitro* (Tsai et al., 2013).

Botanical drugs can reduce the excessive production of ROS; can improve IR, vascular endothelial cell injury, and islet B-cell injury by regulating the PI3K/AKT pathway; and have certain therapeutic effects on DM, AS, NAFLD, and other diseases.

3.3.1.2 TNF-α and other inflammatory factors

ROS production can be stimulated by signal molecules, such as growth factors, cytokines, and circulating exosomes (Sies and Jones, 2020). For example, TNF-α, circular RNA, and interleukin-4 induce ROS production (Lee et al., 2005; Sharma et al., 2008; Saaoud et al., 2021). Meanwhile, the crosstalk between inflammation and OS makes inflammatory factors, other information molecules, and ROS have inextricable relationships (Forrester et al., 2018). All these provide a rationale for reducing ROS production by inhibiting the expression of inflammatory factors and cytokines.

Previous studies have shown that botanical drugs have significant regulatory effects on signaling molecules, such as inflammatory factors in glucolipid metabolism diseases. *In vitro*

TABLE 4 Botanical drugs regulate upstream signal transduction pathways related to ROS.

Disease	Source	Animals/cell lines	Dose	Duration	Detail	Ref.
AS	*Herba Erigerontis*	AS rats	6.25, 25 mg/kg	12 weeks	Regulate the Hippo-FOXO3A, PI3K/AKT pathways	Fu et al. (2019)
		HAECs	50, 100, 200 μmol/L	24 h	Inhibit endothelial cell injury and apoptosis	
AS	*Salvia miltiorrhiza* Bunge	HUVECs	10 nM	1 h	Inhibit TNF-α-induced NOX4 expression	Zhao et al. (2017b)
					Inhibit TNF-α-induced NF-κB activation	
					Inhibit the ICAM-1 expression	
AS	*Gynura bicolor*	EA.hy926 cells	10, 50, 100 μg/ml	8 h	Reduce the role of TNF-α	Hsieh et al. (2020)
AS	*Lithospermum erythrorhizon* Sieb. et Zucc	EA.hy926 cells	0, 0.25, 0.5, 1 mM	16 h	Shikonin protects endothelial	Huang et al. (2015)
					Suppressing ROS/NF-κB-mediated ICAM-1	
					Upregulate PI3K/AKT/Nrf-2 pathway	
AS	*Tribulus terrester* Linn.	HUVECs	3, 30 μg/ml	1 h	Decrease mRNA expression of AKT and AMPK	Jiang et al. (2016)
					Endothelial protective effects	
AS	*Prunella vulgaris* Linn.	HASMC	0, 10, 50, 250 μg/ml	2 h	Protect HASMCs on cell viability and THP-1 cell adhesion	Park et al. (2013)
					Reduce the NF-κB activation	
DM	*Alpinia officinarum* Hance	HepG2 cells		24 h	Regulate the PI3K/AKT/Nrf-2/GSK3β pathways	Zhang et al. (2022b)
DM	*Trigonella foenum-graecum*	3T3-L1 preadipocytes	0–100 μM	48 h	Reduce the expression of adipokines	Luan et al. (2018)
					Activate the AKT/AMPK	
					Maintain the MMP	
					Protect mtDNA	
DM	*Rubus amabilis*	MIN6 β-cells	25, 50, 75 μg/ml	24 h	Activate the PI3K/AKT/FoxO1	Sun et al. (2020)
DM	*Epimedium*	Wistar rats	10 mg/kg	12 weeks	Reduce mitochondrial autophagy	Zhang et al. (2020)
					Activate the PI3K/AKT/mTOR pathway	
DM	*Catharanthus roseus*	Wistar Rats	20 mg/kg	8 weeks	Lower levels of TNF-α	Goboza et al. (2019)
DM	*Syzygium aqueum* Alston	Wistar rats	100, 200 mg/kg	14 days	Activate the Nrf-2 pathway	Mahmoud et al. (2021a)
					Reduce TLR-4 activation	
					Reduce pancreatic islet B cell damage	
DM	*Ginkgo biloba* Linn.	HAEC	100 μg/ml	18 h	Reduce endothelial adhesion	Tsai et al. (2013)

and *in vivo* experiments have shown that botanical drugs can inhibit the overexpression of TNF, vascular adhesion factor (VCAM), and cytokines; reduce ROS generation and vascular endothelial damage caused by them; and have certain therapeutic effects on AS (Gu et al., 2013; Caliceti et al., 2017; Su et al., 2018). TNF-α can activate NOX4 to induce ROS generation, and *S. miltiorrhiza* can inhibit TNF-α-induced ROS generation through hydrogen bond interaction with NOX4, the production of inflammatory factors, and the release of VCAM-1 (Zhao W. W. et al., 2017). *Gynura bicolor* can reduce the effects of TNF-α in several ways (Goboza et al., 2019), and *Catharanthus roseus* can reduce TNF-α levels to reduce ROS (Hsieh et al., 2020). *In vitro* experiments of *Prunella vulgaris* and *in vivo* experiment of *Ligusticum chuanxiong* Hort. showed that both plants suppressed OS and reduced inflammation in human vascular smooth muscle cells following TNF-α treatment (Park et al., 2013; Li et al., 2014). In addition, botanical drugs can interfere with

TABLE 5 Botanical drugs regulate the downstream signal transduction pathways related to ROS.

Disease	Source	Animals/cell lines	Dose	Duration	Detail	Ref.
Obesity	*Zingiber officinale*	C57BL/6 mice	40, 80 mg/kg	8 weeks	Activate the AMPK pathway	Lee et al. (2021)
					Enhance mitochondrial function	
					Inhibit ER stress	
Obesity	*Toxicodendron vernicifluum*	ob/ob mice, ob/+ mice	20, 40, 80 mg/kg	8 weeks	Activate the AMPK pathway	Hoang et al. (2021)
					Inhibit ER stress	
Obesity	*Rubia tinctorum Rubia cordifolia*	3T3-L1 cells	50, 100μM	48 h	Activate the AMPK pathway	Nam et al. (2019)
		C57BL/6 mice	40, 80 mg/kg	10 weeks		
Obesity	*Hibiscus sabdariffa*	3T3-L1 adipocytes		48 h	Activate the AMPK pathway	Herranz-Lopez et al. (2020)
					Reduce mitochondrial autophagy	
NAFLD	*Dillenia indica*	HepG2 cells	5, 10 μg/ml	2 h	Activate the SIRT-1/AMPK pathway	Poornima et al. (2022)
NASH	*Alisma plantago-aquatica* subsp. *orientale*	C57BL/6 mice	15, 30, 60 mg/kg	4 weeks	Regulate autophagy	Wu et al. (2018)
		WRL-68 cells, LX2 cells	1-16 μmol/L	48 h	Activate the AMPK pathway	
NAFLD	*Antrodia Cinnamomea*	HepG2 cells			Inhibit the NLRP3 inflammasome activation	Yen et al. (2020)
		RAW264.7 cells			Inhibit ER stress	
		C57BL/6 mice	100 mg/kg	10 days	Inhibit the NLRP3 inflammasome activation	
NAFLD	*Artemisia capillaris*	FL83B hepatocytes	25–200 μM	24 h	Inhibit apoptosis	Li et al. (2021a)
					Inhibit the NLRP3 inflammasome activation	
		C57BL/6J mice	25, 50, 100 μmol/kg	5 weeks	Inhibit the NLRP3 inflammasome activation	
NAFLD	*Juglans regia*	SPF-grade	300 mg/kg	12 weeks	Inhibit the NLRP3 inflammasome activation	Fang et al. (2022)
NAFLD	*Cannabis sativa*	C57B/6J mice	3 mg/kg	16 weeks	Inhibit the activation of the NF-κB pathway	Jiang et al. (2016)
					Inhibit the NLRP3 inflammasome activation	
					Inhibit pyroptosis	
NAFLD	*Ilex chinensis* Sims	Larval zebrafish	10, 20, 40 μM	13 days	Reduce inflammation	Deng et al. (2021)
		HepG2 cells	10, 15, 20 μM	24 h	Activate the Keap1/Nrf-2 pathway	
	Trigonella foenum-graecum	3T3-L1 Preadipocytes	0–100 μM	48 h	Enhance mitochondrial function	Luan et al. (2018)
					Protect mtDNA	
					Activate the AKT/AMPK pathways	
DM	*Trigonella foenum-graecum*	3T3-L1 Preadipocytes			Activate the AKT/AMPK pathways	Li et al. (2018)
DM	*Premna herbacea*	L6 muscle cells	0.5–10 μM	6 h	Activate the JNK/AKT/mTOR pathway	Kashyap et al. (2021)
		SD rats	250 mg/kg		Improve IR	
DM	*Anoectochilus roxburghii*	HUVECs	10, 20, 30 μg/ml	1 h	Inhibit the NF-κB pathway	Liu et al. (2017)

(Continued on following page)

TABLE 5 (*Continued*) Botanical drugs regulate the downstream signal transduction pathways related to ROS.

Disease	Source	Animals/cell lines	Dose	Duration	Detail	Ref.
		ICR mice	100, 300 mg/kg	15 days		
DM	*Cistanche tubulosa*	LC-540 cells	50 μL/ml	24 h	Inhibit the NF-κB pathway	Kong et al. (2018)
		Sprague–Dawley rats	160, 320 mg/kg	6 weeks	Enhance the activity of antioxidant enzymes	
DM	*Ganoderma lucidum*	INS-1 cells	0–200 μg/ml	4 h	Inhibit NF-κB pathway	Liang et al. (2020)
DN	*Inonotus obliquus*	Human glomerular mesangial cells			Activate Nrf-2 pathway	Li et al. (2021b)
DM	*Hydrangea paniculata*	Wistar rats	15, 30, 45 mg/kg	3 months	Activate the Nrf-2 pathway	Sen et al. (2019)
DM	*Ginkgo biloba*	Wistar rats	20 mg/kg	12 weeks	Activate the Nrf-2 pathway	Su et al. (2022)
DN	*Ginkgo biloba*	DBA/2 mice	50, 200 mg/kg	4 weeks	Activate the Nrf-2/HO-1 pathway	Chang et al. (2021)
DM	*Scutellaria baicalensis*	HUVECs and HAOECs	50 μM	72 h	Activate the Nrf-2 pathway	Chen et al. (2019)
		C57BL/6 mice	50 mg/kg	4 weeks	Reduce endothelial cell apoptosis	
DM	*Anoectochilus roxburghii* Wall. Lindl.	HUVECs	10–30 μg/ml	48 h	Inhibit the expression of RAGE	Liu et al. (2016)
					Decrease intracellular ROS generation	
DM	*Paeonia lactiflora* Pall.	RSC96 cells	1, 10, 100 μM	48 h	Decrease ROS and MDA levels	Yang et al. (2016)
					Increasing GST and GPX activity	
AS	*Cnidium monnieri*, *Angelica gigas Nakai*	HUVECs		30 min	Attenuate vascular inflammation	Choi et al. (2018)
					Activate the Nrf-2/HO-1 pathway	
AS	*Salvia plebeia*	HUVECs	0.1, 1, 10 μM	24 h	Inhibit the NLRP3 inflammasome activation	Qin et al. (2016)
					Inhibit the NF-κB pathway	
AS	*Sophora flavescens*	HUVECs	2, 4, 8 μM	1 h	Activate the SIRT1/Nrf-2 pathway	Jin et al. (2021b)
					Inhibit the NLRP3 inflammasome activation	
					Inhibit the pyroptosis	
AS	*Crataegus aronia*	Wistar rats	200 mg/kg	4, 8 weeks	Inhibit the NLRP3 inflammasome activation	Shatoor and Al Humayed (2020)
AS	*Ligusticum chuanxiong* Hort.	Sprague–Dawley rats	600 mg/kg	12 weeks	Improve serum lipid profiles	Li et al. (2014)
					Reduce the ROS level	
AS	*Scutellaria baicalensis* Georgi	HUVECs	2, 5, 10 μM	6 h	Prevent atherosclerotic lesions	Ku and Bae (2015)
		Mice	2, 5, 10 μM			
AS	*Rubus coreanus* Miq.	RAW264.7 cells		4 h	Improve plasma lipid profile	Kim et al. (2013)
		C57BL/6 J mice	1.67 g/kg	14 weeks	Inhibit inflammation-associated gene	
					Inhibit phase II enzyme function reduces	
AS	*Panax ginseng* C. A. Meyer	Wistar rats	100 mg/kg	10 weeks	Protected endothelial cells	Fan et al. (2016)
					Activate the Nrf-2 pathway	

the activity of TNF-α receptor TRAF to interfere with OS. For example, *Syzygium aqueum* Alston was found to reduce the damage of OS and inflammation on islet B cells by downregulating the expression level of the TRAF6, NF-κB-mediated inflammation, and the effect of TNF-α (Mahmoud et al., 2021a). In addition, berberine and *S. miltiorrhiza* extracts can attenuate TNF-α-induced

TABLE 6 Botanical drugs alleviated inflammation, apoptosis, and IR caused by ROS.

Disease	Source	Animals/cell lines	Dose	Duration	Detail	Ref.
DM	*Crassocephalum crepidioides*	INS-1	50–1,000 μg/ml	24 h	Reduce apoptosis	Bahar et al. (2017)
		Wistar rats	150, 300 mg/kg	48 h		
DM	*Tinospora sinensis*	Wister rats	100–400 mg/kg	4 weeks	Maintain the MMP	Banerjee et al. (2020)
					Reduce apoptosis	
DM	*Nepeta angustifolia* C. Y. Wu	Sprague–Dawley rats	60, 120, 240 mg/kg	56 days	Inhibit inflammation	Huang et al. (2020)
		HBZY-1 cells		12 h	Reduce apoptosis	
DM	*Herba Epimedii*	db/db mice	10, 20, 40 mg/kg	7 weeks	Reduce inflammatory cytokines	Li et al. (2022)
					Inhibit the NF-κB pathway	
DM	*Alpinia officinarum* Hance	HepG2 cells		24 h	Regulate the PI3K/AKT/Nrf-2/GSK3β pathways	Zhang et al. (2022b)
DM	*Syzygium jambos*	Wistar rats	100, 200 mg/kg	14 days	Lower levels of inflammatory cytokines	Mahmoud et al. (2021b)
DM	*Laurus nobilis* Linn.	HepG2 cells	1 μg/ml	24 h	Reduce total intracellular ROS levels	Bourebaba et al. (2021)
DM	*Paeonia suffruticosa* Andr.	HBZY-1 rat mesangial cells	1.25, 2.5, 5 g/kg	24 h	Attenuate MC on macrophages migration	Zhang et al. (2014)
AS	*Morinda citrifolia*	THP-670 cells	1 ng/ml	2 days	Inhibit inflammation	Ishibashi et al. (2017)
AS	*Echinodorus grandiflorus*	Rabbits	10, 30, 100 mg/kg	60 days	Inhibit inflammation	Gasparotto et al. (2019)
AS	*Dendrobium huoshanense*	Zebrafish	0.1, 1, 10 mg/L	10 days	Inhibit inflammation	Fan et al. (2020)
AS	*Dendrobium officinale*	Zebrafish	0.1, 1, 10 mg/L	45 days	Inhibit inflammation	Han et al. (2021)
		HUVECs	0.1, 1, 10 mg/L	24 h	Inhibit inflammation	

NOTE: INS-1: a rat insulinoma cell line.

phosphorylation of IkBα, reduce inflammation and OS, and protect vascular endothelial cells (Zhao W. W. et al., 2017; Caliceti et al., 2017).

3.3.2 Botanical drugs regulate the downstream pathways of ROS and reduce the accumulation of ROS

Botanical drugs can regulate ROS downstream pathways (e.g., the AMPK pathway, the NF-κB/NLRP3 inflammasome pathway, and the Nrf-2 pathway) to reduce cell damage caused by excessive ROS, regulate inflammation, or activate the expression of antioxidant enzymes (Table 5).

3.3.2.1 The AMPK pathway

The AMPK pathway is an important regulator of energy metabolism in the body, which can regulate substance metabolism and synthesis and induce autophagy (Zhao Y. et al., 2017). Currently, all mechanisms by which the AMPK is activated by ROS are not fully understood (Ren and Shen, 2019). However, it has been shown that exogenous or glucose oxidase-generated H_2O_2 induces direct S-glutathionylation of cysteine residues Cys299 and Cys304 on the AMPKα subunit. It has been demonstrated that ROS can directly activate the AMPK pathway (Zmijewski et al., 2010).

Botanical drugs have a certain effect on improving lipid metabolic diseases by regulating the AMPK pathway. For example, the use of *T. vernicifluum* and *Z. officinale* extracts can effectively reduce ROS levels and highly maintain the AMPK/SIRT1 signaling, and *Z. officinale* extracts could inhibit hepatic dyslipidemia and regulate lipid metabolism (Hoang et al., 2021; Lee et al., 2021). *In vitro* and *in vivo* experiments have found that purpurin derived from *Rubia tinctorum* L. and *Rubia cordifolia* can exert anti-obesity effects through the AMPK pathway (Nam et al., 2019). For NAFLD, *Dillenia indica* leaf and *Alisol A 24-acetate* treatment significantly increased the expression of the AMPK/SIRT pathway, reduced ROS production, and had a certain protective effect on hepatocytes (Wu et al., 2018; Poornima et al., 2022).

Moreover, the intervention of AMPK has significant benefits in improving DM. *Trigonella foenum-graecum* reduces the incidence of IR and restores glucose uptake and insulin sensitivity in adipocytes by activating the AKT and AMPK, which are inhibited by dexamethasone. Phosphorylation of AKT/AMPK induced by *Premna herbacea* extract activates AS106 cells and increases glucose uptake by muscle cells (Li et al., 2018; Luan et al., 2018; Kashyap et al., 2021). *In vivo*, *Aspalathus*

linearis was found to increase AMPK phosphorylation, reduce intracellular ROS production in islet B cells, and increase glucose uptake in myocytes (Kamakura et al., 2015). In addition, some botanical drugs can regulate the expression of adipogenic genes, such as PPAR and SREBP-1c, and reduce the production of inflammatory factors while regulating OS and AMPK (Herranz-Lopez et al., 2020; Lee et al., 2021).

3.3.2.2 The NF-κB pathway

NF-κB is an intracellular transcription factor that plays an important role in immunity and inflammation (Vallabhapurapu and Karin, 2009). Studies have shown that high glucose induces the production of ROS and stimulates the phosphorylation of IKKβ and NF-κB p65, but the phosphorylation of IKKβ is inhibited when ROS inhibitors are used, suggesting that ROS can promote the phosphorylation of IKKβ/NF-κB (Qin et al., 2016). Moreover, H_2O_2 can also directly regulate NF-κB (Sies and Jones, 2020).

In AS, NAFLD, and obesity, typical disorders of lipid metabolism, botanical drugs attenuate ROS and inflammation and reduce cell damage and apoptosis by regulating the NF-κB pathway. For example, *in vitro* experiments showed that pretreatment of HUVECs with *Cnidium monnieri* and *Angelica gigas Nakai* inhibited the NF-κB nuclear displacement caused by TNF-α stimulation and downregulated VACM expression and ROS generation (Choi et al., 2018). *In vitro* and *in vivo* experiments have found that the fruits of *Scutellaria baicalensis* Georgi and *Rubus coreanus* Miq. can reduce vascular inflammation by inhibiting ROS production and the NF-κB pathway and have a therapeutic effect on AS (Kim et al., 2013; Ku and Bae, 2015). *Anoectochilus roxburghii* and *Cistanche tubulosa* can protect vascular endothelial cells and reproductive function in diabetic rats by inhibiting the NF-κB pathway (Liu et al., 2017; Kong et al., 2018). For DM, *Ganoderma lucidum* can protect islet B cells by inhibiting NF-κB, JNK, and MAPK pathways (Liang et al., 2020). *In vitro* experiments have found that *Anoectochilus roxburghii* inhibits NF-κB to reduce ROS production and has a certain therapeutic effect on diabetic vasculopathy (Liu et al., 2016).

3.3.2.3 The NLRP3 inflammasome

For NLRP3 inflammasome, ROS and NF-κB activate the NLRP3 inflammasome (Tschopp and Schroder, 2010). Studies have shown that ROS can activate NLRP3 inflammasome via TXNIP, and TXNIP/NLRP3 activation is inhibited after ROS clearance (Dan Dunn et al., 2015; Mai et al., 2020). The NLRP3 inflammasome plays an important role in the development of DM, NAFLD, and AS (Hoseini et al., 2018). Excessive activation of NLRP3 will lead to the excessive release of inflammatory factors and pyrosis of cells (Jin et al., 2021a). It has been documented that AS is a key target for treating NAFLD, AS, and DM (Tang et al., 2019; Wu et al., 2021; Yu et al., 2022). Here, it has been proved that the active ingredients of botanical drugs can treat diabetes and its complications through NLRP3, and the botanical drugs and active ingredients are summarized, so we will not elaborate on this (Bai et al., 2021).

Some botanical drugs can target NLRP3 during AS and exert their effects, as found *in vivo*, *S. plebeia* can improve high glucose-mediated endothelial dysfunction by inhibiting PKCβII-related NLRP3 inflammasome activation and NF-κB signaling (Qin et al., 2016). *Sophora flavescens* can alleviate the NLRP3-mediated apoptosis in HUVECs stimulated by LDL (Jin et al., 2021b). *Crataegus aronia* inhibited the levels of NLRP3, caspase-1, and mature IL-1β in aortic tissues of high-fat diet rats and reduced the nuclear accumulation of NF-κB. Thus, it plays a role in reducing ROS (Shatoor and Al Humayed, 2020). All of the aforementioned plants can alleviate AS, which is worth exploring in depth. For NAFLD, some botanical drugs can target NLRP3 to exert anti-NAFLD effects, such as *Antrodia Cinnamomea* (Yen et al., 2020), *Artemisia capillaris* (Li B. et al., 2021), *Juglans regia* green husk (Fang et al., 2022), and extracts of *Cannabis sativa* (Jiang et al., 2021), which were found to reduce ROS, inhibit the NLRP3 *in vitro* and *in vivo*, and have a certain therapeutic effect on NAFLD.

3.3.2.4 The Nrf-2 pathway

Nrf-2 is also a nuclear factor in the cytoplasm and an important antioxidant factor in the body. It plays an important role in regulating ROS levels (Kaspar et al., 2009). The genes regulated by Nrf-2 include HO-1, GST, and NQO1, and their expressed enzymes have antioxidant effects (Liu et al., 2007). Current studies have found that targeting Nrf-2 has a clear effect on the treatment of cancer, DM and its complications, and AS (Axelsson et al., 2017; Niu et al., 2019; Sivinski et al., 2021).

In DM and its complications, botanical drugs can regulate the expression of antioxidant enzymes through Nrf-2, be antioxidant and anti-inflammatory, and reduce apoptosis. For example, *in vitro* experiments showed that the Nrf-2 expression was significantly enhanced and ROS was significantly reduced under the treatment of *I. obliquus* and *G. biloba* extract, which improved renal podocyte injury caused by DN (Li Y. et al., 2021; Chang et al., 2021). Similarly, the *G. biloba* extract reduced retinal damage in diabetic rats by activating Nrf-2 *in vivo* (Su et al., 2022). *Hydrangea paniculata* can exert beneficial effects on DN by increasing the Nrf-2 expression and inhibiting TGF-smad signaling activation (Zhang et al., 2019). *In vitro* experiments showed that the ethyl acetate fraction of *Penthorum chinense* Pursh stems could directly bind to the Keap1 protein, resulting in nuclear translocation of Nrf-2 and activation of antioxidant-related proteins (Sun et al., 2021). *Scutellaria baicalensis* root improves hyperglycemia-induced vascular endothelial injury by promoting the Nrf-2 nuclear enrichment (Chen et al., 2019). For DM peripheral neuropathy, *Paeonia lactiflora* Pall. pretreatment of RSC96 cells showed that *P. lactiflora* Pall. could inhibit the ROS production induced by high glucose and reduce the apoptosis of RSC96 cells through Nrf-2 (Yang et al., 2016).

The activation of Nrf-2/HO-1 signaling plays an important role in protecting endothelial cells (Zhang et al., 2021). Botanical drugs can treat AS by interfering with Nrf-2, which can protect vascular endothelial cells, anti-inflammation, and anti-oxidation. For example, when *Panax ginseng* and Ginsenoside Rb1 were used to treat AS rats, serum NO and SOD levels were upregulated, and Nrf-2 nuclear translocation and HO-1 activation were observed. Moreover, Ginsenoside Rb1 inhibited oxLDL-induced p38 and VCAM-1 expression and reduced the adhesion of monocytes to vascular endothelial cells (Fan et al., 2016).

In addition, the intervention of botanical drugs on NAFLD can play an anti-inflammatory and anti-oxidative effect by regulating the expression of Nrf-2 and protecting liver cells. As found in *in vivo* and *in vitro* experiments, intervention with *Ilex Chinensis* can increase the expression of the Nrf-2 and Keap1 genes, reduce ROS production, and have a certain improvement effect on NAFLD of zebrafish larvae (Deng et al., 2021).

3.4 Botanical drugs alleviated inflammation, apoptosis, and IR caused by ROS

Abnormal levels of ROS often cause problems other than OS, such as cell apoptosis, IR, and tissue inflammation. These pathological conditions interact with OS and aggravate the development of the disease. Botanical drugs can improve these problems caused by ROS abnormalities. Later, we will explain the effects of related drugs from the perspective of regulating ROS and anti-apoptosis and improving IR and anti-inflammation (Table 6).

3.4.1 Botanical drugs regulate ROS-associated apoptosis and pyroptosis

3.4.1.1 Apoptosis

Excessive accumulation of ROS can lead to impaired mitochondrial function, lipid peroxidation, decreased ATP level, and, finally, cell necrosis (Orrenius et al., 2007). Moreover, ROS can induce apoptosis by mediating the oxidation of cardiolipin and promoting the release of cytochrome C from mitochondria. This demonstrates the bridge-like role of ROS between apoptosis, necrosis, and OS (Kaminskyy and Zhivotovsky, 2014). Botanical drugs can play an anti-apoptotic role by regulating the expression of pro- and anti-apoptotic genes. *In vitro* and *in vivo* studies have found that plants *Crassocephalum crepidioides* and *T. sinensis* can exert antioxidant and anti-apoptotic effects and reduce islet B-cell apoptosis, thereby improving DM (Bahar et al., 2017; Banerjee et al., 2020). *Nepeta angustifolia* C. Y. Wu can improve DN through anti-apoptotic effects (Huang et al., 2020).

3.4.1.2 Pyroptosis

Pyroptosis is a novel mode of programmed cell death characterized by the dependence on inflammatory caspases and the formation of activated gasdermin-D pores in the plasma membrane, eventually leading to cell rupture and the release of cytokines (Shi et al., 2017). NLRP3 plays an important role in pyroptosis (Sutterwala et al., 2006), so the regulation of ROS production and the activation of NLRP3 play an important role in anti-pyroptosis. In *in vitro* and *in vivo* studies of NAFLD, berberine has been shown to reduce pyroptosis by regulating the ROS/NLRP3 pathway (Mai et al., 2020).

3.4.2 Botanical drugs alleviated ROS-mediated IR

As discussed previously, the accumulation of ROS will induce IR, and botanical drugs increase cellular insulin sensitivity. In the hyperglycemia environment, *L. nobilis* Linn. extract could improve the decrease in INSR, AKT, and PI3K protein abundance induced by high insulin in HepG2 cells, and it alleviated IR while reducing ROS (Bourebaba et al., 2021). *Premna herbacea* improved IR, enhanced glucose uptake, and reduced ROS production in rat skeletal muscle cells through JNK/AKT/mTOR signaling (Kashyap et al., 2021). For T2DM, *A. officinarum* Hance can improve IR, as indicated by increased glucose uptake and glucose consumption in HepG2 cells, and this effect occurs through the PI3K/AKT/Nrf-2/GSK3β pathway (Zhang X. G. et al., 2022). *Herba Epimedii* alleviates IR by regulating the IRS1/AKT signal transduction pathway in db-/db-mice (Li et al., 2022). At the same time, this corroborates the importance of PI3K/AKT in combating IR (Huang et al., 2018).

3.4.3 The effects of botanical drugs against the inflammation associated with ROS

OS and inflammation play an important role in the development of abnormal glycolipid metabolic disease. ROS can also induce inflammation (Lei et al., 2015), so the regulation of ROS is also reflected in inflammation. *Syzygium jambos* bark extract protected islet B-cell in DM rats through IRS-2/AKT/GLUT4, ameliorated the elevation of TNF-α and IL-10, and exerted a regulatory effect on inflammation and OS (Mahmoud et al., 2021b). *In vivo* experiments have found that *Dracaena cochinchinensis (Lour.) S. C. Chen* and *Moutan Cortex* play anti-inflammatory and anti-oxidative effects by downregulating inflammatory factors and ROS levels and have certain therapeutic effects on DM (Chen et al., 2013; Zhang et al., 2014). *In vivo* and *in vitro* experiments of botanical drugs for the treatment of NAFLD have shown that berberine can control the release of inflammatory factors through ROS/NLRP3, improve the inflammation of NAFLD, protect hepatocytes, and slow the progression of the disease (Mai et al., 2020). In AS, *Morinda citrifolia* (Ishibashi et al., 2017), *Echinodorus grandiflorus* (Gasparotto et al., 2019), *Dendrobium huoshanense* (Fan et al., 2020), and *Dendrobium officinale* (Han et al., 2021) also play an antioxidant and anti-inflammatory role.

4 Conclusion and prospects

The incidence of glycolipid metabolic disease is gradually increasing, as well as the number of people affected by it, which forces people to find more ways to treat this kind of disease. As discussed earlier, ROS, as a key part of OS, plays an important role in the development of glycolipid metabolic diseases. Therefore, interfering with ROS to treat lipid metabolic diseases is a feasible and effective means in the future. For example, mitoTEMPOL and Q10 target mitochondria to reduce ROS in order to treat diabetes-related vascular damage (Graham et al., 2009; Dikalova et al., 2010) and nanoparticle drugs target ROS to improve AS (Wang et al., 2018; Zhang Z. et al., 2022) and the application of antioxidants in NAFLD (Ma et al., 2021). With this goal in view, this study summarizes the literature on botanical drugs to improve glycolipid metabolic diseases by regulating ROS from 2013 to 2022. In addition, the application parts, types of active ingredients, and extraction methods of botanical drugs involved were summarized. In addition, the mechanism of the intervention of botanical drugs on ROS to treat glycolipid metabolic diseases is briefly described, hoping this study can provide some help for the clinical use of botanical drugs.

However, there are still many deficiencies in the current research on ROS and glycolipid metabolic diseases. For example, many signaling pathways are related to OS, including but not limited to the signaling pathways discussed previously. In the future, if we continue to explore new pathways related to ROS in glycolipid metabolic diseases, or further study the cross-talk of existing pathways and develop drugs that can intervene in multiple pathways, It is believed that these may be helpful to improve OS in glycolipid metabolic diseases. These are going to be interesting new lines of research.

There are also some drawbacks to the study of botanical drugs. For example, there are more studies on the mechanism of botanical drugs or monomer components but fewer studies on adverse

reactions and toxic side effects. There is insufficient clinical trial research and a lack of research on drug metabolism and kinetics. In order to promote the application of botanical drugs in glycolipid metabolic diseases and strengthen the research on the aforementioned problems, the following points are also worth developing: the extraction, processing, and storage of the effective ingredients of botanical drugs. The contents of the same active ingredient in different plants were compared. There is also the combination of a variety of plants, such as the use of herbal decoction and the combination of botanicals and chemical drugs (such as *Erigeron breviscapus* and enalapril to improve diabetic kidney injury; Xu et al., 2013). In addition, plant drugs do not only treat glucose and lipid metabolism diseases by regulating ROS but also include inflammation, apoptosis, cell proliferation, and other mechanisms. Therefore, the study of the combined effects of multiple mechanisms is also a good direction.

In summary, many botanical drugs have shown therapeutic effects on glycolipid metabolic diseases by regulating ROS. However, there are still many deficiencies in the current research. Although botanical drugs are still used empirically in the treatment of glucose and lipid metabolic diseases in many areas, it is obviously of great benefit to the clinical promotion of botanical drugs if a more in-depth study of the mechanism can be carried out. It is believed that better utilization of these widely available, low-cost, and complex botanical drugs will add new and powerful means for the treatment of glycolipid metabolic diseases.

Author contributions

ML, YHZ, and ST reviewed the literature, ML, YHZ, and LG wrote this manuscript. ML and YHZ draught diagrams, YZ and YL guided diagrams modification. RG, JM, and YL draw the tables, RY modified the tables. CZ and PG contributed to the manuscript revision. All authors read and approved the submitted version.

Funding

This study was funded by the fifth batch of training objects of the National TCM Clinical Excellent Talents Training Program (National TCM Renjiao Letter [2022] No. 1); "100 Talent Plan" Project of Hospital of Chengdu University of Traditional Chinese Medicine (Hospital office [2021] 42); and "Tianfu Qingcheng Plan" Tianfu Science and Technology Leading Talent Project (Cheng et al. (2021) No. 1090).

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

Alam, M. B., Kwon, K. R., Lee, S. H., and Lee, S. H. (2017). Lannea coromandelica (houtt) Merr. Induces heme oxygenase 1 (HO-1) expression and reduces oxidative stress via the p38/c-Jun N-terminal kinase-nuclear factor erythroid 2-related factor 2 (p38/JNK-NRF2)-Mediated antioxidant pathway. *Int. J. Mol. Sci* 18 (2), 266. doi:10.3390/ijms18020266

Ando, K., and Fujita, T. (2009). Metabolic syndrome and oxidative stress. *Free Radic. Biol. Med* 47 (3), 213–218. doi:10.1016/j.freeradbiomed.2009.04.030

Apostolova, N., Iannantuoni, F., Gruevska, A., Muntane, J., Rocha, M., and Victor, V. M. (2020). Mechanisms of action of metformin in type 2 diabetes: Effects on mitochondria and leukocyte-endothelium interactions. *Redox Biol.* 34, 101517. doi:10.1016/j.redox.2020.101517

Arroyave-Ospina, J. C., Wu, Z., Geng, Y., and Moshage, H. (2021). Role of oxidative stress in the pathogenesis of non-alcoholic fatty liver disease: Implications for prevention and therapy. *Antioxidants (Basel, Switz.* 10 (2), 174. doi:10.3390/antiox10020174

Axelsson, A. S., Tubbs, E., Mecham, B., Chacko, S., Nenonen, H. A., Tang, Y., et al. (2017). Sulforaphane reduces hepatic glucose production and improves glucose control in patients with type 2 diabetes. *Sci. Transl. Med.* 9 (394), eaah4477. doi:10.1126/scitranslmed.aah4477

Bahar, E., Akter, K. M., Lee, G. H., Lee, H. Y., Rashid, H. O., Choi, M. K., et al. (2017). β-Cell protection and antidiabetic activities of Crassocephalum crepidioides (Asteraceae) Benth. S. Moore extract against alloxan-induced oxidative stress via regulation of apoptosis and reactive oxygen species (ROS). *BMC COMPLEMENTARY Altern. Med.* 17, 179. doi:10.1186/s12906-017-1697-0

Bai, Y., Mu, Q., Bao, X., Zuo, J., Fang, X., Hua, J., et al. (2021). Targeting NLRP3 inflammasome in the treatment of diabetes and diabetic complications: Role of natural compounds from herbal medicine. *Aging Dis.* 12 (7), 1587–1604. doi:10.14336/AD.2021.0318

Banerjee, A., Singh, S., Prasad, S. K., Kumar, S., Banerjee, O., Seal, T., et al. (2020). Protective efficacy of Tinospora sinensis against streptozotocin induced pancreatic islet cell injuries of diabetic rats and its correlation to its phytochemical profiles. *J. Ethnopharmacol.* 248, 112356. doi:10.1016/j.jep.2019.112356

Barquera, S., Pedroza-Tobías, A., Medina, C., Hernández-Barrera, L., Bibbins-Domingo, K., Lozano, R., et al. (2015). Global overview of the epidemiology of atherosclerotic cardiovascular disease. *Archives Med. Res.* 46 (5), 328–338. doi:10.1016/j.arcmed.2015.06.006

Batty, M., Bennett, M. R., and Yu, E. (2022). The role of oxidative stress in atherosclerosis. *Cells* 11 (23), 3843. doi:10.3390/cells11233843

Bedard, K., and Krause, K.-H. (2007). The NOX family of ROS-generating NADPH oxidases: Physiology and pathophysiology. *Physiol. Rev.* 87 (1), 245–313. doi:10.1152/physrev.00044.2005

Begriche, K., Massart, J., Robin, M.-A., Bonnet, F., and Fromenty, B. (2013). Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease. *Hepatol. Baltim. Md)* 58 (4), 1497–1507. doi:10.1002/hep.26226

Bhabak, K. P., and Mugesh, G. (2010). Functional mimics of glutathione peroxidase: Bioinspired synthetic antioxidants. *Accounts Chem. Res.* 43 (11), 1408–1419. doi:10.1021/ar100059g

Bhandary, B., Marahatta, A., Kim, H.-R., and Chae, H.-J. (2012). An involvement of oxidative stress in endoplasmic reticulum stress and its associated diseases. *Int. J. Mol. Sci.* 14 (1), 434–456. doi:10.3390/ijms14010434

Bhattacharya, S., Khan, M. M., Ghosh, C., Bank, S., and Maiti, S. (2017). The role of Dermcidin isoform-2 in the occurrence and severity of Diabetes. *Sci. Rep.* 7 (1), 8252. doi:10.1038/s41598-017-07958-3

Blesia, V., Patel, V. B., Al-Obaidi, H., Renshaw, D., and Zariwala, M. G. (2021). Excessive iron induces oxidative stress promoting cellular perturbations and insulin secretory dysfunction in MIN6 beta cells. *Cells* 10 (5), 1141. doi:10.3390/cells10051141

Blüher, M. (2019). Obesity: Global epidemiology and pathogenesis. *Nat. Rev. Endocrinol.* 15 (5), 288–298. doi:10.1038/s41574-019-0176-8

Bourebaba, N., Kornicka-Garbowska, K., Marycz, K., Bourebaba, L., and Kowalczuk, A. (2021). Laurus nobilis ethanolic extract attenuates hyperglycemia and hyperinsulinemia-induced insulin resistance in HepG2 cell line through the reduction of oxidative stress and improvement of mitochondrial biogenesis - possible implication in pharmacotherapy. *MITOCHONDRION* 59, 190–213. doi:10.1016/j.mito.2021.06.003

Brown, D. I., and Griendling, K. K. (2015). Regulation of signal transduction by reactive oxygen species in the cardiovascular system. *Circulation Res.* 116 (3), 531–549. doi:10.1161/CIRCRESAHA.116.303584

Buettner, G. R. (2011). Superoxide dismutase in redox biology: The roles of superoxide and hydrogen peroxide. *Anti-cancer Agents Med. Chem.* 11 (4), 341–346. doi:10.2174/187152011795677544

Caliceti, C., Rizzo, P., Ferrari, R., Fortini, F., Aquila, G., Leoncini, E., et al. (2017). Novel role of the nutraceutical bioactive compound berberine in lectin-like OxLDL receptor 1-mediated endothelial dysfunction in comparison to lovastatin. *Nutr. METABOLISM Cardiovasc. Dis.* 27 (6), 552–563. doi:10.1016/j.numecd.2017.04.002

Cao, Z. H., Wu, Z. A., Duan, T. T., Tang, C. F., Huang, D., and Hu, X. B. (2022). Curcumin ameliorates HO-induced injury through SIRT1-PERK-CHOP pathway in pancreatic beta cells. *ACTA BIOCHIMICA BIOPHYSICA SINICA* 54 (3), 370–377. doi:10.3724/abbs.2022004

Carnevale, R., Pignatelli, P., Di Santo, S., Bartimoccia, S., Sanguigni, V., Napoleone, L., et al. (2010). Atorvastatin inhibits oxidative stress via adiponectin-mediated NADPH oxidase down-regulation in hypercholesterolemic patients. *Atherosclerosis* 213 (1), 225–234. doi:10.1016/j.atherosclerosis.2010.08.056

Ceriello, A., Novials, A., Ortega, E., Canivell, S., La Sala, L., Pujadas, G., et al. (2013). Glucagon-like peptide 1 reduces endothelial dysfunction, inflammation, and oxidative stress induced by both hyperglycemia and hypoglycemia in type 1 diabetes. *Diabetes Care* 36 (8), 2346–2350. doi:10.2337/dc12-2469

Cervantes Gracia, K., Llanas-Cornejo, D., and Husi, H. (2017). CVD and oxidative stress. *J. Clin. Med.* 6 (2), 22. doi:10.3390/jcm6020022

Chang, T. T., Chen, Y. A., Li, S. Y., and Chen, J. W. (2021). Nrf-2 mediated heme oxygenase-1 activation contributes to the anti-inflammatory and renal protective effects of Ginkgo biloba extract in diabetic nephropathy. *J. Ethnopharmacol.* 266, 113474. doi:10.1016/j.jep.2020.113474

Chatterjee, S., Browning, E. A., Hong, N., DeBolt, K., Sorokina, E. M., Liu, W., et al. (2012). Membrane depolarization is the trigger for PI3K/Akt activation and leads to the generation of ROS. *Am. J. Physiology. Heart Circulatory Physiology* 302 (1), H105–H114. doi:10.1152/ajpheart.00298.2011

Chen, F., Xiong, H., Wang, J., Ding, X., Shu, G., and Mei, Z. (2013). Antidiabetic effect of total flavonoids from Sanguis draxonis in type 2 diabetic rats. *J. Ethnopharmacol.* 149 (3), 729–736. doi:10.1016/j.jep.2013.07.035

Chen, G., Chen, X. J., Niu, C., Huang, X. Z., An, N., Sun, J., et al. (2019). Baicalin alleviates hyperglycemia-induced endothelial impairment via Nrf2. *J. Endocrinol.* 240 (1), 81–98. doi:10.1530/JOE-18-0457

Chen, Z., Tian, R., She, Z., Cai, J., and Li, H. (2020). Role of oxidative stress in the pathogenesis of nonalcoholic fatty liver disease. *Free Radic. Biol. Med.* 152, 116–141. doi:10.1016/j.freeradbiomed.2020.02.025

Cheng, Y.-C., Chiu, Y.-M., Dai, Z.-K., and Wu, B.-N. (2021). Loganin ameliorates painful diabetic neuropathy by modulating oxidative stress, inflammation and insulin sensitivity in streptozotocin-nicotinamide-induced diabetic rats. *Cells* 10 (10), 2688. doi:10.3390/cells10102688

Choi, E. S., Yoon, J. J., Han, B. H., Jeong, D. H., Lee, Y. J., Kang, D. G., et al. (2018). Ligustilide attenuates vascular inflammation and activates Nrf2/HO-1 induction and, NO synthesis in HUVECs. *PHYTOMEDICINE* 38, 12–23. doi:10.1016/j.phymed.2017.09.022

Choi, Y. E., Choi, S. I., Han, X. G., Men, X., Jang, G. W., Kwon, H. Y., et al. (2020). Enhancement of immune activities of mixtures with sasa quelpaertensis Nakai and Ficus erecta var. sieboldii. *ANTIOXIDANTS* 9 (12), 868. doi:10.3390/foods9070868

Dan Dunn, J., Alvarez, L. A., Zhang, X., and Soldati, T. (2015). Reactive oxygen species and mitochondria: A nexus of cellular homeostasis. *Redox Biol.* 6, 472–485. doi:10.1016/j.redox.2015.09.005

De Oliveira, K. A., Araujo, H. N., De Lima, T. I., Oliveira, A. G., Favero-Santos, B. C., Guimaraes, D., et al. (2021). Phytomodulatory proteins isolated from Calotropis procera latex promote glycemic control by improving hepatic mitochondrial function in HepG2 cells. *SAUDI Pharm. J.* 29 (9), 1061–1069. doi:10.1016/j.jsps.2021.07.008

Deng, Y., Ma, J., Weng, X., Wang, Y. Q., Li, M. R., Yang, T. T., et al. (2021). Kaempferol-3-O-Glucuronide ameliorates non-alcoholic steatohepatitis in high-cholesterol-diet-induced larval zebrafish and HepG2 cell models via regulating oxidation stress. *LIFE-BASEL* 11 (5), 445. doi:10.3390/life11050445

Dhounchak, S., Popp, S. K., Brown, D. J., Laybutt, D. R., Biden, T. J., Bornstein, S. R., et al. (2021). Heparan sulfate proteoglycans in beta cells provide a critical link between endoplasmic reticulum stress, oxidative stress and type 2 diabetes. *PloS One* 16 (6), e0252607. doi:10.1371/journal.pone.0252607

Dikalova, A. E., Bikineyeva, A. T., Budzyn, K., Nazarewicz, R. R., McCann, L., Lewis, W., et al. (2010). Therapeutic targeting of mitochondrial superoxide in hypertension. *Circulation Res.* 107 (1), 106–116. doi:10.1161/CIRCRESAHA.109.214601

Dinkova-Kostova, A. T., and Talalay, P. (2008). Direct and indirect antioxidant properties of inducers of cytoprotective proteins. *Mol. Nutr. Food Res.* 52, S128–S138. doi:10.1002/mnfr.200700195

Dröse, S., and Brandt, U. (2012). Molecular mechanisms of superoxide production by the mitochondrial respiratory chain. *Adv. Exp. Med. Biol.* 748, 145–169. doi:10.1007/978-1-4614-3573-0_6

Elsayed, R. H., Kamel, E. M., Mahmoud, A. M., El-Bassuony, A. A., Bin-Jumah, M., Lamsabhi, A., et al. (2020). Rumex dentatus L. phenolics ameliorate hyperglycemia by modulating hepatic key enzymes of carbohydrate metabolism, oxidative stress and PPAR gamma in diabetic rats. *FOOD Chem. Toxicol.* 138, 111202. doi:10.1016/j.fct.2020.111202

Fan, J., Liu, D., He, C., Li, X., and He, F. (2016). Inhibiting adhesion events by Panax notoginseng saponins and Ginsenoside Rb1 protecting arteries via activation of Nrf2 and suppression of p38 - VCAM-1 signal pathway. *J. Ethnopharmacol.* 192, 423–430. doi:10.1016/j.jep.2016.09.022

Fan, X. C., Han, J. C., Zhu, L. J., Chen, Z. P., Li, J. J., Gu, Y., et al. (2020). Protective Activities of Dendrobium huoshanense C. Z. Tang et S. J. Cheng Polysaccharide against High-Cholesterol Diet-Induced Atherosclerosis in Zebrafish. *OXIDATIVE Med. Cell. Longev.* 2020, 8365056. doi:10.1155/2020/8365056

Fang, Q., Li, X., Wang, M., Qiao, X., Huang, F., Hu, C., et al. (2022). Walnut green husk ethanol extract improves gut microbiota and their metabolites associated with NLRP3 in non-alcoholic steatohepatitis. *Food and Funct.* 13 (11), 6387–6403. doi:10.1039/d2fo00012a

Farmer, A., and Fox, R. (2011). Diagnosis, classification, and treatment of diabetes. *BMJ Clin. Res. ed.)* 342, d3319. doi:10.1136/bmj.d3319

Foo, J., Bellot, G., Pervaiz, S., and Alonso, S. (2022). Mitochondria-mediated oxidative stress during viral infection. *Trends Microbiol.* 30 (7), 679–692. doi:10.1016/j.tim.2021.12.011

Forbes-Hernandez, T. Y., Gasparrini, M., Afrin, S., Cianciosi, D., Gonzalez-Paramas, A. M., Santos-Buelga, C., et al. (2017). Strawberry (cv. Romina) methanolic extract and anthocyanin-enriched fraction improve lipid profile and antioxidant status in HepG2 cells. *Int. J. Mol. Sci.* 18 (6), 1149. doi:10.3390/ijms18061149

Forrester, S. J., Kikuchi, D. S., Hernandes, M. S., Xu, Q., and Griendling, K. K. (2018). Reactive oxygen species in metabolic and inflammatory signaling. *Circulation Res.* 122 (6), 877–902. doi:10.1161/CIRCRESAHA.117.311401

Förstermann, U., Xia, N., and Li, H. (2017). Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. *Circulation Res.* 120 (4), 713–735. doi:10.1161/CIRCRESAHA.116.309326

Friedman, S. L., Neuschwander-Tetri, B. A., Rinella, M., and Sanyal, A. J. (2018). Mechanisms of NAFLD development and therapeutic strategies. *Nat. Med.* 24 (7), 908–922. doi:10.1038/s41591-018-0104-9

Fu, Y. F., Sun, S. Y., Sun, H. J., Peng, J. Y., Ma, X. D., Bao, L. C., et al. (2019). Scutellarin exerts protective effects against atherosclerosis in rats by regulating the Hippo-FOXO3A and PI3K/AKT signaling pathways. *J. Cell. PHYSIOLOGY* 234 (10), 18131–18145. doi:10.1002/jcp.28446

Fuchs, C. D., Claudel, T., Scharnagl, H., Stojakovic, T., and Trauner, M. (2017). FXR controls CHOP expression in steatohepatitis. *FEBS Lett.* 591 (20), 3360–3368. doi:10.1002/1873-3468.12845

Gao, Y., Wang, X., and He, C. (2016). An isoflavonoid-enriched extract from Pueraria lobata (kudzu) root protects human umbilical vein endothelial cells against oxidative stress induced apoptosis. *J. Ethnopharmacol.* 193, 524–530. doi:10.1016/j.jep.2016.10.005

Gardner, B. M., and Walter, P. (2011). Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response. *Sci. (New York, N.Y.)* 333 (6051), 1891–1894. doi:10.1126/science.1209126

Gasparotto, F. M., Palozi, R. A. C., da Silva, C. H. F., Pauli, K. B., Donadel, G., Lourenco, B., et al. (2019). Antiatherosclerotic properties of Echinodorus grandiflorus (cham. and schltdl) micheli: From antioxidant and lipid-lowering effects to an anti-inflammatory role. *J. Med. FOOD* 22 (9), 919–927. doi:10.1089/jmf.2019.0017

Germoush, M. O., Elgebaly, H. A., Hassan, S., Kamel, E. M., Bin-Jumah, M., and Mahmoud, A. M. (2020). Consumption of terpenoids-rich Padina pavonia extract attenuates hyperglycemia, insulin resistance and oxidative stress, and Upregulates PPAR gamma in a rat model of type 2 diabetes. *ANTIOXIDANTS* 9 (1), 22. doi:10.3390/antiox9010022

Gier, B., Krippeit-Drews, P., Sheiko, T., Aguilar-Bryan, L., Bryan, J., Düfer, M., et al. (2009). Suppression of KATP channel activity protects murine pancreatic beta cells against oxidative stress. *J. Clin. Investigation* 119 (11), 3246–3256. doi:10.1172/JCI38817

Gimenes, R., Gimenes, C., Rosa, C. M., Xavier, N. P., Campos, D. H. S., Fernandes, A. A. H., et al. (2018). Influence of apocynin on cardiac remodeling in rats with streptozotocin-induced diabetes mellitus. *Cardiovasc. Diabetol.* 17, 15. doi:10.1186/s12933-017-0657-9

Goboza, M., Aboua, Y. G., Chegou, N., and Oguntibeju, O. O. (2019). Vindoline effectively ameliorated diabetes-induced hepatotoxicity by docking oxidative stress, inflammation and hypertriglyceridemia in type 2 diabetes-induced male Wistar rats. *Biomed. Pharmacother.* 112, 108638. doi:10.1016/j.biopha.2019.108638

Graham, D., Huynh, N. N., Hamilton, C. A., Beattie, E., Smith, R. A. J., Cochemé, H. M., et al. (2009). Mitochondria-targeted antioxidant MitoQ10 improves endothelial

function and attenuates cardiac hypertrophy. *Hypertens. (Dallas, Tex, 1979)* 54 (2), 322–328. doi:10.1161/HYPERTENSIONAHA.109.130351

Gu, L., Bai, W., Li, S., Zhang, Y., Han, Y., Gu, Y., et al. (2013). Celastrol prevents atherosclerosis via inhibiting LOX-1 and oxidative stress. *PLoS One* 8 (6), e65477. doi:10.1371/journal.pone.0065477

Hachkova, H., Nagalievska, M., Soliljak, Z., Kanyuka, O., Kucharska, A. Z., Sokol-Letowska, A., et al. (2021). Medicinal plants Galega officinalis L. And Yacon leaves as potential sources of antidiabetic drugs. *ANTIOXIDANTS* 10 (9), 1362. doi:10.3390/antiox10091362

Hamanaka, R. B., and Chandel, N. S. (2010). Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes. *Trends Biochem. Sci.* 35 (9), 505–513. doi:10.1016/j.tibs.2010.04.002

Han, J., Dong, J., Zhang, R., Zhang, X., Chen, M., Fan, X., et al. (2021). Dendrobium catenatum lindl. Water extracts attenuate atherosclerosis. *Mediat. Inflamm.* 2021, 9951946. doi:10.1155/2021/9951946

Herranz-Lopez, M., Olivares-Vicente, M., Gallego, E. R., Encinar, J. A., Perez-Sanchez, A., Ruiz-Torres, V., et al. (2020). Quercetin metabolites from Hibiscus sabdariffa contribute to alleviate glucolipotoxicity-induced metabolic stress *in vitro*. *FOOD Chem. Toxicol.* 144, 111606. doi:10.1016/j.fct.2020.111606

Hoang, T. H., Yoon, Y., Park, S. A., Lee, H. Y., Peng, C., Kim, J. H., et al. (2021). IBF-R, a botanical extract of Rhus verniciflua controls obesity in which AMPK-SIRT1 axis and ROS regulatory mechanism are involved in mice. *J. Funct. FOODS* 87, 104804. doi:10.1016/j.jff.2021.104804

Hoseini, Z., Sepahvand, F., Rashidi, B., Sahebkar, A., Masoudifar, A., and Mirzaei, H. (2018). NLRP3 inflammasome: Its regulation and involvement in atherosclerosis. *J. Cell. Physiology* 233 (3), 2116–2132. doi:10.1002/jcp.25930

Hsieh, S. L., Wang, J. J., Su, K. H., Kuo, Y. L., Hsieh, S. C., and Wu, C. C. (2020). Suppressive effects of the *Gynura bicolor* ether extract on endothelial permeability and leukocyte transmigration in human endothelial cells induced by TNF-α. *EVIDENCE-BASED COMPLEMENTARY Altern. Med.* 2020, 9413724. doi:10.1155/2020/9413724

Hsu, J. Y., Lin, H. H., Chyau, C. C., Wang, Z. H., and Chen, J. H. (2021). Aqueous extract of pepino leaves ameliorates palmitic acid-induced hepatocellular lipotoxicity via inhibition of endoplasmic reticulum stress and apoptosis. *ANTIOXIDANTS* 10 (6), 903. doi:10.3390/antiox10060903

Huang, C. S., Lin, A. H., Yang, T. C., Liu, K. L., Chen, H. W., and Lii, C. K. (2015). Shikonin inhibits oxidized LDL-induced monocyte adhesion by suppressing NFκB activation via up-regulation of PI3K/Akt/Nrf2-dependent antioxidation in EA.hy926 endothelial cells. *Biochem. Pharmacol.* 93 (3), 352–361. doi:10.1016/j.bcp.2014.12.005

Huang, D. Q., El-Serag, H. B., and Loomba, R. (2021). Global epidemiology of NAFLD-related HCC: Trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterology Hepatology* 18 (4), 223–238. doi:10.1038/s41575-020-00381-6

Huang, S., Tan, M., Guo, F., Dong, L. S., Liu, Z. M., Yuan, R. Y., et al. (2020). Nepeta angustifolia C. Y. Wu improves renal injury in HFD/STZ-induced diabetic nephropathy and inhibits oxidative stress-induced apoptosis of mesangial cells. *J. Ethnopharmacol.* 255, 112771. doi:10.1016/j.jep.2020.112771

Huang, X., Liu, G., Guo, J., and Su, Z. (2018). The PI3K/AKT pathway in obesity and type 2 diabetes. *Int. J. Biol. Sci.* 14 (11), 1483–1496. doi:10.7150/ijbs.27173

Ihm, S. H., Park, S. H., Lee, J. O., Kim, O. R., Park, E. H., Kim, K. R., et al. (2021). A standardized Lindera obtusiloba extract improves endothelial dysfunction and attenuates plaque development in hyperlipidemic ApoE-knockout mice. *PLANTS-BASEL* 10 (11), 2493. doi:10.3390/plants10112493

Ishibashi, Y., Matsui, T., Isami, F., Abe, Y., Sakaguchi, T., Higashimoto, Y., et al. (2017). N-butanol extracts of *Morinda citrifolia* suppress advanced glycation end products (AGE)-induced inflammatory reactions in endothelial cells through its anti-oxidative properties. *BMC COMPLEMENTARY Altern. Med.* 17, 137. doi:10.1186/s12906-017-1641-3

Jamwal, S., and Sharma, S. (2018). Vascular endothelium dysfunction: A conservative target in metabolic disorders. *Inflamm. Res. Official J. Eur. Histamine Res. Soc.* 67 (5), 391–405. doi:10.1007/s00011-018-1129-8

Jiang, X., Gu, Y., Huang, Y., Zhou, Y., Pang, N., Luo, J., et al. (2021). CBD alleviates liver injuries in alcoholics with high-fat high-cholesterol diet through regulating NLRP3 inflammasome-pyroptosis pathway. *Front. Pharmacol.* 12, 724747. doi:10.3389/fphar.2021.724747

Jiang, Y. H., Yang, C. H., Li, W., Wu, S., Meng, X. Q., and Li, D. N. (2016). Aqueous extracts of Tribulus terrestris protects against oxidized low-density lipoprotein-induced endothelial dysfunction. *Chin. J. Integr. Med.* 22 (3), 193–200. doi:10.1007/s11655-015-2321-0

Jin, X., Fu, W., Zhou, J., Shuai, N., Yang, Y., and Wang, B. (2021a). Oxymatrine attenuates oxidized low-density lipoprotein-induced HUVEC injury by inhibiting NLRP3 inflammasome-mediated pyroptosis via the activation of the SIRT1/Nrf2 signaling pathway. *Int. J. Mol. Med.* 48 (4), 187. doi:10.3892/ijmm.2021.5020

Jin, X., Fu, W., Zhou, J. X., Shuai, N. N., Yang, Y., and Wang, B. (2021b). Oxymatrine attenuates oxidized low-density lipoprotein-induced HUVEC injury by inhibiting NLRP3 inflammasome-mediated pyroptosis via the activation of the SIRT1/Nrf2 signaling pathway. *Int. J. Mol. Med.* 48 (4), 187. doi:10.3892/ijmm.2021.5020

Juan, C. A., Pérez de la Lastra, J. M., Plou, F. J., and Pérez-Lebeña, E. (2021). The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *Int. J. Mol. Sci.* 22 (9), 4642. doi:10.3390/ijms22094642

Kaim, G., and Dimroth, P. (1999). ATP synthesis by F-type ATP synthase is obligatorily dependent on the transmembrane voltage. *EMBO J.* 18 (15), 4118–4127. doi:10.1093/emboj/18.15.4118

Kajimoto, Y., and Kaneto, H. (2004). Role of oxidative stress in pancreatic beta-cell dysfunction. *Ann. N. Y. Acad. Sci.* 1011, 168–176. doi:10.1007/978-3-662-41088-2_17

Kamakura, R., Son, M. J., de Beer, D., Joubert, E., Miura, Y., and Yagasaki, K. (2015). Antidiabetic effect of green rooibos (Aspalathus linearis) extract in cultured cells and type 2 diabetic model KK-A(y) mice. *Cytotechnology* 67 (4), 699–710. doi:10.1007/s10616-014-9816-y

Kaminskyy, V. O., and Zhivotovsky, B. (2014). Free radicals in cross talk between autophagy and apoptosis. *Antioxidants Redox Signal.* 21 (1), 86–102. doi:10.1089/ars.2013.5746

Kanikowska, D., Kanikowska, A., Swora-Cwynar, E., Grzymisławski, M., Sato, M., Bręborowicz, A., et al. (2021). Moderate caloric restriction partially improved oxidative stress markers in obese humans. *Antioxidants (Basel, Switz.* 10 (7), 1018. doi:10.3390/antiox10071018

Kashyap, B., Barge, S. R., Bharadwaj, S., Deka, B., Rahman, S., Ghosh, A., et al. (2021). Evaluation of therapeutic effect of Premna herbacea in diabetic rat and isoverbascoside against insulin resistance in L6 muscle cells through bioenergetics and stimulation of JNK and AKT/mTOR signaling cascade. *PHYTOMEDICINE* 93, 153761. doi:10.1016/j.phymed.2021.153761

Kaspar, J. W., Niture, S. K., and Jaiswal, A. K. (2009). Nrf2:INrf2 (Keap1) signaling in oxidative stress. *Free Radic. Biol. Med.* 47 (9), 1304–1309. doi:10.1016/j.freeradbiomed.2009.07.035

Kattoor, A. J., Pothineni, N. V. K., Palagiri, D., and Mehta, J. L. (2017). Oxidative stress in atherosclerosis. *Curr. Atheroscler. Rep.* 19 (11), 42. doi:10.1007/s11883-017-0678-6

Khazim, K., Gorin, Y., Cavaglieri, R. C., Abboud, H. E., and Fanti, P. (2013). The antioxidant silybin prevents high glucose-induced oxidative stress and podocyte injury *in vitro* and *in vivo*. *Am. J. Physiol. Ren. Physiol.* 305 (5), F691–F700. doi:10.1152/ajprenal.00028.2013

Kim, S., Kim, C. K., Lee, K. S., Kim, J. H., Hwang, H., Jeoung, D., et al. (2013). Aqueous extract of unripe Rubus coreanus fruit attenuates atherosclerosis by improving blood lipid profile and inhibiting NF-κB activation via phase II gene expression. *J. Ethnopharmacol.* 146 (2), 515–524. doi:10.1016/j.jep.2013.01.016

Kirkman, H. N., and Gaetani, G. F. (2007). Mammalian catalase: A venerable enzyme with new mysteries. *Trends Biochem. Sci.* 32 (1), 44–50. doi:10.1016/j.tibs.2006.11.003

Kong, Z. L., Johnson, A., Ko, F. C., He, J. L., and Cheng, S. C. (2018). Effect of Cistanche tubulosa extracts on male reproductive function in streptozotocin-nicotinamide-induced diabetic rats. *NUTRIENTS* 10 (10), 1562. doi:10.3390/nu10101562

Ku, C. W., Ho, T. J., Huang, C. Y., Chu, P. M., Ou, H. C., and Hsieh, P. L. (2021). Cordycepin attenuates palmitic acid-induced inflammation and apoptosis of vascular endothelial cells through mediating PI3K/Akt/eNOS signaling pathway. *Am. J. Chin. Med.* 49 (07), 1703–1722. doi:10.1142/S0192415X21500804

Ku, S. K., and Bae, J. S. (2015). Baicalin, baicalein and wogonin inhibits high glucose-induced vascular inflammation *in vitro* and *in vivo*. *BMB Rep.* 48 (9), 519–524. doi:10.5483/bmbrep.2015.48.9.017

Lartey, N. L., Asare-Anane, H., Ofori, E. K., Antwi, S., Asiedu-Larbi, J., Ayertey, F., et al. (2021). Antidiabetic activity of aqueous stem bark extract of Annickia polycarpa in alloxan-induced diabetic mice. *J. TRADITIONAL COMPLEMENTARY Med.* 11 (2), 109–116. doi:10.1016/j.jtcme.2020.02.001

Lee, G. H., Peng, C., Jeong, S. Y., Park, S. A., Lee, H. Y., Hoang, T. H., et al. (2021). Ginger extract controls mTOR-SREBP1-ER stress-mitochondria dysfunction through AMPK activation in obesity model. *J. Funct. FOODS* 87, 104628. doi:10.1016/j.jff.2021.104628

Lee, N. K., Choi, Y. G., Baik, J. Y., Han, S. Y., Jeong, D.-W., Bae, Y. S., et al. (2005). A crucial role for reactive oxygen species in RANKL-induced osteoclast differentiation. *Blood* 106 (3), 852–859. doi:10.1182/blood-2004-09-3662

Lee, S. E., Jeong, S. I., Yang, H., Jeong, S. H., Jang, Y. P., Park, C. S., et al. (2012). Extract of Salvia miltiorrhiza (Danshen) induces Nrf2-mediated heme oxygenase-1 expression as a cytoprotective action in RAW 264.7 macrophages. *J. Ethnopharmacol.* 139 (2), 541–548. doi:10.1016/j.jep.2011.11.046

Leenders, F., Groen, N., de Graaf, N., Engelse, M. A., Rabelink, T. J., de Koning, E. J. P., et al. (2021). Oxidative stress leads to β-cell dysfunction through loss of β-cell identity. *Front. Immunol.* 12, 690379. doi:10.3389/fimmu.2021.690379

Lei, X. G., Zhu, J.-H., Cheng, W.-H., Bao, Y., Ho, Y.-S., Reddi, A. R., et al. (2016). Paradoxical roles of antioxidant enzymes: Basic mechanisms and health implications. *Physiol. Rev.* 96 (1), 307–364. doi:10.1152/physrev.00010.2014

Lei, Y., Wang, K., Deng, L., Chen, Y., Nice, E. C., and Huang, C. (2015). Redox regulation of inflammation: Old elements, a new story. *Med. Res. Rev.* 35 (2), 306–340. doi:10.1002/med.21330

Li, B., Wang, R., Wang, L., Zhang, G., and Zhang, Y. (2021a). Capillin protects against non-alcoholic steatohepatitis through suppressing NLRP3 inflammasome activation and oxidative stress. *Immunopharmacol. Immunotoxicol.* 43 (6), 778–789. doi:10.1080/08923973.2021.1984520

Li, C. M., Guo, Y. Q., Dong, X. L., Li, H., Wang, B., Wu, J. H., et al. (2014). Ethanolic extract of rhizome of Ligusticum chuanxiong Hort. (chuanxiong) enhances endothelium-dependent vascular reactivity in ovariectomized rats fed with high-fat diet. *Food Funct.* 5 (10), 2475–2485. doi:10.1039/c4fo00211c

Li, D., and Mehta, J. L. (2000). Upregulation of endothelial receptor for oxidized LDL (LOX-1) by oxidized LDL and implications in apoptosis of human coronary artery endothelial cells: Evidence from use of antisense LOX-1 mRNA and chemical inhibitors. *Arteriosclerosis, Thrombosis, Vasc. Biol.* 20 (4), 1116–1122. doi:10.1161/01.atv.20.4.1116

Li, G., Luan, G. X., He, Y. F., Tie, F. F., Wang, Z. H., Suo, Y. R., et al. (2018). Polyphenol stilbenes from fenugreek (Trigonella foenum-graecum L) seeds improve insulin sensitivity and mitochondrial function in 3T3-L1 adipocytes. *OXIDATIVE Med. Cell. Longev.* 2018, 7634362. doi:10.1155/2018/7634362

Li, Y. Q., Li, Y. L., Chen, N. N., Feng, L. Y., Gao, J. M., Zeng, N., et al. (2022). Icariside II exerts anti-type 2 diabetic effect by targeting pparα/γ: Involvement of ROS/NF-κB/IRS1 signaling pathway. *ANTIOXIDANTS* 11 (9), 1705. doi:10.3390/antiox11091705

Li, Y., Zhou, Y., Wu, J., Li, J. D., and Yao, H. K. (2021b). Phelligridin D from Inonotus obliquus attenuates oxidative stress and accumulation of ECM in mesangial cells under high glucose via activating Nrf2. *J. Nat. Med.* 75 (4), 1021–1029. doi:10.1007/s11418-021-01534-w

Liang, H. H., Pan, Y. N., Teng, Y. L., Yuan, S. L., Wu, X., Yang, H. J., et al. (2020). A proteoglycan extract from Ganoderma Lucidum protects pancreatic beta-cells against STZ-induced apoptosis. *Biosci. Biotechnol. Biochem.* 84 (12), 2491–2498. doi:10.1080/09168451.2020.1805718

Libby, P. (2017). Interleukin-1 beta as a target for atherosclerosis therapy: Biological basis of CANTOS and beyond. *J. Am. Coll. Cardiol.* 70 (18), 2278–2289. doi:10.1016/j.jacc.2017.09.028

Liu, F. F., Ma, H., Wang, G. H., Liu, W. X., Seeram, N. P., Mu, Y., et al. (2018). Phenolics from Eugenia jambolana seeds with advanced glycation endproduct formation and alpha-glucosidase inhibitory activities. *FOOD and Funct.* 9 (8), 4246–4254. doi:10.1039/c8fo00583d

Liu, Q., Qiao, A. M., Yi, L. T., Liu, Z. L., and Sheng, S. M. (2016). Protection of kinsenoside against AGEs-induced endothelial dysfunction in human umbilical vein endothelial cells. *Life Sci.* 162, 102–107. doi:10.1016/j.lfs.2016.08.022

Liu, S.-S. (2010). Mitochondrial Q cycle-derived superoxide and chemiosmotic bioenergetics. *Ann. N. Y. Acad. Sci.* 1201, 84–95. doi:10.1111/j.1749-6632.2010.05632.x

Liu, Y., Kern, J. T., Walker, J. R., Johnson, J. A., Schultz, P. G., and Luesch, H. (2007). A genomic screen for activators of the antioxidant response element. *Proc. Natl. Acad. Sci. U. S. A.* 104 (12), 5205–5210. doi:10.1073/pnas.0700898104

Liu, Z. L., Zhang, J. G., Liu, Q., Yi, L. T., Li, Y. M., and Li, Y. (2017). The vascular protective effects of Anoectochilus roxburghii polysaccharose under high glucose conditions. *J. Ethnopharmacol.* 202, 192–199. doi:10.1016/jjep.2017.03.012

Liu, Z., Xu, S., Huang, X., Wang, J., Gao, S., Li, H., et al. (2015). Cryptotanshinone, an orally bioactive herbal compound from danshen, attenuates atherosclerosis in apolipoprotein E-deficient mice: Role of lectin-like oxidized LDL receptor-1 (LOX-1). *Br. J. Pharmacol.* 172 (23), 5661–5675. doi:10.1111/bph.13068

Luan, G. X., Wang, Y. W., Wang, Z. H., Zhou, W. N., Hu, N., Li, G., et al. (2018). Flavonoid glycosides from fenugreek seeds regulate glycolipid metabolism by improving mitochondrial function in 3T3-L1 adipocytes *in vitro*. *J. Agric. FOOD Chem.* 66 (12), 3169–3178. doi:10.1021/acs.jafc.8b00179

Ma, Y., Lee, G., Heo, S.-Y., and Roh, Y.-S. (2021). Oxidative stress is a key modulator in the development of nonalcoholic fatty liver disease. *Antioxidants (Basel, Switz.* 11 (1), 91. doi:10.3390/antiox11010091

Madamanchi, N. R., and Runge, M. S. (2007). Mitochondrial dysfunction in atherosclerosis. *Circulation Res.* 100 (4), 460–473. doi:10.1161/01.RES.0000258450.44413.96

Mahmoud, M. F., Abdelaal, S., Mohammed, H. O., El-Shazly, A. M., Daoud, R., Abdelfattah, M. A. O., et al. (2021a). Syzygium aqueum (Burm.f) Alston prevents streptozotocin-induced pancreatic beta cells damage via the TLR-4 signaling pathway. *Front. Pharmacol.* 12, 769244. doi:10.3389/fphar.2021.769244

Mahmoud, M. F., Abdelaal, S., Mohammed, H. O., El-Shazly, A. M., Daoud, R., El Raey, M. A., et al. (2021b). Syzygium jambos extract mitigates pancreatic oxidative stress, inflammation and apoptosis and modulates hepatic IRS-2/AKT/GLUT4 signaling pathway in streptozotocin-induced diabetic rats. *Biomed. Pharmacother. = Biomedecine Pharmacother.* 142, 112085. doi:10.1016/j.biopha.2021.112085

Mai, W. J., Xu, Y. Z., Xu, J. H., Zhao, D., Ye, L. Y., Yu, G. X., et al. (2020). Berberine inhibits nod-like receptor family pyrin domain containing 3 inflammasome activation and pyroptosis in nonalcoholic steatohepatitis via the ROS/TXNIP Axis. *Front. Pharmacol.* 11, 185. doi:10.3389/fphar.2020.00185

Mansouri, A., Gattolliat, C.-H., and Asselah, T. (2018). Mitochondrial dysfunction and signaling in chronic liver diseases. *Gastroenterology* 155 (3), 629–647. doi:10.1053/j.gastro.2018.06.083

Martínez-Martínez, E., and Cachofeiro, V. (2022). Oxidative stress in obesity. *Antioxidants (Basel, Switz.* 11 (4), 639. doi:10.3390/antiox11040639

Mihailović, M., Dinić, S., Arambašić Jovanović, J., Uskoković, A., Grdović, N., and Vidaković, M. (2021). The influence of plant extracts and phytoconstituents on antioxidant enzymes activity and gene expression in the prevention and treatment of impaired glucose homeostasis and diabetes complications. *Antioxidants (Basel, Switz.* 10 (3), 480. doi:10.3390/antiox10030480

Muller, F. L., Liu, Y., and Van Remmen, H. (2004). Complex III releases superoxide to both sides of the inner mitochondrial membrane. *J. Biol. Chem.* 279 (47), 49064–49073. doi:10.1074/jbc.M407715200

Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. *Biochem. J.* 417 (1), 1–13. doi:10.1042/BJ20081386

Nagila, A., Permpongpaiboon, T., Tantrarongroj, S., Porapakkham, P., Chinwattana, K., Deakin, S., et al. (2009). Effect of atorvastatin on paraoxonase1 (PON1) and oxidative status. *Pharmacol. Rep. P. R.* 61 (5), 892–898. doi:10.1016/s1734-1140(09)70146-x

Nahdi, A. M. T. A., John, A., and Raza, H. (2017). Elucidation of molecular mechanisms of streptozotocin-induced oxidative stress, apoptosis, and mitochondrial dysfunction in rin-5F pancreatic β-cells. *Oxidative Med. Cell. Longev.* 2017, 7054272. doi:10.1155/2017/7054272

Nakanishi, A., Wada, Y., Kitagishi, Y., and Matsuda, S. (2014). Link between PI3K/AKT/PTEN pathway and NOX proteinin diseases. *Aging Dis.* 5 (3), 203–211. doi:10.14336/AD.2014.0500203

Nam, W., Nam, S. H., Kim, S. P., Levin, C., and Friedman, M. (2019). Anti-adipogenic and anti-obesity activities of purpurin in 3T3-L1 preadipocyte cells and in mice fed a high-fat diet. *BMC COMPLEMENTARY Altern. Med.* 19 (1), 364. doi:10.1186/s12906-019-2756-5

Niu, N., Xu, S., Xu, Y., Little, P. J., and Jin, Z.-G. (2019). Targeting mechanosensitive transcription factors in atherosclerosis. *Trends Pharmacol. Sci.* 40 (4), 253–266. doi:10.1016/j.tips.2019.02.004

Oguntibeju, O. O., Meyer, S., Aboua, Y. G., and Goboza, M. (2016). Hypoxis hemerocallidea significantly reduced hyperglycaemia and hyperglycaemic-induced oxidative stress in the liver and kidney tissues of streptozotocin-induced diabetic male wistar rats. *Evid. Based Complement. Altern. Med.* 2016, 8934362. doi:10.1155/2016/8934362

Ogunyinka, B. I., Oyinloye, B. E., Osunsanmi, F. O., Kolanisi, U., Opoku, A. R., and Kappo, A. P. (2019). Protein isolate from Parkia biglobosa seeds improves dyslipidaemia and cardiac oxidative stress in streptozotocin-induced diabetic rats. *ANTIOXIDANTS* 8 (10), 481. doi:10.3390/antiox8100481

Omodanisi, E. I., Aboua, Y. G., and Oguntibeju, O. O. (2017). Assessment of the anti-hyperglycaemic, anti-inflammatory and antioxidant activities of the methanol extract of Moringa oleifera in diabetes-induced nephrotoxic male wistar rats. *MOLECULES* 22 (4), 439. doi:10.3390/molecules22040439

Orrenius, S., Gogvadze, V., and Zhivotovsky, B. (2007). Mitochondrial oxidative stress: Implications for cell death. *Annu. Rev. Pharmacol. Toxicol.* 47, 143–183. doi:10.1146/annurev.pharmtox.47.120505.105122

Park, J.-G., and Oh, G. T. (2011). The role of peroxidases in the pathogenesis of atherosclerosis. *BMB Rep.* 44 (8), 497–505. doi:10.5483/bmbrep.2011.44.8.497

Park, S. H., Koo, H. J., Sung, Y. Y., and Kim, H. K. (2013). The protective effect of Prunella vulgaris ethanol extract against vascular inflammation in TNF-α-stimulated human aortic smooth muscle cells. *BMB Rep.* 46 (7), 352–357. doi:10.5483/bmbrep.2013.46.7.214

Passos, J. F., Saretzki, G., and von Zglinicki, T. (2007). DNA damage in telomeres and mitochondria during cellular senescence: Is there a connection? *Nucleic Acids Res.* 35 (22), 7505–7513. doi:10.1093/nar/gkm893

Pirillo, A., Casula, M., Olmastroni, E., Norata, G. D., and Catapano, A. L. (2021). Global epidemiology of dyslipidaemias. *Nat. Rev. Cardiol.* 18 (10), 689–700. doi:10.1038/s41569-021-00541-4

Poornima, M. S., Sindhu, G., Billu, A., Sruthi, C. R., Nisha, P., Gogoi, P., et al. (2022). Pretreatment of hydroethanolic extract of Dillenia indica L. attenuates oleic acid induced NAFLD in HepG2 cells via modulating SIRT-1/p-LKB-1/AMPK, HMGCR and PPAR-α signaling pathways. *J. Ethnopharmacol.* 292, 115237. doi:10.1016/j.jep.2022.115237

Qin, W. W., Xi, J. X., He, B. Q., Zhang, B. B., Luan, H. L., and Wu, F. H. (2016). Ameliorative effects of hispidulin on high glucose-mediated endothelial dysfunction via inhibition of PKC beta II-associated NLRP3 inflammasome activation and NF-kappa B signaling in endothelial cells. *J. Funct. FOODS* 27, 392–405. doi:10.1016/j.jff.2016.09.021

Qiu, F., and Zhang, Y.-Q. (2019). Metabolic effects of mulberry branch bark powder on diabetic mice based on GC-MS metabolomics approach. *Nutr. Metabolism* 16, 10. doi:10.1186/s12986-019-0335-x

Quesada, I., de Paola, M., Alvarez, M. S., Hapon, M. B., Gamarra-Luques, C., and Castro, C. (2021). Antioxidant and anti-atherogenic properties of prosopis

strombulifera and Tessaria absinthioides aqueous extracts: Modulation of NADPH oxidase-derived reactive oxygen species. *Front. PHYSIOLOGY* 12, 662833. doi:10.3389/fphys.2021.662833

Ren, Y., and Shen, H.-M. (2019). Critical role of AMPK in redox regulation under glucose starvation. *Redox Biol.* 25, 101154. doi:10.1016/j.redox.2019.101154

Rout, D., Dash, U. C., Kanhar, S., Swain, S. K., and Sahoo, A. K. (2020). The modulatory role of prime identified compounds in the bioactive fraction of Homalium zeylanicum in high-fat diet fed-streptozotocin-induced type 2 diabetic rats. *J. Ethnopharmacol.* 260, 113099. doi:10.1016/j.jep.2020.113099

Saaoud, F., Drummer, I. V, C., Shao, Y., Sun, Y., Lu, Y., Xu, K., et al. (2021). Circular RNAs are a novel type of non-coding RNAs in ROS regulation, cardiovascular metabolic inflammations and cancers. *Pharmacol. Ther.* 220, 107715. doi:10.1016/j.pharmthera.2020.107715

Saifi, M. A., Seal, S., and Godugu, C. (2021). Nanoceria, the versatile nanoparticles: Promising biomedical applications. *J. Control. Release Official J. Control. Release Soc.* 338, 164–189. doi:10.1016/j.jconrel.2021.08.033

Salvamani, S., Gunasekaran, B., Shukor, M. Y., Abu Bakar, M. Z., and Ahmad, S. A. (2016). Phytochemical investigation, hypocholesterolemic and anti-atherosclerotic effects of Amaranthus viridis leaf extract in hypercholesterolemia-induced rabbits. *RSC Adv.* 6 (39), 32685–32696. doi:10.1039/c6ra04827g

Schieber, M., and Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Curr. Biol. CB* 24 (10), R453–R462. doi:10.1016/j.cub.2014.03.034

Schofield, J. H., and Schafer, Z. T. (2021). Mitochondrial reactive oxygen species and mitophagy: A complex and nuanced relationship. *Antioxidants Redox Signal.* 34 (7), 517–530. doi:10.1089/ars.2020.8058

Sekiou, O., Boumendjel, M., Taibi, F., Tichati, L., Boumendjel, A., and Messarah, M. (2021). Nephroprotective effect of Artemisia herba alba aqueous extract in alloxan-induced diabetic rats. *J. Tradit. Complement. Med.* 11 (1), 53–61. doi:10.1016/j.jtcme.2020.01.001

Shadel, G. S., and Horvath, T. L. (2015). Mitochondrial ROS signaling in organismal homeostasis. *Cell* 163 (3), 560–569. doi:10.1016/j.cell.2015.10.001

Shah, A., Gray, K., Figg, N., Finigan, A., Starks, L., and Bennett, M. (2018). Defective base excision repair of oxidative DNA damage in vascular smooth muscle cells promotes atherosclerosis. *Circulation* 138 (14), 1446–1462. doi:10.1161/CIRCULATIONAHA.117.033249

Sharma, P., Chakraborty, R., Wang, L., Min, B., Tremblay, M. L., Kawahara, T., et al. (2008). Redox regulation of interleukin-4 signaling. *Immunity* 29 (4), 551–564. doi:10.1016/j.immuni.2008.07.019

Shatoor, A. S., and Al Humayed, S. (2020). The protective effect of crataegus aronia against high-fat diet-induced vascular inflammation in rats entails inhibition of the NLRP-3 inflammasome pathway. *Cardiovasc. Toxicol.* 20 (1), 82–99. doi:10.1007/s12012-019-09534-9

Shi, J., Gao, W., and Shao, F. (2017). Pyroptosis: Gasdermin-Mediated programmed necrotic cell death. *Trends Biochem. Sci.* 42 (4), 245–254. doi:10.1016/j.tibs.2016.10.004

Shih, D. M., Xia, Y. R., Wang, X. P., Miller, E., Castellani, L. W., Subbanagounder, G., et al. (2000). Combined serum paraoxonase knockout/apolipoprotein E knockout mice exhibit increased lipoprotein oxidation and atherosclerosis. *J. Biol. Chem.* 275 (23), 17527–17535. doi:10.1074/jbc.M910376199

Sies, H., Belousov, V. V., Chandel, N. S., Davies, M. J., Jones, D. P., Mann, G. E., et al. (2022). Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nat. Rev. Mol. Cell Biol.* 23 (7), 499–515. doi:10.1038/s41580-022-00456-z

Sies, H., Berndt, C., and Jones, D. P. (2017). Oxidative stress. *Annu. Rev. Biochem.* 86, 715–748. doi:10.1146/annurev-biochem-061516-045037

Sies, H., and Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.* 21 (7), 363–383. doi:10.1038/s41580-020-0230-3

Sies, H. (2015). Oxidative stress: A concept in redox biology and medicine. *Redox Biol.* 4, 180–183. doi:10.1016/j.redox.2015.01.002

Sivinski, J., Zhang, D. D., and Chapman, E. (2021). Targeting NRF2 to treat cancer. *Seminars Cancer Biol.* 76, 61–73. doi:10.1016/j.semcancer.2021.06.003

Srivastava, A., Bhatt, N. M., Patel, T. P., Dadheech, N., Singh, A., and Gupta, S. (2016). Anti-apoptotic and cytoprotective effect of Enicostemma littorale against oxidative stress in Islets of Langerhans. *Pharm. Biol.* 54 (10), 2061–2072. doi:10.3109/13880209.2016.1141222

Starkov, A. A., and Fiskum, G. (2003). Regulation of brain mitochondrial H2O2 production by membrane potential and NAD(P)H redox state. *J. Neurochem.* 86 (5), 1101–1107. doi:10.1046/j.1471-4159.2003.01908.x

Stiles, B. L. (2009). PI-3-K and AKT: Onto the mitochondria. *Adv. Drug Deliv. Rev.* 61 (14), 1276–1282. doi:10.1016/j.addr.2009.07.017

Su, Q., Dong, J., Zhang, D. L., Yang, L., and Roy, R. (2022). Protective effects of the bilobalide on retinal oxidative stress and inflammation in streptozotocin-induced diabetic rats. *Appl. Biochem. Biotechnol.* 194 (12), 6407–6422. doi:10.1007/s12010-022-04012-5

Su, Q., Sun, Y. H., Ye, Z. L., Yang, H. F., Kong, B. H., and Li, L. (2018). Pinocembrin protects endothelial cells from oxidized LDL-induced injury. *CYTOKINE* 111, 475–480. doi:10.1016/j.cyto.2018.05.033

Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., et al. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res. Clin. Pract.* 183, 109119. doi:10.1016/j.diabres.2021.109119

Sun, M., Zhu, T., Tong, J., Caidan, R., Wang, K., Kai, G., et al. (2020). Screening active components from Rubus amabilis for pancreatic β-cells protection. *Pharm. Biol.* 58 (1), 674–685. doi:10.1080/13880209.2020.1787467

Sun, Y. R., He, L. B., Wang, W., Wang, T. Y., Hua, W., Li, T. T., et al. (2021). Polyphenols from Penthorum chinense Pursh. Attenuates high glucose-induced vascular inflammation through directly interacting with Keap1 protein. *J. Ethnopharmacol.* 268, 113617. doi:10.1016/j.jep.2020.113617

Sutterwala, F. S., Ogura, Y., Szczepanik, M., Lara-Tejero, M., Lichtenberger, G. S., Grant, E. P., et al. (2006). Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of caspase-1. *Immunity* 24 (3), 317–327. doi:10.1016/j.immuni.2006.02.004

Takaki, A., Kawai, D., and Yamamoto, K. (2013). Multiple hits, including oxidative stress, as pathogenesis and treatment target in non-alcoholic steatohepatitis (NASH). *Int. J. Mol. Sci.* 14 (10), 20704–20728. doi:10.3390/ijms141020704

Tang, G., Duan, F., Li, W., Wang, Y., Zeng, C., Hu, J., et al. (2019). Metformin inhibited Nod-like receptor protein 3 inflammasomes activation and suppressed diabetes-accelerated atherosclerosis in apoE-/- mice. *Biomed. Pharmacother. = Biomedecine Pharmacother.* 119, 109410. doi:10.1016/j.biopha.2019.109410

Tang, G., Li, S., Zhang, C., Chen, H., Wang, N., and Feng, Y. (2021). Clinical efficacies, underlying mechanisms and molecular targets of Chinese medicines for diabetic nephropathy treatment and management. *Acta Pharm. Sin. B* 11 (9), 2749–2767. doi:10.1016/j.apsb.2020.12.020

Trnovska, J., Svoboda, P., Pelantova, H., Kuzma, M., Kratochvilova, H., Kasperova, B. J., et al. (2021). Complex positive effects of SGLT-2 inhibitor empagliflozin in the liver, kidney and adipose tissue of hereditary hypertriglyceridemic rats: Possible contribution of attenuation of cell senescence and oxidative stress. *Int. J. Mol. Sci.* 22 (19), 10606. doi:10.3390/ijms221910606

Tsai, H. Y., Huang, P. H., Lin, F. Y., Chen, J. S., Lin, S. J., and Chen, J. W. (2013). Ginkgo biloba extract reduces high-glucose-induced endothelial reactive oxygen species generation and cell adhesion molecule expression by enhancing HO-1 expression via Akt/eNOS and p38 MAP kinase pathways. *Eur. J. Pharm. Sci.* 48 (4-5), 803–811. doi:10.1016/j.ejps.2013.01.002

Tschopp, J., and Schroder, K. (2010). NLRP3 inflammasome activation: The convergence of multiple signalling pathways on ROS production? *Nat. Rev. Immunol.* 10 (3), 210–215. doi:10.1038/nri2725

Vallabhapurapu, S., and Karin, M. (2009). Regulation and function of NF-kappaB transcription factors in the immune system. *Annu. Rev. Immunol.* 27, 693–733. doi:10.1146/annurev.immunol.021908.132641

Vanhaesebroeck, B., Perry, M. W. D., Brown, J. R., André, F., and Okkenhaug, K. (2021). PI3K inhibitors are finally coming of age. *Nat. Rev. Drug Discov.* 20 (10), 741–769. doi:10.1038/s41573-021-00209-1

Vendrov, A. E., Hakim, Z. S., Madamanchi, N. R., Rojas, M., Madamanchi, C., and Runge, M. S. (2007). Atherosclerosis is attenuated by limiting superoxide generation in both macrophages and vessel wall cells. *Arterioscler. Thromb. Vasc. Biol.* 27 (12), 2714–2721. doi:10.1161/ATVBAHA.107.152629

Vezza, T., de Marañón, A. M., Canet, F., Díaz-Pozo, P., Marti, M., D'Ocon, P., et al. (2021). MicroRNAs and oxidative stress: An intriguing crosstalk to Be exploited in the management of type 2 diabetes. *Antioxidants (Basel, Switz.* 10 (5), 802. doi:10.3390/antiox10050802

Vijayaraj, P., Muthukumar, K., Sabarirajan, J., and Nachiappan, V. (2013). Antihyperlipidemic activity of Cassia auriculata flowers in triton WR 1339 induced hyperlipidemic rats. *Exp. Toxicol. Pathol.* 65 (1-2), 135–141. doi:10.1016/j.etp.2011.07.001

Wang, Q., Zhang, M., Torres, G., Wu, S., Ouyang, C., Xie, Z., et al. (2017). Metformin suppresses diabetes-accelerated atherosclerosis via the inhibition of drp1-mediated mitochondrial fission. *Diabetes* 66 (1), 193–205. doi:10.2337/db16-0915

Wang, Y., Li, L., Zhao, W., Dou, Y., An, H., Tao, H., et al. (2018). Targeted therapy of atherosclerosis by a broad-spectrum reactive oxygen species scavenging nanoparticle with intrinsic anti-inflammatory activity. *ACS Nano* 12 (9), 8943–8960. doi:10.1021/acsnano.8b02037

Wu, C. Q., Jing, M. H., Yang, L. J., Jin, L., Ding, Y. C., Lu, J., et al. (2018). Alisol A 24-acetate ameliorates nonalcoholic steatohepatitis by inhibiting oxidative stress and stimulating autophagy through the AMPK/mTOR pathway. *CHEMICO-BIOLOGICAL Interact.* 291, 111–119. doi:10.1016/j.cbi.2018.06.005

Wu, M., Yang, Z., Zhang, C., Shi, Y., Han, W., Song, S., et al. (2021). Inhibition of NLRP3 inflammasome ameliorates podocyte damage by suppressing lipid accumulation in diabetic nephropathy. *Metabolism Clin. Exp.* 118, 154748. doi:10.1016/j.metabol.2021.154748

Xu, X. X., Zhang, W., Zhang, P., Qi, X. M., Wu, Y. G., and Shen, J. J. (2013). Superior renoprotective effects of the combination of breviscapine with enalapril and its mechanism in diabetic rats. *Phytomedicine* 20 (10), 820–827. doi:10.1016/j.phymed.2013.03.027

Yahfoufi, N., Alsadi, N., Jambi, M., and Matar, C. (2018). The immunomodulatory and anti-inflammatory role of polyphenols. *NUTRIENTS* 10 (11), 1618. doi:10.3390/nu10111618

Yang, X., Yao, W., Shi, H., Liu, H., Li, Y., Gao, Y., et al. (2016). Paeoniflorin protects Schwann cells against high glucose induced oxidative injury by activating Nrf2/ARE pathway and inhibiting apoptosis. *J. Ethnopharmacol.* 185, 361–369. doi:10.1016/j.jep.2016.03.031

Yaribeygi, H., Sathyapalan, T., Atkin, S. L., and Sahebkar, A. (2020). Molecular mechanisms linking oxidative stress and diabetes mellitus. *Oxidative Med. Cell. Longev.* 2020, 8609213. doi:10.1155/2020/8609213

Yen, I. C., Lin, J.-C., Chen, Y., Tu, Q.-W., and Lee, S.-Y. (2020). Antrodia Cinnamomea attenuates non-alcoholic steatohepatitis by suppressing NLRP3 inflammasome activation *in vitro* and *in vivo*. *Am. J. Chin. Med.* 48 (8), 1859–1874. doi:10.1142/S0192415X20500937

Yoshitomi, T., and Nagasaki, Y. (2014). Reactive oxygen species-scavenging nanomedicines for the treatment of oxidative stress injuries. *Adv. Healthc. Mater.* 3 (8), 1149–1161. doi:10.1002/adhm.201300576

Yu, E., Calvert, P. A., Mercer, J. R., Harrison, J., Baker, L., Figg, N. L., et al. (2013). Mitochondrial DNA damage can promote atherosclerosis independently of reactive oxygen species through effects on smooth muscle cells and monocytes and correlates with higher-risk plaques in humans. *Circulation* 128 (7), 702–712. doi:10.1161/CIRCULATIONAHA.113.002271

Yu, L., Hong, W., Lu, S., Li, Y., Guan, Y., Weng, X., et al. (2022). The NLRP3 inflammasome in non-alcoholic fatty liver disease and steatohepatitis: Therapeutic targets and treatment. *Front. Pharmacol.* 13, 780496. doi:10.3389/fphar.2022.780496

Zeeshan, H. M. A., Lee, G. H., Kim, H.-R., and Chae, H.-J. (2016). Endoplasmic reticulum stress and associated ROS. *Int. J. Mol. Sci.* 17 (3), 327. doi:10.3390/ijms17030327

Zeng, Z., He, X., Li, C., Lin, S., Chen, H., Liu, L., et al. (2021). Oral delivery of antioxidant enzymes for effective treatment of inflammatory disease. *Biomaterials* 271, 120753. doi:10.1016/j.biomaterials.2021.120753

Zhang, J., Li, S., Li, S., Zhang, S. Q., Wang, Y. H., Jin, S. P., et al. (2020). Effect of icariside II and metformin on penile erectile function, glucose metabolism, reaction oxygen species, superoxide dismutase, and mitochondrial autophagy in type 2 diabetic rats with erectile dysfunction. *Transl. Androl. UROLOGY* 9 (2), 355–366. doi:10.21037/tau.2020.02.07

Zhang, M. H., Feng, L., Zhu, M. M., Gu, J. F., Jiang, J., Cheng, X. D., et al. (2014). The anti-inflammation effect of Moutan Cortex on advanced glycation end products-induced rat mesangial cells dysfunction and High-glucose-fat diet and streptozotocin-induced diabetic nephropathy rats. *J. Ethnopharmacol.* 151 (1), 591–600. doi:10.1016/j.jep.2013.11.015

Zhang, Q., Liu, J., Duan, H., Li, R., Peng, W., and Wu, C. (2021). Activation of Nrf2/HO-1 signaling: An important molecular mechanism of herbal medicine in the treatment of atherosclerosis via the protection of vascular endothelial cells from oxidative stress. *J. Adv. Res.* 34, 43–63. doi:10.1016/j.jare.2021.06.023

Zhang, S., Wang, W. D., Ma, J., Sheng, L., Zhang, D. M., and Chen, X. G. (2019). Coumarin glycosides from Hydrangea paniculata slow down the progression of diabetic nephropathy by targeting Nrf2 anti-oxidation and smad2/3-mediated profibrosis. *PHYTOMEDICINE* 57, 385–395. doi:10.1016/j.phymed.2018.12.045

Zhang, X. G., Liu, A. X., Zhang, Y. X., Zhou, M. Y., Li, X. Y., Fu, M. H., et al. (2022a). A diarylheptanoid compound from Alpinia officinarum Hance ameliorates high glucose-induced insulin resistance by regulating PI3K/AKT-Nrf2-GSK3 beta signaling pathways in HepG2 cells. *J. Ethnopharmacol.* 295, 115397. doi:10.1016/j.jep.2022.115397

Zhang, Z., Dalan, R., Hu, Z., Wang, J.-W., Chew, N. W., Poh, K.-K., et al. (2022b). Reactive oxygen species scavenging nanomedicine for the treatment of ischemic heart disease. *Adv. Mater. Deerf. Beach, Fla.)* 34 (35), e2202169. doi:10.1002/adma.202202169

Zhao, W. W., Feng, H. T., Guo, S. H., Han, Y. T., and Chen, X. P. (2017a). Danshenol A inhibits TNF-alpha-induced expression of intercellular adhesion molecule-1 (ICAM-1) mediated by NOX4 in endothelial cells. *Sci. Rep.* 7, 12953. doi:10.1038/s41598-017-13072-1

Zhao, W. W., Yuan, Y., Zhao, H. Y., Han, Y. T., and Chen, X. P. (2019). Aqueous extract of Salvia miltiorrhiza Bunge-Radix Puerariae herb pair ameliorates diabetic vascular injury by inhibiting oxidative stress in streptozotocin-induced diabetic rats. *FOOD Chem. Toxicol.* 129, 97–107. doi:10.1016/j.fct.2019.04.018

Zhao, Y., Hu, X., Liu, Y., Dong, S., Wen, Z., He, W., et al. (2017b). ROS signaling under metabolic stress: Cross-talk between AMPK and AKT pathway. *Mol. Cancer* 16 (1), 79. doi:10.1186/s12943-017-0648-1

Zhou, L., Liu, Y., Wang, Z., Liu, D., Xie, B., Zhang, Y., et al. (2021a). Activation of NADPH oxidase mediates mitochondrial oxidative stress and atrial remodeling in diabetic rabbits. *Life Sci.* 272, 119240. doi:10.1016/j.lfs.2021.119240

Zhou, Y., Zhou, C., Zhang, X., Vong, C. T., Wang, Y., and Cheang, W. S. (2021b). Coptisine attenuates diabetes-associated endothelial dysfunction through inhibition of endoplasmic reticulum stress and oxidative stress. *Mol. (Basel, Switz.* 26 (14), 4210. doi:10.3390/molecules26144210

Zhu, Z. S., Li, J. Y., and Zhang, X. R. (2019). Astragaloside IV protects against oxidized low-density lipoprotein (ox-LDL)-Induced endothelial cell injury by reducing oxidative stress and inflammation. *Med. Sci. Monit.* 25, 2132–2140. doi:10.12659/MSM.912894

Zmijewski, J. W., Banerjee, S., Bae, H., Friggeri, A., Lazarowski, E. R., and Abraham, E. (2010). Exposure to hydrogen peroxide induces oxidation and activation of AMP-activated protein kinase. *J. Biol. Chem.* 285 (43), 33154–33164. doi:10.1074/jbc.M110.143685

Glossary

AGE	Advanced glycation
AMPK	AMP-activated protein kinase
AS	Atherosclerosis
ATP	Adenosine triphosphate
BBR	Berberine
DA	Danshenol A
DM	Diabetes mellitus
DN	Diabetic nephropathy
ER	Endoplasmic reticulum
ETC	Electron transport chain
ETF	Electron transfer flavoprotein
FOXO	Forkhead box O
GLUT-4	Glucose transporter-4
GPX	Glutathione peroxidase
GSH	Glutathione
H2O2	Hydrogen peroxide
HDL	High-density lipoprotein
HUVECs	Human umbilical vein endothelial cells
ICAM-1	Intercellular adhesion molecule-1
IR	Insulin resistance
IRS	Insulin receptor substrate
JNK	C-Jun N-terminal kinase
LDL	Low-density lipoprotein
LOE	*Lindera obtusiloba* extract
LOX	Lipoxygenase
MMP	Mitochondrial membrane potential
mtDNA	Mitochondrial DNA
mTOR	Mammalian target of rapamycin
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NAFLD	Non-alcoholic fatty liver disease
NOX	Nicotinamide adenine dinucleotide phosphate hydrogen oxidases
O2-	Superoxide
OS	Oxidative stress
PARP	Polymerase
PH	Hydrogen ion concentration
PI3K	Phosphatidylinositol 3-kinases
PKC	Protein kinase c
REDOX	Reduction and oxidation
ROS	Radical oxygen species
SOD	Superoxide dismutase
TNF	Tumor necrosis factor
VCAM-1	Vascular cell adhesion molecule-1
VLDL	Very low-density lipoprotein
VSMC	Vascular smooth muscle cell

frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 11 July 2023
DOI 10.3389/fphar.2023.1184572

OPEN ACCESS

EDITED BY
Yongmei Xie,
Sichuan University, China

REVIEWED BY
Xichun Pan,
Army Medical University, China
Chongbo Zhao,
Shaanxi University of Chinese Medicine, China
Mingxing Hu,
Sichuan University, China

*CORRESPONDENCE
Si-Jing Liu,
liusijing@cdutcm.edu.cn
Jin-Lin Guo,
guo596@cdutcm.edu.cn

RECEIVED 12 March 2023
ACCEPTED 04 July 2023
PUBLISHED 11 July 2023

CITATION
He L-Y, Li Y, Niu S-Q, Bai J, Liu S-J and Guo J-L (2023), Polysaccharides from natural resource: ameliorate type 2 diabetes mellitus via regulation of oxidative stress network.
Front. Pharmacol. 14:1184572.
doi: 10.3389/fphar.2023.1184572

Polysaccharides from natural resource: ameliorate type 2 diabetes mellitus via regulation of oxidative stress network

Li-Ying He[1], Yong Li[1], Shu-Qi Niu[2,3], Jing Bai[2], Si-Jing Liu[2,3]* and Jin-Lin Guo[1,2,3]*

[1]Key Laboratory of Characteristic Chinese Medicine Resources in Southwest China, College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]College of Medical Technology, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [3]Chongqing Key Laboratory of Sichuan-Chongqing Co Construction for Diagnosis and Treatment of Infectious Diseases Integrated Traditional Chinese and Western Medicine, Chongqing, China

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia that can occur in children, adults, elderly people, and pregnant women. Oxidative stress is a significant adverse factor in the pathogenesis of DM, especially type 2 diabetes mellitus (T2DM), and metabolic syndrome. Natural polysaccharides are macromolecular compounds widely distributed in nature. Some polysaccharides derived from edible plants and microorganisms were reported as early as 10 years ago. However, the structural characterization of polysaccharides and their therapeutic mechanisms in diabetes are relatively shallow, limiting the application of polysaccharides. With further research, more natural polysaccharides have been reported to have antioxidant activity and therapeutic effects in diabetes, including plant polysaccharides, microbial polysaccharides, and polysaccharides from marine organisms and animals. Therefore, this paper summarizes the natural polysaccharides that have therapeutic potential for diabetes in the past 5 years, elucidating their pharmacological mechanisms and identified primary structures. It is expected to provide some reference for the application of polysaccharides, and provide a valuable resource for the development of new diabetic drugs.

KEYWORDS

diabetes mellitus, natural polysaccharide, oxidative stress, metabolism, mechanism

1 Introduction

Diabetes mellitus (DM) is a metabolic disease with symptoms of hyperglycemia. It can be caused by the deficiency of insulin secretion or insulin action in islet β cells (Galicia-Garcia et al., 2020). In 2015, approximately 415 million people worldwide had diabetes; of them, more than 90% had type 2 DM (T2DM). This number is projected to increase to 642 million in 2040 (Chen, 2015; Chatterjee et al., 2017). In addition, the onset of T2DM is often associated with conditions such as chronic hyperglycemia, hyperlipidemia and hypertension, placing a great burden on the healthcare system (Gerich, 2003; Taylor, 2013; Chatterjee et al., 2017). Physical activity has been reported to improve symptoms in diabetic patients with OS (Leeuwenburgh et al., 1994; Venkatasamy et al., 2013), but people seem to lack the time to exercise. Nowadays, unhealthy lifestyles and dietary factors are aggravating this condition

(Dos Santos et al., 2019; Salazar-Garcia and Corona, 2021). Diabetes is still a key topic worthy of continued attention of scholars.

Insulin resistance is a major feature in T2DM development (Gerich, 2003) and may be caused by an insulin signalling defect, inflammatory cytokines (Halim and Halim, 2019), lipotoxicity (Kelly et al., 2019; Poznyak et al., 2020), glucose transporter defect, amyloid formation for β-cell dysfunction (Eizirik et al., 2020), OS (Dos Santos et al., 2019), mitochondrial dysfunction, excess fatty acid, or lack of the cretin effect (Galicia-Garcia et al., 2020). At present, according to the manifestations of diabetes, many treatment methods are available for T2DM. In addition to injectable preparations such as insulin and insulin analogs, glucagon-like peptide 1and oral hypoglycemic drugs, such as peptidyl peptidase-4 (DPP-4) inhibitors and sodium-glucose co-transporter 2 (SGLT2) inhibitor, are commonly used to treat diabetes (Chatterjee et al., 2017). New methods have recently been used to treat diabetes, including islet transplantation, gene therapy (Green et al., 2018), and combination therapy. Islet transplantation can effectively control blood sugar and prevent long-term complications by increasing the number of islet cells in patients, to reduce their dependence on exogenous insulin and rebuild their physiological regulation of blood sugar (Bertuzzi et al., 2018; Rickels and Robertson, 2019). However, reducing inflammation and adverse effects around transplantation is a problem associated with this method that needs to be urgently resolved. Stem cell transplantation can help increase β cells content. However, the medical evidence of the efficacy and safety of stem cell therapy to support its routine use for T2DM is insufficient (Chatterjee et al., 2017). Moreover, when single drug therapy is insufficient for controlling T2DM, drug combinations can be used to achieve rapid improvement in blood glucose levels. For example, better outcomes can be achieved with a combination of the DPP-4 inhibitor and metformin or TZD pioglitazone, and insulin and metformin, glyburide, and pioglitazone (Cersosimo et al., 2018). Combination therapy appears to be more effective than single-agent therapy, however, complex multi-agent regimens, higher costs, and reduced patient compliance make it difficult to determine the efficacy of combination therapies (Miccoli et al., 2011). Meanwhile, many adverse reactions associated with these drugs, including hypoglycemia and gastrointestinal problems, can cause extremely painful experiences for patients (Ganesan and Xu, 2019). The development of safer and more economical hypoglycemic drugs has become the need of the hour urgent problem for researchers.

As the advantages of traditional Chinese medicine (TCM) in chronic diseases continue to manifest, several TCMs are used in the treatment of diabetes with mild side effects (Ganesan and Xu, 2019; Zheng et al., 2019). Polysaccharide is a type of bioactive macromolecule present in almost every TCM and food, and plays crucial role in new drug development. It have been found to have antioxidant, antitumor, and immune, regulatory properties and can improve intestinal microorganisms and hypoglycemic effects (Wang et al., 2016; Zheng et al., 2019). Several studies have reported that polysaccharides from natural products may improve diabetes symptoms mainly through regulating the OS (Dos Santos et al., 2019; Zheng et al., 2019), inhibiting α-amylase and α-glucosidase (Qiu et al., 2022), enhancing insulin secretion and improving glucose metabolism in a non-insulin-dependent manner. OS has been recognized as a potential causative factor for diabetes (Maritim et al., 2003; Thakur et al., 2018; Kang and Yang, 2020), and therefore, we tried to summarize natural polysaccharides that can act on T2DM through the OS pathway, hoping to provide a data reference for developing of new therapeutic agents for diabetes.

2 Relationship between oxidative stress and diabetes mellitus

Hyperglycemia is a chronic T2DM manifestation, prolonged hyperglycaemia and hyperlipidaemia in T2DM can lead to OS (Nishikawa et al., 2000; Giacco and Brownlee, 2010). OS may aggravate insulin resistance in diabetic patients (Dos Santos et al., 2019). Impaired insulin signalling disrupts the glucose flow into fat cells (Yaribeygi et al., 2019). As the result reactive oxygen species (ROS) and reactive nitrogen species (RNS) overproduction in the cytosol or mitochondria, which counteract the cellular redox balance and induces more OS (Petersen et al., 2003; Ceriello et al., 2009; Salazar-García and Corona, 2021; Liu et al., 2022). Simultaneously, ROS overproduction can damage lipids, proteins, and DNA, leading to the deterioration of β cell function (Omolola et al., 2014; Ezraty et al., 2017). Another momentous reason for OS is the overproduction of free radicals due to mitochondrial dysfunction (Schieber and Chandel, 2014). Furthermore, the levels of plasma free fatty acids (FFAs) are higher in T2DM patients. The increased FFAs levels also affects mitochondrial function, resulting in impaired insulin signalling pathways and lower nitric oxide (NO) levels (Ghosh et al., 2017), and higher ROS contents. Eventually, at high glucose levels, OS induction through regulating the protein kinase C (PKC), glycolysis, hexosamine, polyols pathway, and advanced glycation end products (AGE). In summary, OS plays a pivotal role in the mechanism of DM and related complications (Maritim et al., 2003; Hu et al., 2018). The mechanism of OS in diabetic patients is presented in Figure 1.

2.1 Hyperglycemic condition-induced oxidative stress in diabetes

Constant high glucose levels activate the polyol pathway, and formation of advanced glycosylation end products (AGEs); upregulate the hexosamine pathway (Kang and Yang, 2020); and activate PKC isoforms, leading to OS and eventually worsening of T2DM (Ighodaro, 2018; Thakur et al., 2018). Hyperglycemia influx to the glycolytic pathway and activation of the polyol pathway result in NADH overproduction and higher ROS levels (Mima, 2016). Furthermore, NADPH overproduced leads to a decrease in the intracellular concentration of glutathione (GSH), an antioxidant (Giannini et al., 2011). Moreover, NADPH plays an important role in AGEs-RAGE activation and OS (Ceriello et al., 2009). AGEs are associated with several molecules that enhance oxidative activity, and the activated AGE pathway also ultimately results in increased ROS production (Yamagishi and Matsui, 2010). The higher NADH/NAD^+ ratio also leads to low-density lipoprotein (LDL) oxidation, cytotoxic effects, decrease in NO levels, and increase of O^{2-} in cells (Eftekharpour and Fernyhough, 2022) (Kessler et al., 1998; Giannini et al., 2011). Excessive production of free radicals damages the body's antioxidant defense system, and has toxic effects, which cause

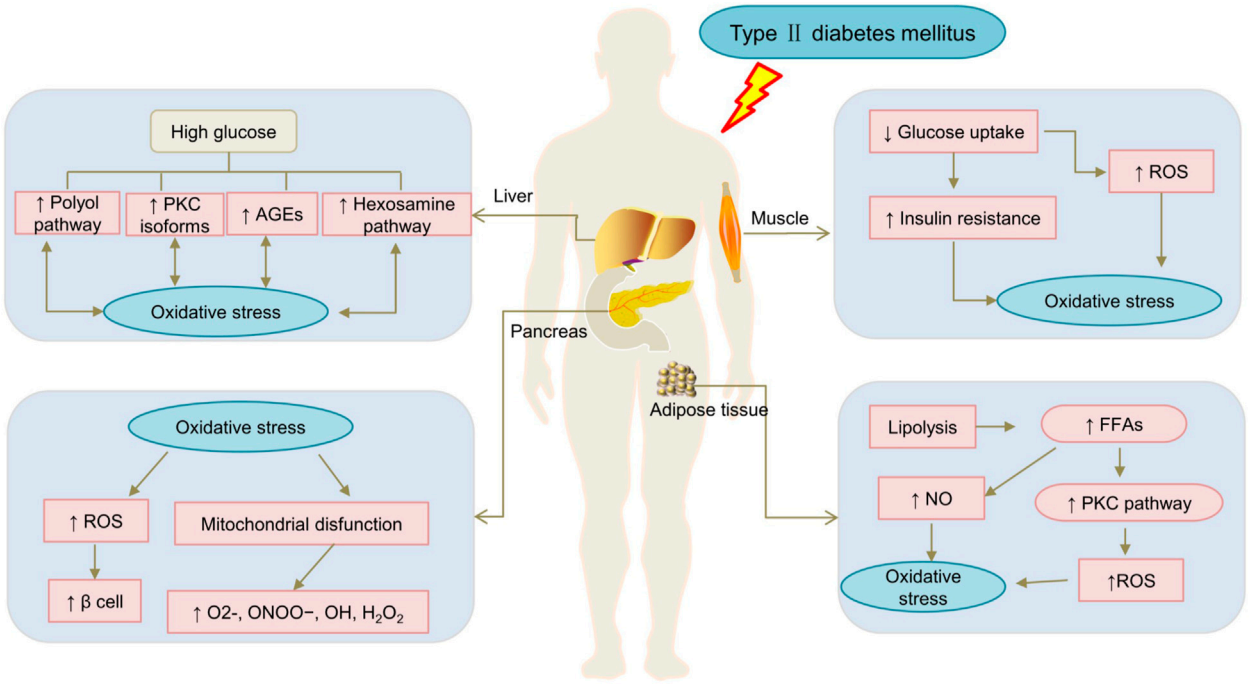


FIGURE 1
The mechanism of oxidative stress in diabetic patients. AGE, advanced glycosylation end products; FFAs, Free fatty acids. NO, nitric oxide; PKC, protein kinase C; ROS, reactive oxygen species; ↓: downregulated; ↑:upregulated.

OS (Halim and Halim, 2019) (Spinelli and Haigis, 2018) (Sergi et al., 2019). The activated polyol pathway also leads to immoderate and continuous activation of several PKC isoforms, which prevents NO release and thus a reduction in NO levels (Goncalves et al., 2022). The mechanism of hyperglycemic condition-induced OS in diabetes is shown in Figure 2.

2.2 Mitochondrial dysfunction caused by oxidative stress in diabetes

Mitochondria, also known as the "energy house" [39], are highly dynamic double-membrane organelles that produce adenosine triphosphate (ATP), the energy that the body requires (Newmeyer and Ferguson-Miller, 2003). Cellular energy production mainly occurs through two pathways: glycolysis and oxidative phosphorylation. Abnormal metabolism in diabetes can lead to excess production of mitochondrial superoxide in endothelial cells (Kaneto et al., 2016), thus damaging the mitochondrial respiratory chain (Maritim et al., 2003; Hu et al., 2018). Mitochondrial dysfunction that occurs in T2DM is inseparable from the glycolytic pathway, presumably it involves increased ROS production and energy expenditure (Qi et al., 2017). It also leads to the production of more free radicals (O^{2-}, $ONOO^{-}$, OH, and H_2O_2) (Erejuwa, 2012), ROS production (Wu et al., 2018), and ultimately decrease glucose homeostasis and insulin sensitivity in the liver (Yang et al., 2010). In addition to their role in energy production, mitochondria are responsible for FFA synthesis, which is an important consideration in DM (Yao L. et al., 2022). Especially in some obese diabetic patients, FFAs increased significantly (Ghosh et al., 2017). Excessive exposure to FFAs results in mitochondrial dysfunction and the increased formation of the toxic product MDA (Wu et al., 2018). In conclusion, mitochondrial dysfunction accelerates T2DM the development as shown in Figure 3.

3 Application of natural polysaccharides in treating diabetes by reducing oxidative stress

As a kind of complex biological macromolecule, polysaccharides have many structural characteristics, such as molecular weight, monosaccharide composition, glucoside bond, branching degree and high chain conformation, which are important factors affecting their functional properties. Due to their near non-toxic properties (Ganesan and Xu, 2019), the activity of natural polysaccharides has received more attention. With the deepening of the research, the chemical structure of polysaccharide and its mechanism of action in the treatment of diabetes have been reported more. It can control diabetes and reduce its complications through various mechanisms. For example, polysaccharides purified from saffron, curcumin, cinnamon and garlic have clear antioxidant properties, and those purified from pumpkin, wolfberry, sea cucumber, mushroom, tea, and beans have good effects on glucose homeostasis, reduce diabetic complications, and ultimately improve insulin sensitivity through the anti-OS damage defense mechanism (Liu et al., 2016). Thus, natural polysaccharides have great potential in improving OS and

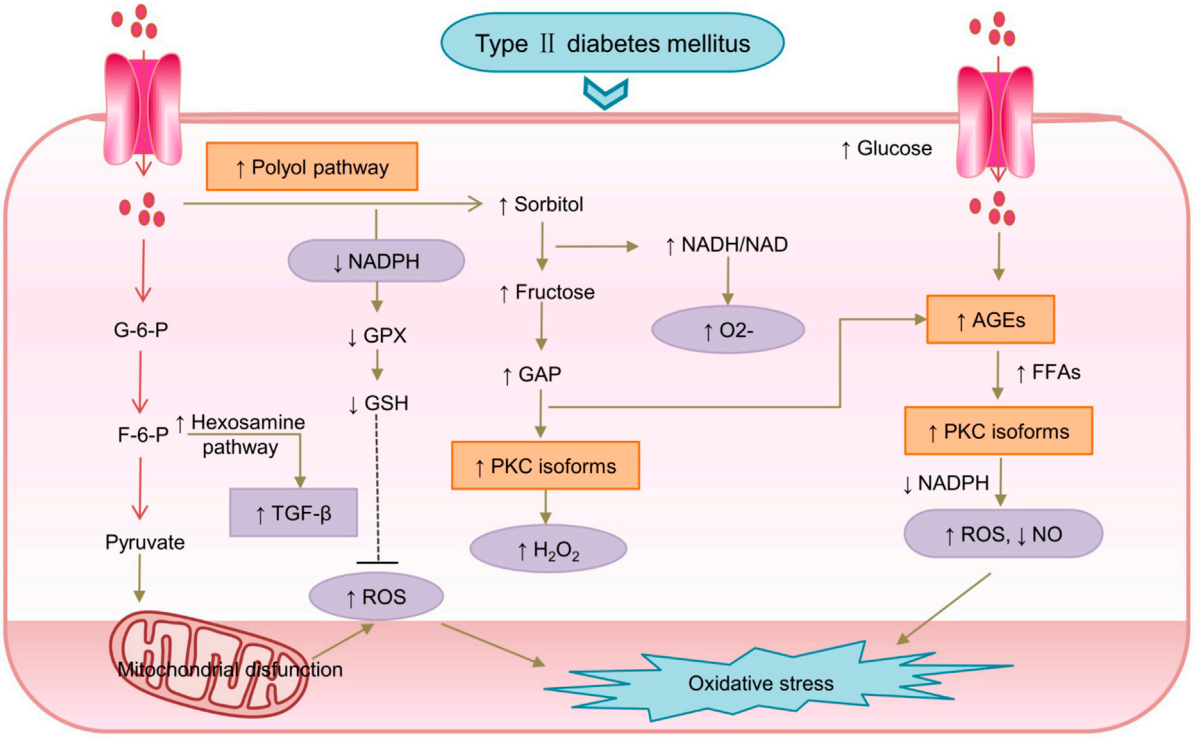


FIGURE 2
The mechanism of hyperglycemic conditions induced-oxidative stress in diabetes.F-6-P, Fructose-6-P; G-6-P, Glucose-6-P; GSH, glutathione; NO, nitric oxide; TGF-β, transforming growth factor beta; ↓: downregulated; ↑:upregulated.

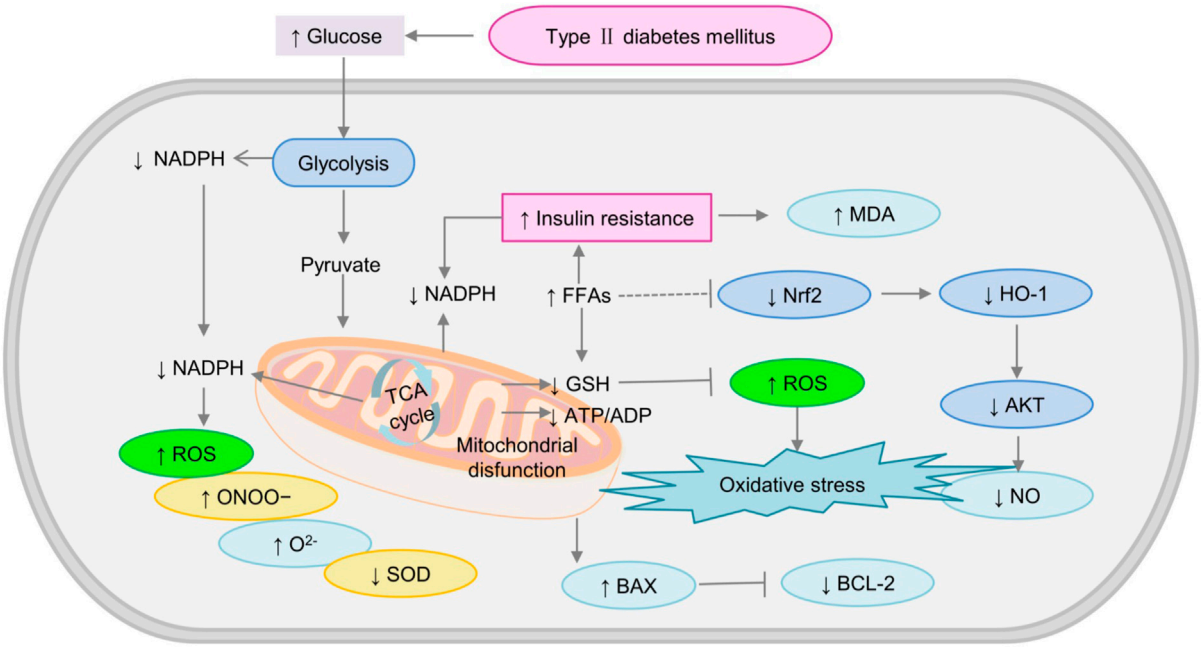


FIGURE 3
The mechanism of mitochondrial dysfunction induced-oxidative stress in diabetes. AKT, Phosphorylated protein kinase B; BAX, Bcl-2-associated X protein (BAX); HO-1, heme oxygenase-1; MDA, malondialdehyde; Nrf2, nuclear factor erythroid-2; SOD, superoxide dismutase; ↓: downregulated; ↑: upregulated.

treating diabetes. In order to accelerate the study of the structure and therapeutic mechanism of natural polysaccharides, we classified the antioxidant polysaccharides with the potential to treat diabetes into 4 categories according to the sources of natural polysaccharides, such as plant polysaccharides, microbial polysaccharides, Marine polysaccharides, and animal polysaccharides.

3.1 Polysaccharides derived from plants

3.1.1 Medicinal plant

The *Abelmoschus esculentus* polysaccharides (AEP) were reported to exhibit anti-oxidative and hypoglycemic activities. On the one hand, AEP can significantly increase the content of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), SOD2, GSH, and catalase (CAT), whereas decreases the content of ROS and malondialdehyde (MDA). In particular, It may alleviate T2DM by improving the phosphoinositide 3-kinase (PI3K)/Phosphorylated protein kinase B (AKT)/glycogen synthase kinase 3 beta (GSK3β) pathway, and activating nuclear factor erythroid-2/heme oxygenase-1(Nrf2/HO-1) pathway. Furthermore, AEP regulated mitochondrial dysfunction by inhibiting NOX2 activation in HFD and STZ treated mice (Liao et al., 2019a). Moreover, AEP obviously increased adenosine monophosphate-activated protein kinase (p-AMPK/AMPK), and exhibited anti-apoptotic effects by diminishing the levels of cleaved caspase-3, and bcl-2-associated X protein (Bax), and enhanced Bcl-2 expression in the same models (Liao et al., 2019b). These indicate that AEP can not only improve the content of antioxidant enzymes, but also improve mitochondrial function and oxidation-related pathways to relieve diabetic.

The *Cyclocarya paliurus* polysaccharide (CCPP) could alleviate T2DM symptoms by boosting GSH-px, SOD, GSH, and CAT levels in diabetic rats (Zhang et al., 2021). In addition, compared with T2DM group, CCPP could upregulate SOD, CAT, GSH-Px, and GSH levels, whereas downregulated AGE and transforming growth factor-beta1 (TGF-β1) levels (Xia et al., 2020; Lin C. et al., 2021). Besides, CCPP significantly improved pathways closely with the nutrition metabolism (amino acids and purine) and energy metabolism (TCA cycle) to improve mitochondrial function (Li et al., 2021). Therefore, CCPP may improve the OS of diabetic patients by regulating AGE pathway to increase the content of antioxidant enzymes. In addition, in the same article, it was also reported to have a role in regulating inflammation and mitochondrial function.

Polysaccharides from the Chinese medicine *Fructus Corni* (FCPs) showed hypoglycemic, hypolipidemic and antioxidative effects in the STZ-induced diabetic rats by increased insulin secretion and promoted pancreatic β cell proliferation. In same study, FCPs have been found to increase the levels of antioxidant enzymes, such as SOD and GSH in STZ injected rats to alleviate diabetic (Wang D. et al., 2019). In a word, the potential mechanism of the hypoglycemic and hypolipidemic activities of FCP in diabetic related to OS. FCPC might have potential applications in regulating OS and T2DM.

The *Astragalus membranaceus* polysaccharide (AMP), as a main bioactive macromolecules of *A. membranaceus* (Fisch.), alleviates OS to relieve liver damage in T2DM mice (Chen et al., 2022). One possible therapeutic mechanism was observed in experimental mice that exhibited augmented SOD, GSH, and CAT levels, and decreased MDA levels after AMP treatment. In STZ-treated mice and heterozygous ($SOD2^{+/-}$) knockout mice, astragalus polysaccharides (APS) improved the damage of the cardiac stem and progenitor cells by inhibiting of apoptosis (Chen et al., 2018). In particular, APS has a beneficial effect on SOD2 enzyme activities. Notably, APS reduced cellular ROS levels, and inhibited OS injury indicators, that are 8-Hydroxy-2′-deoxyguanosine (8-OH-dG) and nitrotyrosine levels, in high glucose-induced H9C2 cells (Sun et al., 2019). Moreover, APS can promote SOD, and GSH-Px levels and diminish ROS, MDA, and NO levels in an AGE-induced H9C2 cell model, thereby performing the anti-oxidative function (Chang et al., 2018). Otherwise, APS may display a protective function by activating NGR1/ErbB signalling and the downstream AKT/PI3K signalling pathway (Chang et al., 2018). APS can regulate multiple pathways to improve the etiology and complications of DM (Zhang Z. et al., 2019).

Polysaccharides from *Hedysarum alpinum* (HPS) are an active ingredient for DM treatment. HPS markedly revises OS damage in DM mouse models and cell model. Mechanistically, it inhibits Keap1 signalling and upregulates Nrf2 signalling pathway. Besides, HPS significantly suppressed MDA concentration and enhanced the concentration of antioxidant enzymes in HG-induced Schwann cells. Moreover, HPS stimulated Nrf2 signalling, whereas decreased Keap1 expression in SCs (He et al., 2022). Therefore, HPS has been demonstrated to have antioxidant activity both *in vivo* and *in vitro* and can be considered as a potential diabetes treatment.

The *Morus alba* polysaccharide (MLP) can ameliorate diabetes by improving OS injury and the mitochondrial function of islet cells in HFD- and STZ-induced diabetic rats. Furthermore, MLP inhibits MDA production, but promotes SOD and SDH activities (Liu et al., 2017). In 2015, MLP was found to activate the PI3K/AKT pathway and mitigate OS in HFD- and STZ-induced rats (Ren et al., 2015). These results suggest that MLP has the potential to treat diabetes. MLP could improve OS by reducing MDA content, enhancing SOD, CAT, and GPx activities, and regulating amino acid and lipid metabolism by decreasing TG and TC levels. Additionally, blood urea nitrogen (BUN), albumin (ALB), Creatinine (CRE), and UA (uric acid) levels, and the expression of PI3K of insulin receptor substrate 2 (IRS2) and the PKB/AKT pathway relative to insulin signalling were significantly increased with the MLPs. Finally, glucose levels were restored in T2DM rats (Li et al., 2022).

The *Dendrobium officinale* polysaccharide (DOP) can ameliorate hyperglycemia, improve β-cell function inflammation, regulate lipid concentration, inhibit insulin resistance, and regulate OS to ameliorate diabetes (Yang J. et al., 2020). Specifically, compared with normal C57BL/6 male mice, SOD and CAT concentrations in the liver were significantly increased after DOP administration in T2DM rats (Yang J. et al., 2020). DOP can also improve AKT signalling pathways (Liu et al., 2020b).

Polygonatum sibiricum polysaccharides (PSP), as an antioxidant activity component, have been found to have anti-diabetic effects. PSP attenuates diabetes by decreasing ROS production and MDA levels, and augmenting SOD and GPx activities. PSP also scavenged the activation of the Nrf2/HO-1 pathway in HG-induced ARPE-19 cells. Moreover, PSP significantly regulated apoptosis by

diminishing bax and caspase-3 expression, and promoting Bcl-2 production (Wang et al., 2022). In another *in vivo* study, PSP was advantageous in controlling polydipsia, polyuria, polyphagia, and weight loss in DM rats. With PSP, MDA content was lowered, and cataract progression was delayed. Thus, PSP may alleviate the progression of diabetes by alleviating OS (Wang et al., 2017d). Simultaneously, PSP pre-treatment significantly alleviated the IR effect in IR-3T3-L1 adipocytes by inhibiting IL-6, IL-1β, and TNF-α levels and promoting Nrf2 and HO-1 expression (Cai et al., 2019).

The polysaccharide (CLP) of *Codonopsis lanceolata*, as an important bio-active, has been used to treat diabetes. It improves insulin sensitivity by regulating Akt pathway and hyperphosphorylation of IRS-1, decrease MDA levels, elevates SOD, and CAT levels, and the GSH/oxidized glutathione ratio, and activates Nrf2 signalling in chronic high fat/high sucrose (HFHS) diet-fed mice (Zhang et al., 2020). CLP through activating Nrf2 signaling pathway and increased antioxidant oxidase content regulated HFHS diet-induced insulin resistance, offers the possibility that the CLP can be used as a treatment for diabetes.

The Polysaccharide (DAP) from the roots of *Dipsacus asper* has been used in STZ-induced diabetic rats. After administration with DAP, the fasting blood glucose and glycosylated haemoglobin (HbA1c) levels were decreased, but the body weight increase in STZ-induced diabetic rats. Moreover, advanced glycation end products-receptor accumulation were nearly reversed to the normal levels along with inhibition of AGE-RAGE expression in STZ-treated diabetic rat by DAP (Li T. et al., 2019).

The *Ophiopogon japonicus* polysaccharide (OJP) exhibited antioxidant effects by efficiently diminishing the levels of free radicals (DPPH and ABTS), markedly enhancing the NO production, and diminishing the mRNA expressions of iNOS, COX2 in nickel-induced RAW264.7 cells (Chen et al., 2013; Lin et al., 2020). Moreover, with OJP treatment, the levels of TG, TC, HDL-C and LDL-C were obviously reversed in diabetic rats. Altogether, these results suggest that OJP could be a natural antioxidant and can improve diabetes (Chen et al., 2013).

The polysaccharide isolated from *Rehmannia glutinosa* (RGP), as a main active ingredient exhibited hypoglycemic effects (Zhou et al., 2015a). In STZ-induced mice, RGP dispiayed anti-oxidative activities by elevating serum GPx and SOD levels, whereas decreasing blood levels of IL-6, MDA, TNF-α, and monocyte chemoattractant protein-1 (MCP-1) (Zhou et al., 2015a). Moreover, with RGP administration, high levels of TG, TC, LDL-C were reversed and HDL-C increased in diabetic mice.

Polysaccharides obtained from guava (*Psidium guajava* L.) Leaves (GLP) showed antioxidant and antidiabetic activities. In a STZ + HFD induced diabetic mice. These polysaccharides exhibited good OH, ABTS free-radical, and DPPH scavenging abilities, and significantly ameliorated fasting blood sugar, Total cholesterol (TC), MDA, creatinine, and Triglycerides (TG) levels. Meanwhile, it significantly increased SOD enzyme activity (Luo et al., 2019). Thus, GLP might be a promising candidate for the development of a new anti-diabetic or antioxidant agents. However, its clinical efficacy needs to be further verified.

Holothurian glycosaminoglycan from *Apostichopus japonicus* (AHG) can suppress hepatic glucose production for the development of an anti-hyperglycemic agent. In HFD-fed mice, AHG supplementation apparently reduced conventional index, such as body weight, blood glucose level, and serum insulin content. In same model, AHG elevated phosphorylation levels of insulin receptor substrate-1(IRS1), Akt, and AMPK levels that were decreased by HFD treatment (Chen Y. et al., 2019).

The *Pueraria lobata* polysaccharide (PLP) improved diabetic by scavenging blood glucose levels and alleviating the lipid metabolic function of db/db mice. In particular, PLP boosted the expression of G6PC, FOXO1, PEPCK, SREBP-1, and ACC, whereas diminished the mRNA expression of antioxidant-related indicators, such as glycogen synthase, phosphatidylinositol-3-kinase, Akt2, PPARa, and glucose transporter 2 (GLUT2) in the liver of db/db mice (Luo et al., 2021).

Lycium barbarum polysaccharides (LBPs) extracted from L. barbarum, markedly increased insulin secretion and insulin sensitizing activities to improve insulin resistance. They also regulated glucose metabolism through hypoglycemic and antioxidative activities to relieve diabetes (Li, 2007; Cheng et al., 2015; Shi et al., 2017). Compared with the diabetic group, LBPs promoted the production of antioxidant enzymes such as SOD, CAT, and GPx, while down-regulating Caspase-3 expression and up-regulating the ratio of Bcl-2/Bax in STZ-included diabetes mice (Shi et al., 2017). Simultaneously, LBPs activated the PI3K/Akt/mTOR pathway, and Nrf2/HO-1 pathway, whereas inhibited the AGEs-RAGE signalling pathway to mitigate OS (Li, 2007; Tian et al., 2019).

Euryale ferox polysaccharides (EFP) exhibited protective effects against alloxan-induced oxidative injury in mice. Furthermore, EFPPs can enhance CAT, SOD and GSH-Px expression and scavenging of MDA content in the liver and kidney of alloxan-induced hyperglycemic mice. In addition, EFPP was beneficial enhancing oral glucose tolerance, and hepatic glycogen content, but reversed blood glucose levels and relieved diabetes (Wu C. Y. et al., 2017).

Physalis alkekengi polysaccharides (PAP) have been reported to have an antidiabetic effect in mice. The PAP intervention decreased blood glucose and glycated serum protein levels. Moreover, PAP protected and reversed β-cell necrosis and upregulated the mRNA expression of PI3K, GLUT4, and Akt in alloxan-induced diabetic mice (Guo et al., 2017). Consequently, PAP could be explored as a potential for the development of a new treatment diabetic agent.

Schisandra sphenanthera polysaccharides (SSPs) regulated hyperglycemia, improved the glucose tolerance, reduced FBG, and elevated the levels of FINS and the value of ISI in STZ induced rats. After SSPs administration, MDA levels were also repressed and SOD, GSH-PX and CAT activities were enhanced (Niu et al., 2017).

The polysaccharide from Moutan Cortex (MC-P) ameliorates diabetes by down-regulating the AGEs pathway. It also decreased ROS production induced by AGEs in HG- and STZ-induced rats and the AGEs-stimulated human umbilical vein endothelial cells. Moreover, MC-P can regulate the production of inflammatory factors (VCAM-1 and ICAM-1) and improve TGF-β1 levels (Lian et al., 2021).

Arctium lappa polysaccharides (ALP) displayed anti-diabetes effect by regulating lipid metabolism and OS through the PKC/NF-κB pathway in diabetic rats. Administration with these polysaccharides ALP *in vivo*, led to upregulation of antioxidant

TABLE 1 Polysaccharides from medicinal plant with antioxidation and anti-diabetic effects.

Polysaccharide source	Monosaccharides composition	Models	Administration	Mechanism	Positive control	Reference
Abelmoschus esculentus (L.) Moench	Man: rha: glucuronic acid, galactosal acid, gal: ara = 3.4:3.76:24.19:6.27:8.73:3.13	HFD combined with STZ induced mice	200 or 400 mg/kg	↓: TG, TC, LDL-C; MDA, body weight, and ROS ↑: HDL-C, SOD, GSH-Px and CAT, Nrf2, HO-1, SOD2, PI3K/AKT/GSK3β pathway	Metformin	Liao et al. (2019a)
Abelmoschus esculentus (L.) Moench	Rha: Gal: Gal A = 1.87:3.58:1.00	HFD and STZ induced mice	200, 400 mg/kg	↓: ROS, MDA, caspase-3, and Bax ↑: SOD, Bcl-2 and CAT, Nrf2, HO-1, SOD2, AMPK/AMPK pathway	Metformin	Liao et al. (2019b)
Cyclocarya paliurus (Batal.) Iljinskaja	Glc: ara: gal: man: xyl: rha: galacturonic acid: glucuronic acid: fuc: rib = 27.90:9.68:7.67:1.93:1.67:1.26:0.72:0.66:0.17:0.16	STZ induced diabetic rats	1, 10 and 100 mg/kg/·d for 12 weeks	↑: SOD, CAT, GSH-Px, and GSH ↓: AGEs and TGF-β1	Metformin hydrochloride	Xia et al. (2020)
Cyclocarya paliurus (Batal.) Iljinskaja	Rha: ara: xyl: man: glu: gal	STZ induced rats	1, 10, 100 mg/kg/d for 12 weeks	↑:SOD, CAT, GSH-Px, GSH ↓: AGEs, ROS, and TGF-β1		Zhang et al. (2021)
Fructus Corni	Man: gal A, glc: xyl: gal: ara = 0.95:0.08:3.17:1.88:1.23:0.25	STZ-induced diabetic rats	800 mg/kg on 42nd day	↑: ACT, SOD, and GSH	Metformin hydrochloride	Wang et al. (2019a)
Astragali radix	Glc, gal A, ara, gal, glu A xyl	STZ induced male C57BL/6J mice	400 mg/kg/d	↑: SOD, GSH, CAT ↓: MDA levels		(Chen et al., 2022) (Chen et al., 2018)
Hedysarum alpinum L.		ob/ob mice	50, 100, and 200 mg/kg/d	↓: Keap1 signalling ↑: Nrf2 signalling	lipoic acid	He et al. (2022)
Hedysarum alpinum L.		HG induced Schwann cells	30, 60, 120, and 240 mg/L	↓: MDA ↑: GCLC, GR		He et al. (2022)
Morus alba L.		STZ+HFD induced rats		↓: MDA ↑: SOD, CCO and SDH		Liu et al. (2017)
Mulberry leaves		STZ induced diabetic rats	50–200 mg/kg for 5weeks	↓: MDA ↑: SOD		Li et al. (2022)
Dendrobium officinale Kimura & Migo	D-Glcp: D-Manp = 1.00:4.41. 190 kDa	male wistar rats	20, 40, 80, and 160 mg/kg/d for 8 weeks	↑: SOD, CAT, and T-AOC	Metformin	Yang et al. (2020a)
Polygonatum sibiricum Redouté		STZ induced rats	200, 400 and 800 mg/kg	↑: insulin ↓: MDA, blood glucose		Wang et al. (2017d)
Polygonatum sibiricum Redouté		HG-induced ARPE-19 cells		↓: MDA, ROS, bcl-2 ↑: SOD and GPx, Bax, caspase-3 activity, Nrf2/HO-1 pathway		Wang et al. (2022)

(Continued on following page)

TABLE 1 (*Continued*) Polysaccharides from medicinal plant with antioxidation and anti-diabetic effects.

Polysaccharide source	Monosaccharides composition	Models	Administration	Mechanism	Positive control	Reference
Polygonatum sibiricum Redouté		1 μmol/L insulin and 25 mmol/L glucose induced 3T3-L1 adipocytes	50, 100, 250, 500 μg/mL	↑: Nrf2,HO-1 ↓: IL-6, IL-1βand TNF-α		Cai et al. (2019)
Codonopsis lanceolata (Siebold & Zucc.) Benth. & Hook.f. ex Trautv	Rha: Ara:xyl:man: gal: glc: Gal A: glu A = 0.17:1:0.12:0.05:0.26:2.32:0.19:0.95	HFHS diet-fed mice	100 mg/kg	↓: MDA ↑:GSH/GSSG ratio, SOD, CAT, Nrf2 signalling		Zhang et al. (2020)
Dipsacus asper Wall. ex DC.	D-glu	STZ-induced diabetic rats	100 and 300 mg/kg for 4 weeks	Downregulated oxidative stress and glycation end products-receptor	Metformin	Li et al. (2019a)
Ophiopogon japonicus (Thunb.) Ker Gawl	Ara: Glu: gal = 1:16:8	STZ induced mice	150 mg/kg/d for 28 days	↑: GPx and SOD	Metformin	Chen et al. (2013)
Ophiopogon japonicus (Thunb.) Ker Gawl		RAW264.7 cells induced by nickel	10, 50, and 100 μg/mL for 24 h	↓: DPPH, ABTS, NO, iNOS, COX2		Lin et al. (2020)
Rehmannia glutinosa (Gaertn.) DC.	Rha: ara: man: glc: gal = 1.00:1.26:0.73: 16.45:30.40; 63.5 kDa	STZ-induced diabetic mice	20, 40 and 80 mg/kg/day	↑: GPx and SOD activities ↓: MDA, IL-6, TNF-α, and MCP-1	Metformin	Zhou et al. (2015a)
Psidium guajava L	polysaccharides (≥80.99 kDa) and low molecular weight polysaccharides (3.64 kDa)	STZ and HFD induced mice	100 mg/kg, 200 mg/kg/d	↑: GPx and SOD activities	Acarbose	Luo et al. (2019)
Apostichopus japonicus	→4GlcA (Fuc2S,4Sα1→3)β1→3GalNAc4S6Sβ1→	HFD for 12 weeks	50 mg/kg/day	↑: P- IRS1, Akt, and AMPK	Metformin	Chen et al. (2019b)
Pueraria lobata (Willd.) Ohwi (Fabaceae)		db/db mice	100 or 200 mg/kg for 6 weeks	↓: PEPCK, G6PC, FOXO1, SREBP-1, and ACC ↑: GS, Akt2, PI3K, GLUT2, PPARa, and LDLR	Rosiglitazone	Luo et al. (2021)
Lycium barbarum L	ara, glc, gal, man, rha, xyl, gal A	STZ-induced diabetic mice	50, 100, 200 mg/kg for 30 days	↓: MDA ↑: SOD		Li (2007)
		STZ-included mice	10, 20, and 40 mg/kg	↓: Caspase-3 ↑: Bcl-2/Bax	Sildenafil citrate	Shi et al. (2017)
Euryale ferox Salisb.	Man, GlcA, Rha, Glc, Gal and Ara at a molar ratio of 0.12:0.01:9.57:0.41:1.00:0.24	alloxan-induced hyperglycemic mice	100,200,400 mg/kg/d	↑: CAT, SOD, oral glucose tolerance, hepatic glycogen content and GSH-Px ↓: MDA, glucose level increase	metformin hydrochloride	Wu et al. (2017a)
Physalis alkekengi var. franchetii Makino		Alloxan-induced hyperglycemic mice	200, 400, and 800 mg/kg	↑: PI3K, Akt and GLUT4 mRNA; Hypoglycaemic; protecting and reverse β-cells from necrosis	Acarbose	Guo et al. (2017)
Schisandra sphenanthera Rehder & E.H.Wilson	Rha:Ara:Man:Gal:Glc = 13.52:5.69:3.92: 41.28:35.59	STZ induced rats	100,200,400 mg/kg/d	↑: GLUT-4; mitigate the insulin resistance; hpyerglycemic; improving the glucose tolerance	glibenclamide	Niu et al. (2017)

(Continued on following page)

TABLE 1 (*Continued*) Polysaccharides from medicinal plant with antioxidation and anti-diabetic effects.

Polysaccharide source	Monosaccharides composition	Models	Administration	Mechanism	Positive control	Reference
Paeonia × suffruticosa Andrews	D-glucose: L-arabinose = 3.31:2.25	high-sugar diet and STZ treatment rats	80, 160 mg kg	↓: serum creatinine, AGEs/RAGE		Lian et al. (2021)
Moutan Cortex		200 μg/mL AGEs induced human umbilical vein endothelial cells for 24 h	64.5 μg/mL	↓: ROS	Aminoguanidine	Lian et al. (2021)
Arctium lappa L	Gal: Man: Glc: Rha: Xyl: GlcN: GlcA = 6: 6.4:26.5: 3.3:26.2:7 2	high-sugar, HFD, and STZ	100, 200 mg/kg for 40 days	↓: PKC expression, MDA, and NF-κB pathway ↑: SOD and GSH px		Li et al. (2019b)
Anoectochilus roxburghii (Wall.) Lindl		HFD induced mice	100, 200, and 400 mg/kg	↑: GST, GPX, GST and SOD ↓: MDA, ATP		Chen et al. (2021)
Buddleja officinalis Maxim	glucose, galactose, fucose, glucuronic acid, and galacturonic acid in a ratio of 6.75 : 3.33 : 1.79 : 1.42 : 1.00	db/db mice	40,80,160 μg/mL	↑: HO-1, NQO1, SOD and Nrf2/ARE signalling pathway ↓: CD34, ROS		Zhu et al. (2022)
Opuntia milpa alta	Xylose: arabinose: galactose = 23:36:15	alloxan-treated INS-1 cells	50, 100, and 200 μg/mL	↑: Bcl-2, MDA, and NO ↓: Bax, ROS, caspase-3, caspase-9		Li et al. (2020b)
Astragalus mongholicus Bunge	D-glu: D-gal: L-ara = 1.75:1.63:1	HG induced H9C2 cells for 24 h	0.1,1,10,100 μg/mL	↑: SOD2, GSH-Px ↓: ROS, MDA, and NO		Sun et al. (2019)

enzymes such as SOD and GSH-Px, whereas a decrease in MDA levels decreased in the rat livers. Moreover, the expression of P-select, PKC-α, and PKC-β was obviously decreased. Meanwhile, the PKC/NF-κB signalling pathway was inhibited (Li X. et al., 2019).

The *Anoectochilus roxburghii* polysaccharide (ARP) has been reported to regulate OS. After the ARP intervention in HFD-induced male C57BL/6 mice for 12 weeks, the fatty acid pathway regulating lipogenesis was inhibited, the levels of antioxidant enzymes, such as GPX, glutathione S-transferase (GST) and SOD were increased, and MDA levels were decreased. Furthermore, the Nrf2-mediated phase II enzyme system (Nrf2/HO-1/NQO1) was activated to improve OS. Moreover, the mitochondrial complex and PGC-1α were activated to promote mitochondrial function. Furthermore, ARP also had some effects on reducing ROS levels and increasing ATP levels, thereby significantly improving diet-induced OS (Chen et al., 2021).

Buddleja officinalis (BOP) polysaccharides could improve diabetes by improving levels of blood glucose, blood lipids, and insulin and further improving CD34 expression. Furthermore, BOP diminished ROS levels, whereas promoted SOD, NQO1, Nrf2, and HO-1 expression levels in db/db mice to ameliorate diabetes (Zhu et al., 2022).

Opuntia milpa alta polysaccharides (MAPs) contain xylose (23%), arabinose (36%), and galactose (15%) and have been reported exhibit anti-diabetic effects. *In vitro* research, treatment with MAPs restored oxidative enzyme activities and cell viability improved NO, MDA and ROS, and regulated antioxidant PARP and Nrf2 pathways in alloxan-induced INS-1 cell. Moreover, MAPs regulated proteins related to apoptosis, such as the expression of Bcl-2, Bax, caspase-3, and caspase-9 (Li W. et al., 2020). These findings indicated that MAPs has the potential to regulate OS.

Astragalus mongholicus Bunge polysaccharides (APS) also repressed apoptosis by enhancing Bcl-2 levels and diminished Bax, caspase-3, and caspase-9 levels (Chang et al., 2018). In both STZ-induced diabetic mice and nondiabetic SOD2+/−mice, APS reduced the apoptosis and enhanced the proliferation of cardiac stem and progenitor cells (CSPCs). Furthermore, APS enhanced SOD2 protein levels and enzyme activities, and downregulated ROS formation and oxidative damage of CSPCs (Chen et al., 2018). In HG-induced or SOD2-silenced H9C2 cells, APS improved cell apoptosis and higher levels of ROS, and regulated the levels of oxidative stress injury indicators 8-OH-dG and nitrotyrosine (Sun et al., 2019). More polysaccharides from medicinal plant with antioxidation and anti-diabetic effects show in Table 1.

3.1.2 Food

The *Siraitia grosvenorii* polysaccharide (SGP-1) alleviated inflammation by reducing the cytokines IL-6 and TNF-α. Moreover, it regulated oxidation by stimulating SOD production of and diminished MDA in DN mouse models (Gong et al., 2021).

Polysaccharides obtained from the dried pumpkin pulp (PPs) have hypoglycemic effects (Wang S. et al., 2017). After PPs treatment OS was improved through the upregulation of the Nrf2, HO-1, NOS, MDA and PI3K levels (Chen X. et al., 2019). They also can reduce TG, TC, and LDL-C levels and improve HDL-C, SOD, PI3K levels in the hypoglycemic mechanism (Chen X. et al., 2019).

The *Ficus pumila Linn* polysaccharide (FPLP) improved glycogen metabolism by activating the phosphoenolpyruvate carboxykinase and glucose-6-phosphatase expression, regulating the IRS-1/PI3K/Akt/GSK3β/GS and AMPK/GSK3β/GS signalling pathway and glucokinase in C57BL/KsJ db/db mice (Wu J. et al., 2017). Mechanistically, the PI3K/Akt pathway and AMPK signalling pathway are closely related to the oxidation reaction process of the body. This suggests that FPLP may have the potential to ameliorate diabetes by regulating OS (Wu J. et al., 2017).

The peach [*Prunus persica* (L.)] tic gum polysaccharide (PGP) could restore the postprandial blood glucose levels in STZ-induced diabetic mice by recovering pancreaislets, and activating the expression of HO-1 and insulin, which are all beneficial for improving diabetes (Wang et al., 2017c).

The mulberry fruit polysaccharide (MFP) also possesses numerous bioactivities. In STZ induced diabetic mice deal with STZ, MFP can promote pancreatic β cells proliferation and thus increase insulin secretion. Moreover, MFP can improve diabetes by regulating OS, which is mainly manifested in inhibiting MDA content, and increasing SOD, GPx, and CAT levels (Chen et al., 2017).

The polysaccharide from the sweet potato (SPP) significantly alterated the lipid metabolism index (TC, TG, and LDL), while remarkably decreasing HDL levels in STZ-induced rats. Compared with the diabetic rats, the level of MDA decreased along with an increase in SOD, GSH-Px, GSH and CAT levels after SPP treatment (Yuan et al., 2017).

Momordica charantia L. has been reported to exhibit antiobesity and antidiabetes activities (Zhang et al., 2019). In an *in vitro* study, this bitter gourd polysaccharide improved diabetes mice by enhancing the concentration of CAT, SOD, and GSH-px antioxidant enzymes and decreasing the content of MDA in mice (Zhang et al., 2019).

The *Dipsacusa sper* polysaccharide (DAP) regulated hyperlipidemia, downregulated the formation of the advanced glycated end-product receptor (AGE-RAGE), and improved OS in STZ-treated diabetic rats (Li et al., 2019).

A novel polysaccharide (RTFP-3), extracted from *Rosa roxburghii* fruit, has been found have antioxidant activity. In H_2O_2-induced INS-1 cells, RTFP-3 possessed higher protective and suppressive activities against H_2O_2-induced apoptosis of INS-1 cells in comparison with normal. Additional, after treatment with RTFP-3, ROS was decreased in INS-1 cells, which indicated that RTFP-3 via attenuating oxidative stress to prevent dibaetes (Wang et al., 2019). More polysaccharides from foods with antioxidation and anti-diabetic effects show in Table 2.

3.2 Polysaccharides derived from Microorganisms

The *Agrocybe Cylindracea* polysaccharide (ACP) can decrease blood glucose levels, heal liver and colon injuries, and repress inflammation (Wu L. et al., 2022). It also can increase SOD, GSH-Px, and CAT levels and improve lipid metabolism in HFD combined STZ induced mice (Sun et al., 2022).

Cordyceps militaris (CM), as a TCM, has been reported have anti-diabetes effects (Chen, 2015). In animal experiments, it was found that CM polysaccharide (CMP) could downregulate the contents of lipid peroxidation, blood lipid and blood sugar. Besides, it could improve insulin resistance and blood glucose levels. In particular, CMP increased the activities of antioxidant enzymes, such as glutathione peroxidase (GSH-px), superoxide dismutase (SOD), and catalase (CAT) in HFD and STZ induced mice (Zhao et al., 2018). Similarly, in another research, a polysaccharide-enriched fraction obtained from CM also produced hypoglycemic effects in STZ-induced rats (Zhang et al., 2006).The anti-diabetic effects of C. cicadae polysaccharide (SHF) were evaluated in alloxan-induced diabetic rats. SHF administered repressed the body weights of rats. Additionally, GSH, SOD, and HDL levels were upregulated, whereas MDA, urea, LDL, TG, CREA, TC, ALP, AST, and ALT levels were downregulated with SHF treatment (Zhang et al., 2018).

Mushroom (*Cynomorium coccineum* L.) polysaccharides (MPs), derived from the mycelium and the fermentation broth, have a role in anti-diabetes treatment (Zhao et al., 2014; Chen, 2015; Liu et al., 2022). MP can enhance the levels of antioxidant enzymes such as SOD, GSH-Px, and CAT in diabetes models *in vivo* (Chen, 2015; Zhang et al., 2015; Zhang et al., 2017; Liu et al., 2022). The polysaccharide hispidin from *Phellinus linteus* (Berk. and M.A. Curtis) improved the β-cell activities by inhibiting H_2O_2 induced apoptosis and improving insulin levels (Maity et al., 2021).

Ganoderma lingzhi polysaccharides (GLPs) exhibited outstanding antioxidant, hypoglycemic, and hypolipidemic activities in STZ-induced T2DM rats (Liu, 2019; Xu et al., 2019; Chen et al., 2020). More data on the role of MP in diabetes treatment can be obtained from another review (Arunachalam et al., 2022). Furthermore, GLP improved the OS and inhibited apoptosis by increasing the expression of TGF-β1, GSH, SOD, and NOS and suppressing the phosphorylation of MDA, JNK, eNOS and ERK in DM rats (Yao et al., 2022). In addition, GLP-treatment reversed the levels of fasting plasma lipids, hepatic lipid accumulation, and higher blood glucose levels (Li et al., 2020), increased SOD, CAT, and GSH-Px expression, as well as promoted Nrf2 and HO-1expression in db/db mice, by contrast, the content of MDA and TNF-a decreased in these mice.

Auricularia auricular (L.et Hook.) Underw polytricha (AAP) are popular edible fungi. At a low dose (100 mg/kg/day), AAP generally increased the vitality of antioxidant enzymes in STZ-induced diabetes mice, and might regulate the NF-κB pathway and the associated signalling pathway to alleviate diabetes (Xiang et al., 2021).

Lentinus edodes polysaccharides (LEPs) have been found to exhibit hypoglycemic ability by improving the Nrf2/HO-1 pathway. The LEP can increase SOD and GSH activity, as well as reduce MDA levels in STZ induced DM mice (Gong et al., 2022).

TABLE 2 Polysaccharides from foods with antioxidation and anti-diabetic effects.

Polysaccharide source	Monosaccharides composition	Models	Administration	Mechanism	Positive control	Reference
Siraitia grosvenorii	Ara: Rib: GalAc: Gal: Man: Glc = 1.00:1.72:90: 2.24:3.64:3.89: 22.77	HFD and HSD induced mice	50, 100, and 200 mg kg/d	↓: IL-6, TNF-α and MDA ↑: SOD		Gong et al. (2021)
Pumpkin		STZ + HFD induced rats		↓: blood glucose, insulin, TC, TG, LDL-C, and MDA ↑: HDL-C, SOD, and CAT		Ti et al. (2022)
pumpkin	Man: rib: glc: glu A:gal A: glc: gal: xyl: fuc = 142.92:42.89: 1.03:17.83: 2.6: 125.75:0.85 : 112.34:73.25	HFD and STZ to induce T2DM	400 mg/kg/day	↑: Nrf2; HO-1, PI3K		Chen et al. (2019a)
Ficus pumila Linn	A linear (1,4)-α-D-galacturonic acid binding 1.30% branched chain hexenuronic acid with 23.34% methyl esterification	C57BL/KsJ db/db mice	100,200 mg/kg/d	activated the IRS-1/PI3K/Akt/GSK3β/GS and AMPK/GSK3β/GS signalling pathway	rosiglitazone	Wu et al. (2017b)
Peach	Ara:Xyl:Gal = 5.98:1:3.55	STZ-treated mice	200, 400 and 800 mg/kg	↓: blood glucose ↑: pancreatic duodenal homeobox-1, insulin and hexokinase1	Metformin hydrochloride	Wang et al. (2017c)
Mulberry fruit	Ara:Gal:Glc:Xyl:Man = 19.19:31.4: 26.31:5.98:7.12	STZ-induced diabetic mice	200,600,1000 mg/kg/d	↓: MDA; α-amylase, and α-glucosidase ↑: SOD, GPx, CAT, insulin secretion ameliorate insulin resistance; PI3 K/Akt pathway activators	metformin	Chen et al. (2017)
Sweet potato	Rha:Gal:Glc = 3.2:1.6:6.5	STZ rats	100 mg/kg/d	↓: MDA	glibenclamide	Yuan et al. (2017)
Momordica charantia L	Rha: ara: xyl: man: glc: gal: fuc = 15.7,23.6,11.9,.6,31.22,12,1.1	STZ-induced DM mice	1,500 mg/kg	↑: CAT, SOD, GSH-px ↓: MDA	metformin	Zhang et al. (2019a)
Rosa roxburghii fruit	Ara: gal: fuc: glu:man: xyl = 37.20: 34.14: 18.30: 10.02:0.15:0.17	H_2O_2-induced INS-1 cells	0.25, 0.5, 0.75, 1, 1.5, 2 mg/mL	↓: ROS; mitochondrial damage ↑: caspase-3, caspase-8, and caspase-9		Wang et al. (2019b)

Furthermore, in an *vivo* study, LEP reduced cell damage by increasing SOD activity, decreasing ROS content, and revising HG-induced MDA levels in MIN6 cells (Cao et al., 2019). In addition, LEP increased the viability of HG-induced human umbilical vein endothelial cells, decreased ROS production, and reversed the inhibition of α-glucosidase activity. It especially inhibited AGE formation in cells (Cao et al., 2020).

The polysaccharide from the caps of *Suillellus luridus* (SLP) exhibited excellent antidiabetic activities by improving Nrf2/HO-1-mediated OS (Liu et al., 2020a) in the STZ-induced diabetic mice. SLP was composed of galactose (gal), glucose (glu), arabinose (ara), and mannose (man). It had a backbone principally composed of 1,3 linked α-D-Galp, 1,3 linked β-D-Glcp and 1,6 linked β-D-Glcp with the branches mainly composed of T-linked α-D-Galp, 1,3 linked α-L-Arap, 1,3 linked β-D-Glcp, and 1,3 linked α-D-Manp. Moreover, SLP regulated the mRNA levels and protein expression in NF-kB signaling pathways. In short, SLP can be treated as a potential agent for preventing and treating diabetes via regulating oxidative stress (Liu et al., 2020a).

Polysaccharides from *Armillariella tabescens* mycelia diminished the levels of blood glucose, and repressed OS cytokine. They increased SOD and GSH concentrations, and decreased MDA production in diabetic mice to improve T2DM (Yang et al., 2020).

Polysaccharides separated from *Inonotus obliquusvia* (IOP) modulated OS in STZ-induced mice (Wang et al., 2017). Simultaneously, it remarkably regulated DPPH scavenging activity and ferric-reducing power in H_2O_2-induced hepatic L02 cells (Wang et al., 2018).

Polysaccharides from the mycelia of *Coprinus comatus* (CMP) reduced insulin resistance in STZ-induced mice models. It is mainly manifested in the many aspects, they improved energy metabolism, suppressed OS and inflammation, and modulated the Wnt-1/β-catenin and PTEN/PI3K/Akt pathways (Gao et al., 2021).

TABLE 3 Polysaccharides from microorganisms with antioxidation and anti-diabetic effects.

Polysaccharide source	Monosaccharides composition	Models	Administration	Mechanism	Positive control	Reference
Agrocybe Cylindracea	Man, rib, rha, glu A, gal A, glu, gal, xyl, ara, and fuc	HFD and STZ-induced mice	100, 200, and 400 mg/kg/d for 4 weeks	↓: Blood glucose, liver and colon injuries, and inflammation ↑: SOD, GSH-Px, CAT, and lipid metabolism		Sun et al. (2022)
Cordyceps sinensis	Fuc: rib: ara: xyl: man: gal: glc = 1.23: 0.57: 0.29:2.12: 2.73:4.66: 88.4	HFD and STZ induced mice	100 and 400 mg/kg, for 4 weeks	↑: GSH-Px, SOD, and CAT ↓: MDA, LDL, TC, TG, urea, CREA, ALT, AST, and ALP		Zhao et al. (2018)
Cordyceps cicadae		alloxan monohydrate induced rats	100, 200 and 400 mg/kg for 30 days	↑: HDL, SOD, and GSH ↓: TC, TG, LDL, MDA, urea, CREA, ALT, AST, and ALP	glibenclamide	Zhang et al. (2018c)
Cynomorium coccineum *L*		Male SD rats	1.0, 2.0 g/kg for 4 weeks	↓: α-glucosidase, blood glc, MDA, LDL-C, TC, and TG ↑: SOD, GSH-Px, and HDL-C		Zhang et al. (2017)
Phellinus linteus		alloxan-induced mice	100 mg/kg	↓: blood glc level	metformin	Zhao et al. (2014)
Mycelium zinc		STZ induced mice	800, 400, 200 mg/kg	↑:GSH-Px, SOD, CAT, and HDL-C ↓:MDA, ALT, AST, BUN, CRE,TC,ALB, LDL-C, and VLDL-C		Zhang et al. (2015)
Ganoderma lucidum	Rha:xyl:fru: Gal:man:glu = 0.793: 0.964: 2.944: 0.167: 0.384: 7.94	HFD induced male db/db mice	100 mg/kg/d	improving the Nrf2/HO-1 signalling pathway	metformin	Li et al. (2020a)
Auricularia auricular (L.et Hook.) Underw	Fuc, glu, gal, xyl, Rha, man; 17.1 kDa	STZ-induced diabetic mice	100, 300 mg/kg, For 4 weeks	↓: Blood glucose, TNF-α, and MDA ↑: serum insulin, SOD		Xiang et al. (2021)
Lentinus edodes (Berk.) sing	Rha: Fuc: Ara: GLC-UA:Gal: Man: Glc = 1 : 6.13 : 1.28 : 1.79 : 20.62 : 4.74 : 842.17	STZ mice	50, 100, and 200 mg/kg	↑: Nrf2/HO-1 pathway		Gong et al. (2022)
Suillellus luridus	Gal: Glc: Car: Man = 44.9:27.6:14.7: 12.8; 9.4 kDa	STZ-induced diabetic mice	100 mg/kg/d for 30 days	↑: Hepatic glycogen, CAT, insulin, SOD, GSH-Px, and HDL-C ↓:blood glucose, TC, TG, and LDL-C	glibenclamide	Liu et al. (2020a)
Suillellus luridus	Fuc: glc: gal: xyl: rha: man; 173 kDa	STZ-induced diabetic mice	100,300 mg/kg for 4 weeks	↓: Blood glucose and MDA ↑: serum insulin, CAT	glibenclamide	Zhang et al. (2018b)
Armillariella tabescens mycelia	Man, ara, fuc	HFD and STZ induced mice	200, 400 mg/kg	↑: SOD, GSH ↓: ROS, LPO and MDA		Yang et al. (2020b)
Inonotus obliquusvia		H_2O_2-induced hepatic L02 cells	50, 250 and 500 μg/mL	↓: DPPH, ferric reducing power	Vc	Wang et al. (2018)
Coprinus comatus mycelium	Gal,α-pyranose; 495.8 kDa	HFD-STZ fed mice	100, 200, 400 mg/kg/d	modulating the PTEN/PI3K/Akt and Wnt-1/β-catenin pathways	metformin	Gao et al. (2021)

(Continued on following page)

TABLE 3 (*Continued*) Polysaccharides from microorganisms with antioxidation and anti-diabetic effects.

Polysaccharide source	Monosaccharides composition	Models	Administration	Mechanism	Positive control	Reference
Pleurotus eryngii SI-04	Ara: Man:Gal:Glu = 1:1.7:1:4.3	STZ induced mice	600 and 300 mg/kg	↓: GLU, ALB, BUN, CRE, UA levels, TC, MDA, TG, VLDL-C and LDL-C ↑:GSH-Px, SOD, and CAT	glibenclamide	Zhang et al. (2018a)
Paecilomyces hepialid	D-Xylose (D-Xyl), D-Mannose (D-Man), D-Glc and D-Gal	db/db mice	10, 20, and 40 mg/kg	↑: CAT, GSH-Px, and SOD ↓: MDA, ROS	metformin	Hu et al. (2020)
Grifola frondosa (Dicks.) Gray	Glc:Man:Gala:Xyl:Ara:Rha:Ribose = 26.74:22.79:16.76:16.02:14.29:2.05: 1.35/Ribose:Ara:Xyl = 74.73:14.20: 11.08/Rha:Ara:Xyl:Man:Glc:Gal = 4.74:5:1:3.42:31.29:6.89	STZ induced diabetic rats	200 mg/kg	↑: CAT, SOD, GSH-px ↓: ROS	metformin	Kou et al. (2019)
Pholiota nameko		methylglyoxal -induced Hs68 cell		↓: ROS, AGEs		Lin et al. (2021b)

Extracellular polysaccharides (EPS) from *Pleurotus eryngii* SI-04 exhibited anti-glycated activities in STZ-regulated diabetic mice. EPS significantly suppressed GLU levels; decreased BUN, ALB, CRE and UA levels; downregulated serum lipid levels; modified the content of antioxidant enzymes, such as CAT, GSH-Px, SOD, and MDA (Zhang et al., 2018).

Paecilomyces hepiali polysaccharides (PHP) exerted anti-diabetic properties by regulating Nrf2-meadited NF-κB signalling in db/db mice. They not only decreased ROS and MDA concentrations, but also increased GSH-Px, CAT, and SOD contents in the serum and kidney (Hu et al., 2020).

Grifolafrondosa frondosa polysaccharides exert hypoglycemic, anti-diabetic, and nephritic properties by modulating the content of antioxidant enzymes in HFD- and STZ-induced diabetic rats. When CAT, SOD, and GSH-px levels increased, ROS levels decreased in the serum (Kou et al., 2019).

Pholiota nameko polysaccharides (PNPs) exhibited antiglycation ability by improving methylglyoxal -induced Hs68 cell damage. Preprocessing of Hs68 cells with PNPs increased the cell survival rate and inhibited intracellular ROS content. Furthermore, PNPs significantly decreased AGEs and inhibited ROS production, thus alleviating cell damage and OS (Lin et al., 2021). Representative polysaccharides from microorganisms with antioxidation and anti-diabetic effects show in Table 3.

3.3 Polysaccharides derived from marine life.

Sargassum thunbergii polysaccharides (STP), as a promising natural antioxidant and hypoglycemic agent, can be used to improve diabetes. They not only exhibited strong antioxidant and α-glucosidase inhibitory activities, but also improved the glucose uptake and radical-scavenging (DPPH, ROS) rates in insulin-resistant HepG2 cells (Ren et al., 2017).

The polysaccharide from *Laminaria japonica* (LGP) displays various pharmacological functions, especially in oxidative stress. LGP significantly improved mitochondrial dysfunction, leading to higher ATP and SOD content and lower ROS content. Furthermore, LGP upregulated Nrf2 pathway and repaired dysfunction in H_2O_2-induced β-cell (Wu et al., 2022).

3.4 Polysaccharides derived from animal

Field cricket (*Gryllus bimaculatus*), as an edible insect, has been reported to have multiple effects (Table 3). Field cricket glycosaminoglycan has regulated diabetes by significantly increased CAT, SOD and GSH-Px content in db mice (Ahn et al., 2020).

The *Misgurnus anguillicaudatus* polysaccharide (MAP) has a potential hyperglycemic effect (Table 3). It repressed the levels of inflammatory factor, such as IL-6, TNF-α, MDA, and MCP-1, whereas boosted SOD and GPx activities in STZ-induced mice (Zhou et al., 2015b). Representative polysaccharides from marine life and animal with antioxidation and anti-diabetic effects show in Table 4.

3.5 Characteristics of polysaccharides in improving oxidative stress in diabetes mellitus

As we all know, excessive sugar intake is a contraindication in diabetic patients, which will cause increased insulin secretion, resulting in damage to islet beta cells, causing elevated blood sugar. But natural polysaccharides, as a special class of compounds, have been found not only to improve high blood sugar, but also to treat diabetes through other routes. Acting as an antioxidant, natural polysaccharides may control OS-induced diabetes mainly in two ways. They improved type 2 diabetes by inhibiting the production of reactive oxygen species, improving mitochondrial function impairment, and regulating signalling pathways such as oxidative pathway AGE, which is closely related to hyperglycemia patients. They may ameliorate OS by controlling

TABLE 4 Polysaccharides from marine life and animal with antioxidation and anti-diabetic effects.

Polysaccharide source	Monosaccharides composition	Models	Administration	Mechanism	Positive control	Reference
Sargassum thunbergii	Ara: gal: glu: xyl:man:gal A: glu A = 1.94: 30.7: 4.54: 23.2: 17.6: 8.11:13.9	Insulin-resistant HepG2 cells	0.1, 0.5, and 1.0 mg/mL) for 24 h	↓: α-glucosidase; improving the glucose uptake in insulin-resistant		Ren et al. (2017)
Laminaria japonica		MIN6 cells exposed 125 mM H2O2 for 2 h	25, 50, 100, or 200 mg/mL	↓: ROS ↑: SOD, SIRT1-PGC1-α pathway, Nrf2		Wu et al. (2022b)
Field cricket (*Gryllus bimaculatus*)	Rha: rib: ara: fru: glu = 81.10: 7.16:5.91:1.91:1.62	Cg-m+/+Leprdb, heterozygous (db/+) and homozygotes (db/db)	5 mg/kg	↑: CAT, SOD and GSH-Px activities	metformin	Ahn et al. (2020)
Misgurnus anguillicaudatus (Cantor)	Gal:Fuc:man = 5:4:1	STZ induced diabetic mice	50, 100 and 200 mg/kg	↓: TNF-α, IL-6, MCP-1, and MDA ↑: SOD and GPx	metformin	Zhou et al. (2015b)

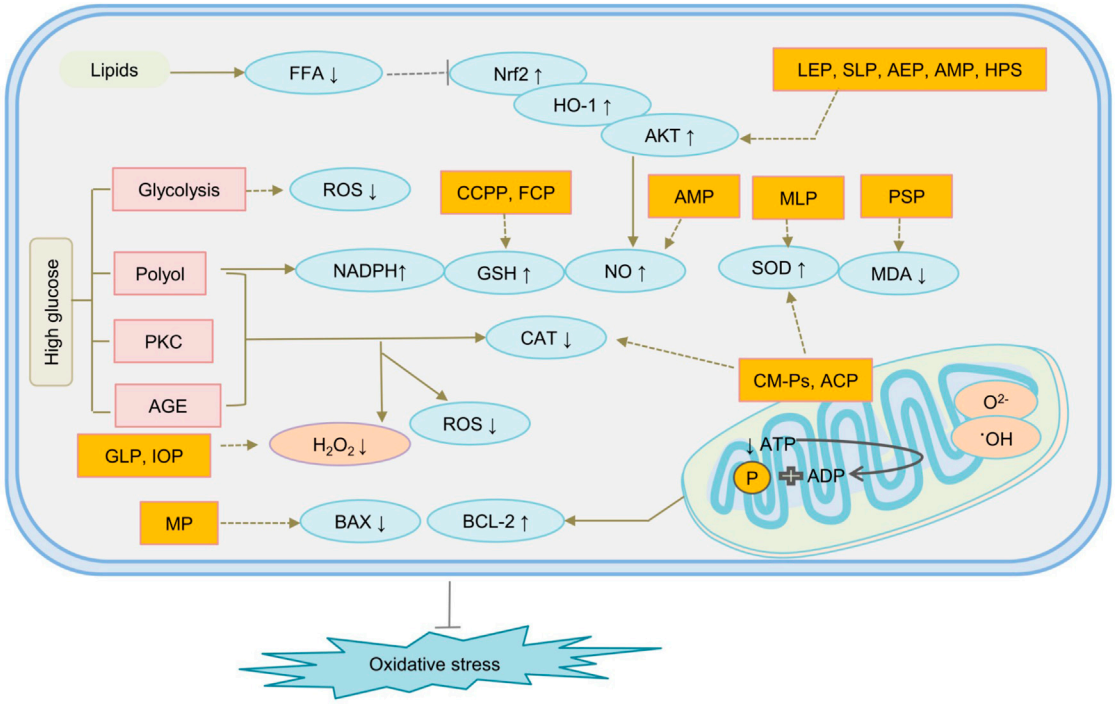


FIGURE 4
The mechanism of natural polysaccharides treat diabetes through oxidative stress is shown follows. ACP, Agrocybe Cylindracea Polysaccharide; AEP, Abelmoschus esculentus polysaccharide; AMP, Astragalus membranaceus polysaccharide; AMP, Astragalus membranaceus polysaccharide; CCPP, Cyclocarya paliurus polysaccharide; CM-Ps, acidic-extractable polysaccharides; FCP, Fructus Corni Polysaccharides; GLP, Ganoderma lucidum polysaccharide; HPS, Hedysarum polysaccharide; IOP, Polysaccharides separated from Inonotus obliquusvia; LEP, Lentinus edodes polysaccharides; MLP, mulberry leaf polysaccharide; MP, Mushroom polysaccharides; PSP, Polygonatum sibiricum polysaccharides; SLP, Suillellus luridus Polysaccharide; STP, Sargassum thunbergii Polysaccharides.

the production of ROS, improving mitochondrial dysfunction of β cells, scavenge free radicals, or enhance antioxidant defense enzymes and their related pathways, such as Nrf2 pathway, oxygenase-1 (HO-1) pathway, Kelch-like ECH-associated protein 1 (Keap1) pathway, and antioxidant response elements (ARE) pathway to improve OS-induced insulin resistance (Nishikawa et al., 2000; Giacco and Brownlee, 2010; Yaribeygi et al., 2020). In addition, polysaccharides may ameliorate diabetes by regulating the cascade of molecular events in different metabolic pathways, such as glycolysis, hexosamine, PKC, polyols, and advanced AGE pathway. The mechanism of representative polysaccharides underlying diabetes treatment by OS is given in Figure 4.

3.6 Boundedness of polysaccharides in diabetes improvement

Although numerous natural polysaccharides have been shown to have a therapeutic effect on diabetes and oxidative stress inhibition, their pharmacological effects is closely linked to the structure of these compounds. Currently, the primary structure analysis is the main method used to determine the structure of natural polysaccharides, and the structure-activity relationship of natural polysaccharides cannot be accurately predicted. Moreover, the structural analysis of natural polysaccharides from food, marine organisms, and animals is relatively limited. The characterization of the advanced structure of natural polysaccharides is still in the exploration stage, and there are few studies on the functional fragments of natural polysaccharides that can clarify the activity orientation. Although many scholars have investigated the efficacy of natural polysaccharides in improving diabetic oxidative stress and attempted to speculate on the possible mechanism of action, there is a lack of innovation and breakthrough, and few in-depth studies have been conducted. Again, because the precise structure of polysaccharides is not clear, the application of polysaccharides is still only in animal or cell models, and there is not much clinical trial evidence. Whether the role of polysaccharides *in vivo* and *in vitro* models can fully reflect the efficacy of polysaccharides in diabetic patients in the future is a question worthy of consideration for every scholar.

4 Conclusion and future perspectives

More than 50 natural polysaccharides have been documented to improve oxidative stress, by regulating mitochondrial function, free radical levels, oxidase content, and Nrf2/HO-1, and AGEs/RAGE pathways in diabetes. However, intervention in diabetes and its complications is the result of multi-target, multi-pathway synergies. For example, polysaccharides extracted from fermented Momordica charantia significantly increased intestinal flora diversity and elevated the levels of short-chain fatty acids (SCFAs), thus exhibiting antidiabetic effects in HFD + STZ-induced rats (Gao et al., 2018). Furthermore, polysaccharides obtained from L. barbarum increased SCFAs levels, elevated gut microbiota diversities, and improved the intestinal barrier in HFD mice to mitigate diabetes (Yang et al., 2021). Bupleurum polysaccharides not only decreased blood creatinine, blood glucose, and urine albumin levels but also repaired the gut barrier and regulated inflammatory responses (Feng et al., 2019). Natural polysaccharides may also improve diabetes by regulating the function of glucose receptors. For example, SSP could promote GLUT-4 levels in BRL cells (Jin et al., 2016). Natural polysaccharides may have many potential antidiabetic activities waiting for us to explore. At the same time, we should also consider how to identify the key signalling pathways and core targets involved in the regulation of natural polysaccharides. It is worth exploring whether network pharmacology can be used to predict potential core targets of natural polysaccharides, and if the signalling pathway of significant enrichment of core targets can be used as the research pathway. Finally, animal experiments or *in vitro* cell experiments can be conducted to provide evidence.

The study of natural polysaccharides is currently limited to animal and *in vitro* cell experiments. Animal and cellular models have been used to explore mechanisms of oxidative damage in diabetes, such as streptozotocin, high-fat diets, and alloxan induced mice or rat, ob/ob mice, zucker diabetic fatty rat, as well as otsuka long-evans tokushima fatty rats (King, 2012). A review of polysaccharide applications for diabetes treatment for the past 5 years, revealed that the dose of polysaccharides ranged from 1 to 1,500 mg/kg/d in STZ or HFD induced diabetic models. Moreover, the polysaccharide and was mostly administered through intragastric administration. For example, the polysaccharide from *C. paliurus* was used as of 1, 10, and 100 mg/kg/d in STZ-induced mice (Zhang et al., 2021). After the administration of 1,500 mg/kg polysaccharide from *M. charantia* L (Zhang et al., 2019), diabetes symptoms improved. *In vivo* experiments of mice, polysaccharide doses were mainly distributed in the range of 100–800 mg/kg/d. How dosing is determined is unclear in some papers, but ultimately the results of these non-clinical trials seem to be very promising. We found that the dose of polysaccharides administered *in vitro* and *in vivo* seems to differ greatly, which seems to make it difficult to explain the specific effective dose of polysaccharides. In order to develop new treatments for diabetes with natural product characteristics that meet international standards, further clinical studies and evaluation of natural products, as well as structural and mechanism of action, as well as the relationship between biological outcomes and therapeutic effects, are needed. Using modern technology to develop more effective and economical methods to extract and separate polysaccharides, and expand the application of polysaccharides.

Author contributions

L-YH, conceived and designed the study and wrote the manuscript, YL and JB, collected data, S-QN and S-JL, revised the manuscript. J-LG supervised the project. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Sciences Foundation of China (Nos. 81872959, 81373920, 30801522, 82003900). “Xinglin Scholar” Talent Research Promotion Plan of Chengdu University of Traditional Chinese Medicine (No. ZYTS2019022). Young Science and Technology Innovation Team of Sichuan Province (No. 2019CXTD0055).

Acknowledgments

The authors would like to thank MJEditor (www.mjeditor.com) for its linguistic assistance during the preparation of this manuscript.

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

Ahn, M. Y., Kim, B. J., Kim, H. J., Jin, J. M., Yoon, H. J., Hwang, J. S., et al. (2020). Anti-diabetic activity of field cricket glycosaminoglycan by ameliorating oxidative stress. *Bmc. Complement. Med. Ther.* 20 (1), 232. doi:10.1186/s12906-020-03027-x

Arunachalam, K., Sreeja, P. S., and Yang, X. (2022). The antioxidant properties of mushroom polysaccharides can potentially mitigate oxidative stress, beta-cell dysfunction and insulin resistance. *Front. Pharmacol.* 13, 874474. doi:10.3389/fphar.2022.874474

Bertuzzi, F., Colussi, G., Lauterio, A., and De Carlis, L. (2018). Intramuscular islet allotransplantation in type 1 diabetes mellitus. *Eur. Rev. Med. Pharmacol. Sci.* 22, 1731–1736. doi:10.26355/eurrev_201803_14588

Cai, J., Zhu, Y., Zuo, Y., Tong, Q., Zhang, Z., Yang, L., et al. (2019). Polygonatum sibiricum polysaccharide alleviates inflammatory cytokines and promotes glucose uptake in high glucose and high insulin induced 3T3 L1 adipocytes by promoting Nrf2 expression. *Mol. Med. Rep.* 20, 3951–3958. doi:10.3892/mmr.2019.10626

Cao, X., Liu, D., Xia, Y., Cai, T., He, Y., and Liu, J. (2019). A novel polysaccharide from Lentinus edodes mycelia protects MIN6 cells against high glucose-induced damage via the MAPKs and Nrf2 pathways. *Food. Nutr. Res.* 63. doi:10.29219/fnr.v63.1598

Cao, X., Xia, Y., Liu, D., He, Y., Mu, T., Huo, Y., et al. (2020). Inhibitory effects of Lentinus edodes mycelia polysaccharide on α-glucosidase, glycation activity and high glucose-induced cell damage. *Carbohydr. Polym.* 246, 116659. doi:10.1016/j.carbpol.2020.116659

Ceriello, A., Ihnat, M. A., and Thorpe, J. E. (2009). Clinical review 2: The "metabolic memory": Is more than just tight glucose control necessary to prevent diabetic complications? *J. Clin. Endocrinol. Metab.* 94, 410–415. doi:10.1210/jc.2008-1824

Cersosimo, E., Johnson, E. L., Chovanes, C., and Skolnik, N. (2018). Initiating therapy in patients newly diagnosed with type 2 diabetes: Combination therapy vs a stepwise approach. *Diabetes. Obes. Metab.* 20, 497–507. doi:10.1111/dom.13108

Chang, X., Lu, K., Wang, L., Lv, M., and Fu, W. (2018). Astraglaus polysaccharide protects diabetic cardiomyopathy by activating NRG1/ErbB pathway. *Biosci. Trends.* 12, 149–156. doi:10.5582/bst.2018.01027

Chatterjee, S., Khunti, K., and Davies, M. J. (2017). Type 2 diabetes. *Lancet* 389, 2239–2251. doi:10.1016/S0140-6736(17)30058-2

Chen, C., Huang, Q., Li, C., and Fu, X. (2017). Hypoglycemic effects of a Fructus mori polysaccharide *in vitro* and *in vivo*. *Food & Funct.* 8, 2523–2535. doi:10.1039/c7fo00417f

Chen, C., Kang, M., Wang, Q., Liu, W., Yang, M., Liang, S., et al. (2021). Combination of Anoectochilus roxburghii polysaccharide and exercise ameliorates diet-induced metabolic disorders in obese mice. *Front. Nutr.* 8, 735501. doi:10.3389/fnut.2021.735501

Chen, M., Xiao, D., Liu, W., Song, Y., Zou, B., Li, L., et al. (2020). Intake of Ganoderma lucidum polysaccharides reverses the disturbed gut microbiota and metabolism in type 2 diabetic rats. *Int. J. Biol. Macromol.* 155, 890–902. doi:10.1016/j.ijbiomac.2019.11.047

Chen, P. H., Weng, Y. M., Lin, S. M., Yu, Z. R., and Wang, B. J. (2015). Molecular weight affected antioxidant, hypoglycemic and hypotensive activities of cold water extract from Pleurotus citrinopileatus. *J. Food. Sci.* 82 (10), 2456–2461. doi:10.1111/1750-3841.13851

Chen, W., Ju, J., Yang, Y., Wang, H., Chen, W., Zhao, X., et al. (2018). Astragalus polysaccharides protect cardiac stem and progenitor cells by the inhibition of oxidative stress-mediated apoptosis in diabetic hearts. *Drug. Des. devel. Ther.* 12, 943–954. doi:10.2147/DDDT.S155686

Chen, X., Chen, C., and Fu, X. (2022). Hypoglycemic activity *in vitro* and vivo of a water-soluble polysaccharide from Astragalus membranaceus. *Food & Funct.* 13 (21), 11210–11222. doi:10.1039/d2fo02298b

Chen, X., Qian, L., Wang, B., Zhang, Z., Liu, H., Zhang, Y., et al. (2019a). Synergistic hypoglycemic effects of pumpkin polysaccharides and puerarin on type II diabetes mellitus mice. *Molecules* 24 (5), 955. doi:10.3390/molecules24050955

Chen, X., Tang, J., Xie, W., Wang, J., Jin, J., Ren, J., et al. (2013). Protective effect of the polysaccharide from Ophiopogon japonicus on streptozotocin-induced diabetic rats. *Carbohydr. Polym.* 94 (1), 378–385. doi:10.1016/j.carbpol.2013.01.037

Chen, Y., Wang, Y., Yang, S., Yu, M., Jiang, T., and Lv, Z. (2019b). Glycosaminoglycan from Apostichopus japonicus improves glucose metabolism in the liver of insulin resistant mice. *Mar. Drugs.* 18 (1), 1. doi:10.3390/md18010001

Cheng, J., Zhou, Z. W., Sheng, H. P., He, L. J., Fan, X. W., He, Z. X., et al. (2015). An evidence-based update on the pharmacological activities and possible molecular targets of Lycium barbarum polysaccharides. *Drug Des. devel. Ther.* 9, 33–78. doi:10.2147/DDDT.S72892

Cho, E. J., Hwang, H. J., Kim, S. W., Oh, J. Y., Baek, Y. M., Choi, J. W., et al. (2007). Hypoglycemic effects of exopolysaccharides produced by mycelial cultures of two different mushrooms Tremella fuciformis and Phellinus baumii in ob/ob mice. *Appl. Microbiol. Biotechnol.* 75, 1257–1265. doi:10.1007/s00253-007-0972-2

Dos Santos, J. M., Tewari, S., and Mendes, R. H. (2019). The role of oxidative stress in the development of diabetes mellitus and its complications. *J. Diabetes. Res.* 2019, 4189813. doi:10.1155/2019/4189813

Eftekharpour, E., and Fernyhough, P. (2022). Oxidative stress and mitochondrial dysfunction associated with peripheral neuropathy in type 1 diabetes. *Antioxid. Redox. Signal.* 37, 578–596. doi:10.1089/ars.2021.0152

Eizirik, D. L., Pasquali, L., and Cnop, M. (2020). Pancreatic β-cells in type 1 and type 2 diabetes mellitus: Different pathways to failure. *Endocrinology.* 16, 349–362. doi:10.1038/s41574-020-0355-7

Erejuwa, O. O. (2012). *Oxidative stress in diabetes mellitus: Is there a role for hypoglycemic drugs and/or antioxidants? (Oxidative stress and diseases).* doi:10.5772/32741

Ezraty, B., Gennaris, A., Barras, F., and Collet, J. F. (2017). Oxidative stress, protein damage and repair in bacteria. *Microbiology.* 15, 385–396. doi:10.1038/nrmicro.2017.26

Feng, Y., Weng, H., Ling, L., Zeng, T., Zhang, Y., Chen, D., et al. (2019). Modulating the gut microbiota and inflammation is involved in the effect of Bupleurum polysaccharides against diabetic nephropathy in mice. *Int. J. Biol. Macromol.* 132, 1001–1011. doi:10.1016/j.ijbiomac.2019.03.242

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., et al. (2020). Pathophysiology of type 2 diabetes mellitus. *Int. J. Mol. Sci.* 21, 6275. doi:10.3390/ijms21176275

Ganesan, K., and Xu, B. (2019). Anti-diabetic effects and mechanisms of dietary polysaccharides. *Molecules* 24 (14), 2556. doi:10.3390/molecules24142556

Gao, H., Wen, J. J., Hu, J. L., Nie, Q. X., Chen, H. H., Xiong, T., et al. (2018). Polysaccharide from fermented Momordica charantia L. with Lactobacillus plantarum NCU116 ameliorates type 2 diabetes in rats. *Carbohydr. Polym.* 201, 624–633. doi:10.1016/j.carbpol.2018.08.075

Gao, Z., Kong, D., Cai, W., Zhang, J., and Jia, L. (2021). Characterization and anti-diabetic nephropathic ability of mycelium polysaccharides from Coprinus comatus. *Carbohydr. Polym.* 251, 117081. doi:10.1016/j.carbpol.2020.117081

Gerich, J. E. (2003). Contributions of insulin-resistance and insulin-secretory defects to the pathogenesis of type 2 diabetes mellitus. *Mayo. Clin. Proc.* 78, 447–456. doi:10.4065/78.4.447

Ghosh, A., Gao, L., Thakur, A., Siu, P. M., and Lai, C. W. K. (2017). Role of free fatty acids in endothelial dysfunction. *J. Biomed. Sci.* 24, 50. doi:10.1186/s12929-017-0357-5

Giacco, F., and Brownlee, M. (2010). Oxidative stress and diabetic complications. *Circulation. Res.* 107, 1058–1070. doi:10.1161/CIRCRESAHA.110.223545

Giannini, C., Mohn, A., Chiarelli, F., and Kelnar, C. J. (2011). Macrovascular angiopathy in children and adolescents with type 1 diabetes. *Metab. Res. Rev.* 27, 436–460. doi:10.1002/dmrr.1195

Goncalves, J. S., Seica, R. M., Laranjinha, J., and Lourenco, C. F. (2022). Impairment of neurovascular coupling in the hippocampus due to decreased nitric oxide bioavailability supports early cognitive dysfunction in type 2 diabetic rats. *Free. Radic. Biol. Med.* 193, 669–675. doi:10.1016/j.freeradbiomed.2022.11.009

Gong, P., Cui, D., Guo, Y., Wang, M., Wang, Z., Huang, Z., et al. (2021). A novel polysaccharide obtained from Siraitia grosvenorii alleviates inflammatory responses in a diabetic nephropathy mouse model via the TLR4-NF-κB pathway. *Food & Funct.* 12, 9054–9065. doi:10.1039/d1fo01182k

Gong, P., Wang, X., Liu, M., Wang, M., Wang, S., Guo, Y., et al. (2022). Hypoglycemic effect of a novel polysaccharide from Lentinus edodes on STZ-induced diabetic mice via metabolomics study and Nrf2/HO-1 pathway. *Food & Funct.* 13, 3036–3049. doi:10.1039/d1fo03487a

Green, A. D., Vasu, S., and Flatt, P. R. (2018). Cellular models for beta-cell function and diabetes gene therapy. *Acta. Physiol. (Oxf).* 222, e13012. doi:10.1111/apha.13012

Guo, Y., Li, S., Li, J., Ren, Z., Chen, F., and Wang, X. (2017). Anti-hyperglycemic activity of polysaccharides from calyx of Physalis alkekengi var. franchetii Makino on alloxan-induced mice. *Int. J. Biol. Macromol.* 99, 249–257. doi:10.1016/j.ijbiomac.2017.02.086

Halim, M., and Halim, A. (2019). The effects of inflammation, aging and oxidative stress on the pathogenesis of diabetes mellitus (type 2 diabetes). *Diabetes. Metab. Syndr.* 13, 1165–1172. doi:10.1016/j.dsx.2019.01.040

He, L., Huan, P., Xu, J., Chen, Y., Zhang, L., Wang, J., et al. (2022). Hedysarum polysaccharide alleviates oxidative stress to protect against diabetic peripheral neuropathy via modulation of the keap1/Nrf2 signaling pathway. *J. Chem. Neuroanat.* 126, 102182. doi:10.1016/j.jchemneu.2022.102182

Hu, J. L., Nie, S. P., and Xie, M. Y. (2018). Antidiabetic mechanism of dietary polysaccharides based on their gastrointestinal functions. *J. Agric. Food. Chem.* 66, 4781–4786. doi:10.1021/acs.jafc.7b05410

Hu, W., Wang, J., Guo, W., Liu, Y., Guo, Z., Miao, Y., et al. (2020). Studies on characteristics and anti-diabetic and -nephritic effects of polysaccharides isolated from Paecilomyces hepiali fermentation mycelium in db/db mice. *Carbohydr. Polym.* 232, 115766. doi:10.1016/j.carbpol.2019.115766

Ighodaro, O. M. (2018). Molecular pathways associated with oxidative stress in diabetes mellitus. *Biomed. Pharmacother.* 108, 656–662. doi:10.1016/j.biopha.2018.09.058

Jin, D., Zhao, T., Feng, W. W., Mao, G. H., Zou, Y., Wang, W., et al. (2016). Schisandra polysaccharide increased glucose consumption by up-regulating the expression of GLUT-4. *Int. J. Biol. Macromol.* 87, 555–562. doi:10.1016/j.ijbiomac.2016.03.028

Kaneto, H., Matsuoka, T. A., Kimura, T., Obata, A., Shimoda, M., Kamei, S., et al. (2016). Appropriate therapy for type 2 diabetes mellitus in view of pancreatic beta-cell glucose toxicity: "the earlier, the better. *J. Diabetes.* 8, 183–189. doi:10.1111/1753-0407.12331

Kang, Q., and Yang, C. (2020). Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications. *Redox. Biol.* 37, 101799. doi:10.1016/j.redox.2020.101799

Kelly, M. S., Lewis, J., Huntsberry, A. M., Dea, L., and Portillo, I. (2019). Efficacy and renal outcomes of SGLT2 inhibitors in patients with type 2 diabetes and chronic kidney disease. *Postgrad. Med.* 131, 31–42. doi:10.1080/00325481.2019.1549459

Kessler, L., Wiesel, M. L., Attali, P., Mossard, J. M., Cazenave, J. P., and Pinget, M. (1998). Von Willebrand factor in diabetic angiopathy. *Diabetes. Metab.* 24, 327–336.

King, A. J. (2012). The use of animal models in diabetes research. *Br. J. Pharmacol.* 166 (3), 877–894. doi:10.1111/j.1476-5381.2012.01911.x

Kou, L., Du, M., Liu, P., Zhang, B., Zhang, Y., Yang, P., et al. (2019). Anti-diabetic and anti-nephritic activities of grifola frondosa mycelium polysaccharides in diet-streptozotocin-induced diabetic rats via modulation on oxidative stress. *Appl. Biochem. And. Biotechnol.* 187, 310–322. doi:10.1007/s12010-018-2803-6

Leeuwenburgh, C., Fiebig, R., Chandwaney, R., and Ji, L. L. (1994). Aging and exercise training in skeletal muscle: Responses of glutathione and antioxidant enzyme systems. *Am. J. Physiol.* 267, R439–R445. doi:10.1152/ajpregu.1994.267.2.R439

Li, H. N., Zhao, L. L., Zhou, D. Y., and Chen, D. Q. (2020a). Ganoderma lucidum polysaccharides ameliorates hepatic steatosis and oxidative stress in db/db mice via targeting nuclear factor E2 (Erythroid-Derived 2)-related factor-2/heme oxygenase-1 (HO-1) pathway. *Med. Sci. Monit.* 26, e921905. doi:10.12659/MSM.921905

Li, J. S., Ji, T., Su, S. L., Zhu, Y., Chen, X. L., Shang, E. X., et al. (2022). Mulberry leaves ameliorate diabetes via regulating metabolic profiling and AGEs/RAGE and p38 MAPK/NF-κB pathway. *J. Ethnopharmacol.* 283, 114713. doi:10.1016/j.jep.2021.114713

Li, Q., Hu, J., Nie, Q., Chang, X., Fang, Q., Xie, J., et al. (2021). Hypoglycemic mechanism of polysaccharide from Cyclocarya paliurus leaves in type 2 diabetic rats by gut microbiota and host metabolism alteration. *Sci. China. Life. Sci.* 64, 117–132. doi:10.1007/s11427-019-1647-6

Li, T., Hua, Q., Li, N., Cui, Y., and Zhao, M. (2019a). Protective effect of a polysaccharide from Dipsacus asper Wall on streptozotocin (STZ)-induced diabetic nephropathy in rat. *Int. J. Biol. Macromol.* 133, 1194–1200. doi:10.1016/j.ijbiomac.2019.04.069

Li, W., Lin, K., Zhou, M., Xiong, Q., Li, C., and Ru, Q. (2020b). Polysaccharides from Opuntia milpa alta alleviate alloxan-induced INS-1 cells apoptosis via reducing oxidative stress and upregulating Nrf2 expression. *Nutr. Res.* 77, 108–118. doi:10.1016/j.nutres.2020.02.004

Li, X. M. (2007). Protective effect of Lycium barbarum polysaccharides on streptozotocin-induced oxidative stress in rats. *Int. J. Biol. Macromol.* 40, 461–465. doi:10.1016/j.ijbiomac.2006.11.002

Li, X., Zhao, Z., Kuang, P., Shi, X., Wang, Z., and Guo, L. (2019b). Regulation of lipid metabolism in diabetic rats by Arctium lappa L. polysaccharide through the PKC/NF-κB pathway. *Int. J. Biol. Macromol.* 136, 115–122. doi:10.1016/j.ijbiomac.2019.06.057

Lian, Y., Zhu, M., Chen, J., Yang, B., Lv, Q., Wang, L., et al. (2021). Characterization of a novel polysaccharide from Moutan Cortex and its ameliorative effect on AGEs-induced diabetic nephropathy. *Diabetes. Metab. J.* 176, 589–600. doi:10.1016/j.ijbiomac.2021.02.062

Liao, Z., Zhang, J., Liu, B., Yan, T., Jia, Y. J. M., Xiao, F., et al. (2019a). Polysaccharide from okra (Abelmoschus esculentus (L) moench) improves antioxidant capacity via PI3K/AKT pathways and Nrf2 translocation in a type 2 diabetes model. *Molecules* 24, 1906. doi:10.3390/molecules24101906

Liao, Z., Zhang, J., Wang, J., Yan, T., Xu, F., Wu, B., et al. (2019b). The anti-nephritic activity of a polysaccharide from okra (Abelmoschus esculentus (L) Moench) via modulation of AMPK-Sirt1-PGC-1α signaling axis mediated anti-oxidative in type 2 diabetes model mice. *Int. J. Biol. Macromol.* 140, 568–576. doi:10.1016/j.ijbiomac.2019.08.149

Lin, C., Kuo, T. C., Lin, J. C., Ho, Y. C., and Mi, F. L. (2020). Delivery of polysaccharides from Ophiopogon japonicus (OJPs) using OJPs/chitosan/whey protein co-assembled nanoparticles to treat defective intestinal epithelial tight junction barrier. *Int. J. Biol. Macromol.* 160, 558–570. doi:10.1016/j.ijbiomac.2020.05.151

Lin, C., Lin, Y., Xiao, J., Lan, Y., Cao, Y., and Chen, Y. (2021a). Effect of Momordica saponin- and Cyclocarya paliurus polysaccharide-enriched beverages on oxidative stress and fat accumulation in *Caenorhabditis elegans*. *J. Sci. Food. Agric.* 101, 3366–3375. doi:10.1002/jsfa.10966

Lin, H., Lin, T. Y., Lin, J. A., Cheng, K. C., Santoso, S. P., Chou, C. H., et al. (2021b). Effect of pholiota nameko polysaccharides inhibiting methylglyoxal-induced glycation damage *in vitro*. *Antioxidants. (Basel)* 10, 1589. doi:10.3390/antiox10101589

Liu, C. G., Ma, Y. P., and Zhang, X. J. (2017). Effects of mulberry leaf polysaccharide on oxidative stress in pancreatic β-cells of type 2 diabetic rats. *Eur. Rev. Med. Pharmacol. Sci.* 21, 2482–2488.

Liu, C., Song, J., Teng, M., Zheng, X., Li, X., Tian, Y., et al. (2016). Antidiabetic and antinephritic activities of aqueous extract of cordyceps militaris fruit body in diet-streptozotocin-induced diabetic sprague dawley rats. *Oxid. Med. Cell. Longev.* 2016, 9685257. doi:10.1155/2016/9685257

Liu, M., Li, Y., Zhang, W., Sun, M., and Zhang, Z. (2019). Hypoglycemic effect of inulin combined with ganoderma lucidum polysaccharides in T2DM rats. *J. Funct.Foods.* 55, 381–390. doi:10.1016/j.jff.2019.02.036

Liu, X., Luo, D., Guan, J., Chen, J., and Xu, X. (2022). Mushroom polysaccharides with potential in anti-diabetes: Biological mechanisms, extraction, and future perspectives: A review. *A Rev. Front. Nutr.* 9, 1087826. doi:10.3389/fnut.2022.1087826

Liu, Y., Liu, Y., Zhang, M., Li, C., Zhang, Z., Liu, A., et al. (2020a). Structural characterization of a polysaccharide from Suillellus luridus and its antidiabetic activity via Nrf2/HO-1 and NF-κB pathways. *Int. J. Biol. Macromol.* 162, 935–945. doi:10.1016/j.ijbiomac.2020.06.212

Liu, Y., Yang, L., Zhang, Y., Liu, X., Wu, Z., Gilbert, R. G., et al. (2020b). Dendrobium officinale polysaccharide ameliorates diabetic hepatic glucose metabolism via glucagon-mediated signaling pathways and modifying liver-glycogen structure. *J. Ethnopharmacol.* 248, 112308. doi:10.1016/j.jep.2019.112308

Luo, D., Dong, X., Huang, J., Huang, C., Fang, G., and Huang, Y. (2021). Pueraria lobata root polysaccharide alleviates glucose and lipid metabolic dysfunction in diabetic db/db mice. *Pharm. Biol.* 59, 382–390. doi:10.1080/13880209.2021.1898648

Luo, Y., Peng, B., Wei, W., Tian, X., and Wu, Z. (2019). *Antioxidant and anti-diabetic activities of polysaccharides from guava Leaves. Molecules*. Basel, Switzerland, 24. doi:10.3390/molecules24071343

Maity, P., Sen, I. K., Chakraborty, I., Mondal, S., Bar, H., Bhanja, S. K., et al. (2021). Biologically active polysaccharide from edible mushrooms: A review. *Int. J. Biol. Macromol.* 172, 408–417. doi:10.1016/j.ijbiomac.2021.01.081

Maritim, A. C., Sanders, R. A., and Watkins, J. B., 3rd (2003). Diabetes, oxidative stress, and antioxidants: A review. *J. Biochem. Mol. Toxicol.* 17, 24–38. doi:10.1002/jbt.10058

Miccoli, R., Penno, G., and Del Prato, S. (2011). Multidrug treatment of type 2 diabetes: A challenge for compliance. *Diabetes Care* 34 (2), S231–S235. doi:10.2337/dc11-s235

Mima, A. (2016). Incretin-Based therapy for prevention of diabetic vascular complications. *J. Diabetes. Res.* 2016, 1379274. doi:10.1155/2016/1379274

Newmeyer, D. D., and Ferguson-Miller, S. J. C. (2003). Mitochondria: Releasing power for life and unleashing the machineries of death. *Cell* 112, 481–490. doi:10.1016/s0092-8674(03)00116-8

Nie, C., Zhu, P., Wang, M., Ma, S., and Wei, Z. (2017). Optimization of water-soluble polysaccharides from stem lettuce by response surface methodology and study on its characterization and bioactivities. *Int. J. Biol. Macromol.* 105, 912–923. doi:10.1016/j.ijbiomac.2017.07.125

Nishikawa, T., Edelstein, D., Du, X. L., Yamagishi, S., Matsumura, T., Kaneda, Y., et al. (2000). Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 404, 787–790. doi:10.1038/35008121

Niu, J., Xu, G., Jiang, S., Li, H., and Yuan, G. (2017). *In vitro* Antioxidant activities and anti-diabetic effect of a polysaccharide from Schisandra sphenanthera in rats with type 2 diabetes. *Int. J. Biol. Macromol.* 94, 154–160. doi:10.1016/j.ijbiomac.2016.10.015

Omolola, R., Nicole, L., and Oluwafemi, O. (2014). *Oxidative stress and diabetic complications: The role of antioxidant vitamins and flavonoids*. doi:10.5772/57282

Petersen, K. F., Befroy, D., Dufour, S., Dziura, J., Ariyan, C., Rothman, D. L., et al. (2003). Mitochondrial dysfunction in the elderly: Possible role in insulin resistance. *Sci. N. Y. N.Y.).* 300, 1140–1142. doi:10.1126/science.1082889

Poznyak, A., Grechko, A. V., Poggio, P., Myasoedova, V. A., Alfieri, V., and Orekhov, A. N. (2020). The diabetes mellitus-atherosclerosis connection: The role of lipid and

glucose metabolism and chronic inflammation. *Int. J. Mol. Sci.* 21, 1835. doi:10.3390/ijms21051835

Qi, W., Keenan, H. A., Li, Q., Ishikado, A., Kannt, A., Sadowski, T., et al. (2017). Pyruvate kinase M2 activation may protect against the progression of diabetic glomerular pathology and mitochondrial dysfunction. *Nat. Med.* 23, 753–762. doi:10.1038/nm.4328

Qiu, A., Wang, Y., Zhang, G., and Wang, H. (2022). Natural polysaccharide-based nanodrug delivery systems for treatment of diabetes. *Polym. (Basel)* 14, 3217. doi:10.3390/polym14153217

Ren, B., Chen, C., Li, C., Fu, X., You, L., and Liu, R. H. (2017). Optimization of microwave-assisted extraction of Sargassum thunbergii polysaccharides and its antioxidant and hypoglycemic activities. *Carbohydr. Polym.* 173, 192–201. doi:10.1016/j.carbpol.2017.05.094

Ren, C., Zhang, Y., Cui, W., Lu, G., Wang, Y., Gao, H., et al. (2015). A polysaccharide extract of mulberry leaf ameliorates hepatic glucose metabolism and insulin signaling in rats with type 2 diabetes induced by high fat-diet and streptozotocin. *Int. J. Biol. Macromol.* 72, 951–959. doi:10.1016/j.ijbiomac.2014.09.060

Rickels, M. R., and Robertson, R. P. (2019). Pancreatic islet transplantation in humans: Recent progress and future directions. *Endocr. Rev.* 40, 631–668. doi:10.1210/er.2018-00154

Salazar-Garcia, M., and Corona, J. C. (2021). The use of natural compounds as a strategy to counteract oxidative stress in animal models of diabetes mellitus. Int. J. Mol. *Sci.* 22 (13), 7009. doi:10.3390/ijms22137009

Schieber, M., and Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. Curr. Biol. *CB* 24, R453–R462. doi:10.1016/j.cub.2014.03.034

Sergi, D., Naumovski, N., Heilbronn, L. K., Abeywardena, M., O'Callaghan, N., Lionetti, L., et al. (2019). Mitochondrial (Dys)function and insulin resistance: From pathophysiological molecular mechanisms to the impact of diet. *Front. physiology.* 10, 532. doi:10.3389/fphys.2019.00532

Shi, G. J., Zheng, J., Wu, J., Qiao, H. Q., Chang, Q., Niu, Y., et al. (2017). Beneficial effects of Lycium barbarum polysaccharide on spermatogenesis by improving antioxidant activity and inhibiting apoptosis in streptozotocin-induced diabetic male mice. *Food & Funct.* 8, 1215–1226. doi:10.1039/c6fo01575a

Spinelli, J. B., and Haigis, M. C. (2018). The multifaceted contributions of mitochondria to cellular metabolism. *Nat. Cell. Biol.* 20, 745–754. doi:10.1038/s41556-018-0124-1

Sun, Q., Wu, X., Wang, H., Chen, W., Zhao, X., Yang, Y., et al. (2019). Protective effects of Astragalus polysaccharides on oxidative stress in high glucose-induced or SOD2-silenced H9C2 cells based on PCR array analysis. *Diabetes. Metab. Syndr. Obes.* 12, 2209–2220. doi:10.2147/DMSO.S228351

Sun, W., Zhang, Y., and Jia, L. J. F. B. (2022). Polysaccharides from Agrocybe cylindracea residue alleviate type 2-diabetes-induced liver and colon injuries by p38 MAPK signaling pathway. *Food. Biosci.* 47, 101690. doi:10.1016/j.fbio.2022.101690

Taylor, R. (2013). Type 2 diabetes: Etiology and reversibility. *Diabetes. Care.* 36, 1047–1055. doi:10.2337/dc12-1805

Thakur, P., Kumar, A., and Kumar, A. (2018). Targeting oxidative stress through antioxidants in diabetes mellitus. *J. Drug. Target.* 26, 766–776. doi:10.1080/1061186X.2017.1419478

Ti, Y., Wang, W., Wang, X., Ban, Y., Wang, P., Zhang, Y., et al. (2022). Pumpkin polysaccharide extracted by subcritical water: Physicochemical characterization and anti-diabetic effects in T2DM rats. *Mol. Nutr. Food. Res.* 66, e2200160. doi:10.1002/mnfr.202200160

Tian, X., Liang, T., Liu, Y., Ding, G., Zhang, F., and Ma, Z. (2019). Extraction, structural characterization, and biological functions of lycium barbarum polysaccharides: A review. *Biomolecules* 9 (9), 389. doi:10.3390/biom9090389

Venkatasamy, V. V., Pericherla, S., Manthuruthil, S., Mishra, S., and Hanno, R. (2013). Effect of physical activity on insulin resistance, inflammation and oxidative stress in diabetes mellitus. *JCDR* 7, 1764–1766. doi:10.7860/JCDR/2013/6518.3306

Wang, C., Gao, X., Santhanam, R. K., Chen, Z., Chen, Y., Xu, L., et al. (2018). Effects of polysaccharides from Inonotus obliquus and its chromium (III) complex on advanced glycation end-products formation, α-amylase, α-glucosidase activity and H_2O_2-induced oxidative damage in hepatic L02 cells. *Food. Chem. Toxicol.* 116, 335–345. doi:10.1016/j.fct.2018.04.047

Wang, D., Li, C., Fan, W., Yi, T., Wei, A., and Ma, Y. (2019a). Hypoglycemic and hypolipidemic effects of a polysaccharide from Fructus Corni in streptozotocin-induced diabetic rats. *Int. J. Biol. Macromol.* 133, 420–427. doi:10.1016/j.ijbiomac.2019.04.160

Wang, J., Hu, W., Li, L., Huang, X., Liu, Y., Wang, D., et al. (2017a). Antidiabetic activities of polysaccharides separated from Inonotus obliquus via the modulation of oxidative stress in mice with streptozotocin-induced diabetes. PloS. *one* . 12, e0180476. doi:10.1371/journal.pone.0180476

Wang, L., Li, C., Huang, Q., and Fu, X. (2019b). Biofunctionalization of selenium nanoparticles with a polysaccharide from Rosa roxburghii fruit and their protective effect against H(2)O(2)-induced apoptosis in INS-1 cells. *Food & Funct.* 10, 539–553. doi:10.1039/c8fo01958d

Wang, P. C., Zhao, S., Yang, B. Y., Wang, Q. H., and Kuang, H. X. (2016). Anti-diabetic polysaccharides from natural sources: A review. *Carbohydr. Polym.* 148, 86–97. doi:10.1016/j.carbpol.2016.02.060

Wang, S., Lu, A., Zhang, L., Shen, M., Xu, T., Zhan, W., et al. (2017b). Extraction and purification of pumpkin polysaccharides and their hypoglycemic effect. *Int. J. Biol. Macromol.* 98, 182–187. doi:10.1016/j.ijbiomac.2017.01.114

Wang, W., Li, S., and Song, M. (2022). Polygonatum sibiricum polysaccharide inhibits high glucose-induced oxidative stress, inflammatory response, and apoptosis in RPE cells. *J. Recept. Signal. Transduct. Res.* 42, 189–196. doi:10.1080/10799893.2021.1883061

Wang, Y., Lin, D., Wang, X., Zhu, W., Ye, J., Li, G., et al. (2017c). The impact of a novel peach gum-derived polysaccharide on postprandial blood glucose control in streptozotocin-induced diabetic mice. *Int. J. Biol. Macromol.* 98, 379–386. doi:10.1016/j.ijbiomac.2017.01.085

Wang, Y., Qin, S., Pen, G., Chen, D., Han, C., Miao, C., et al. (2017d). Original Research: Potential ocular protection and dynamic observation of Polygonatum sibiricum polysaccharide against streptozocin-induced diabetic rats' model. *Exp. Biol. Med. (Maywood).* 242, 92–101. doi:10.1177/1535370216663866

Wu, C. Y., Wang, H., He, X. X., Wu, D. W., Yue, W., and Wu, Q. N. (2017a). The hypoglycemic and antioxidant effects of polysaccharides from the petioles and pedicels of Euryale ferox Salisb. on alloxan-induced hyperglycemic mice. *Food & Funct.* 8, 3803–3813. doi:10.1039/c7fo01035d

Wu, J., Chen, M., Shi, S., Wang, H., Li, N., Su, J., et al. (2017b). Hypoglycemic effect and mechanism of a pectic polysaccharide with hexenuronic acid from the fruits of Ficus pumila L. in C57BL/KsJ db/db mice. *Carbohydr. Polym.* 178, 209–220. doi:10.1016/j.carbpol.2017.09.050

Wu, L., Liu, X., Hu, R., Chen, Y., Xiao, M., Liu, B., et al. (2022a). Prebiotic Agrocybe cylindracea crude polysaccharides combined with Lactobacillus rhamnosus GG postpone aging-related oxidative stress in mice. *Food & Funct.* 13, 1218–1231. doi:10.1039/d1fo02079j

Wu, M. Y., Yiang, G. T., Lai, T. T., and Li, C. J. (2018). The oxidative stress and mitochondrial dysfunction during the pathogenesis of diabetic retinopathy. *Oxid. Med. Cell. Longev.* 2018, 3420187. doi:10.1155/2018/3420187

Wu, N., Jin, W., Zhao, Y., Wang, H., He, S., Zhang, W., et al. (2022b). Sulfated fucogalactan from *Laminaria japonica* ameliorates β-cell failure by attenuating mitochondrial dysfunction *via* SIRT1-PGC1-α signaling pathway activation. *Front. Endocrinol. (Lausanne).* 13, 881256. doi:10.3389/fendo.2022.881256

Xia, X., Mao, D., Dai, H., Wu, X., Zhang, Z., Wang, H., et al. (2020). Effect of Cyclocarya paliurus polysaccharides on streptozotocin-induced diabetic nephropathy in rats. *J. Tradit. Chin. Med.* 40, 956–964. doi:10.19852/j.cnki.jtcm.2020.06.007

Xiang, H., Sun-Waterhouse, D., and Cui, C. J. (2021). Hypoglycemic polysaccharides from Auricularia auricula and Auricularia polytricha inhibit oxidative stress, NF-κB signalling and proinflammatory cytokine production in streptozotocin-induced diabetic mice. *Food. Sci. Hum. Health.* 001,010.

Xu, Y., Zhang, X., Yan, X. H., Zhang, J. L., Wang, L. Y., Xue, H., et al. (2019). Characterization, hypolipidemic and antioxidant activities of degraded polysaccharides from Ganoderma lucidum. *Int. J. Biol. Macromol.* 135, 706–716. doi:10.1016/j.ijbiomac.2019.05.166

Yamagishi, S., and Matsui, T. (2010). Advanced glycation end products, oxidative stress and diabetic nephropathy. *Oxid. Med. Cell. Longev.* 3, 101–108. doi:10.4161/oxim.3.2.11148

Yang, J., Chen, H., Nie, Q., Huang, X., and Nie, S. (2020a). Dendrobium officinale polysaccharide ameliorates the liver metabolism disorders of type II diabetic rats. *Int. J. Biol. Macromol.* 164, 1939–1948. doi:10.1016/j.ijbiomac.2020.08.007

Yang, L., Li, P., Fu, S., Calay, E. S., and Hotamisligil, G. S. (2010). Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. *Cell. Metab.* 11, 467–478. doi:10.1016/j.cmet.2010.04.005

Yang, R., Li, Y., Mehmood, S., Yan, C., Huang, Y., Cai, J., et al. (2020b). Polysaccharides from Armillariella tabescens mycelia ameliorate renal damage in type 2 diabetic mice. *Int. J. Biol. Macromol.* 162, 1682–1691. doi:10.1016/j.ijbiomac.2020.08.006

Yang, Y., Chang, Y., Wu, Y., Liu, H., Liu, Q., Kang, Z., et al. (2021). A homogeneous polysaccharide from Lycium barbarum: Structural characterizations, anti-obesity effects and impacts on gut microbiota. *Int. J. Biol. Macromol.* 183, 2074–2087. doi:10.1016/j.ijbiomac.2021.05.209

Yao, L., Liang, X., Qiao, Y., Chen, B., Wang, P., and Liu, Z. (2022a). Mitochondrial dysfunction in diabetic tubulopathy. *Metabolism* 131, 155195. doi:10.1016/j.metabol.2022.155195

Yao, X., Yuan, Y., Jing, T., Ye, S., Wang, S., and Xia, D. (2022b). Ganoderma lucidum polysaccharide ameliorated diabetes mellitus-induced erectile dysfunction in rats by regulating fibrosis and the NOS/ERK/JNK pathway. *Transl. Androl. Urol.* 11, 982–995. doi:10.21037/tau-22-428

Yaribeygi, H., Farrokhi, F. R., Butler, A. E., and Sahebkar, A. (2019). Insulin resistance: Review of the underlying molecular mechanisms. *J. Cell. Physiol.* 234, 8152–8161. doi:10.1002/jcp.27603

Yaribeygi, H., Sathyapalan, T., Atkin, S. L., and Sahebkar, A. (2020). Molecular mechanisms linking oxidative stress and diabetes mellitus. *Oxid. Med. Cell. Longev.* 2020, 8609213. doi:10.1155/2020/8609213

Yuan, B., Yang, X. Q., Kou, M., Lu, C. Y., Wang, Y. Y., Peng, J., et al. (2017). Selenylation of polysaccharide from the sweet potato and evaluation of antioxidant, antitumor, and antidiabetic activities. *J. Agric. Food. Chem.* 65, 605–617. doi:10.1021/acs.jafc.6b04788

Zhang, C., Huang, M., Hong, R., and Chen, H. (2019a). Preparation of a Momordica charantia L. polysaccharide-chromium (III) complex and its anti-hyperglycemic activity in mice with streptozotocin-induced diabetes. *Int. J. Biol. Macromol.* 122, 619–627. doi:10.1016/j.ijbiomac.2018.10.200

Zhang, C., Zhang, L., Liu, H., Zhang, J., Hu, C., and Jia, L. (2018a). Antioxidation, anti-hyperglycaemia and renoprotective effects of extracellular polysaccharides from Pleurotus eryngii SI-04. *Int. J. Biol. Macromol.* 111, 219–228. doi:10.1016/j.ijbiomac.2018.01.009

Zhang, G., Huang, Y., Bian, Y., Wong, J. H., Ng, T. B., Wang, H. J. A. M., et al. (2006). Hypoglycemic activity of the fungi Cordyceps militaris, Cordyceps sinensis, Tricholoma mongolicum, and Omphalia lapidescens in streptozotocin-induced diabetic rats. *Appl. Microbiol. Biotechno.* l72, 1152–1156. doi:10.1007/s00253-006-0411-9

Zhang, J., Meng, G., Zhang, C., Lin, L., Xu, N., Liu, M., et al. (2015). The antioxidative effects of acidic-alkalic-and enzymatic-extractable mycelium zinc polysaccharides by Pleurotus djamor on liver and kidney of streptozocin-induced diabetic mice. *Bmc. Complement. Altern. Med.* 15, 440. doi:10.1186/s12906-015-0964-1

Zhang, J., Zhao, X., Zhao, L. Q., Zhao, J., Qi, Z., and Wang, L. A. (2017). A primary study of the antioxidant, hypoglycemic, hypolipidemic, and antitumor activities of ethanol extract of Brown slimecap mushroom, chroogomphus rutilus (agaricomycetes). *Int. J. Med. Mushrooms.* 19, 905–913. doi:10.1615/IntJMedMushrooms.2017024564

Zhang, K., Qi, J. X., Li, Y. Y., Gao, H. S., Shao, X. Y., Su, C. Y., et al. (2021). Antioxidative and immunological effects of Cyclocarya paliurus polysaccharides on the spleen injury of diabetic rat. *J. Tradit. Chin. Med.* 41, 739–746. doi:10.19852/j.cnki.jtcm.20210203.001

Zhang, L., Liu, Y., Ke, Y., Liu, Y., Luo, X., Li, C., et al. (2018b). Antidiabetic activity of polysaccharides from Suillellus luridus in streptozotocin-induced diabetic mice. *Int. J. Biol. Macromol.* 119, 134–140. doi:10.1016/j.ijbiomac.2018.07.109

Zhang, Q., Olatunji, O. J., Chen, H., Tola, A. J., and Oluwaniyi, O. O. (2018c). Evaluation of the anti-diabetic activity of polysaccharide from cordyceps cicadae in experimental diabetic rats. *Chem. Biodivers.* 15, e1800219. doi:10.1002/cbdv.201800219

Zhang, Y., Wang, H., Zhang, L., Yuan, Y., and Yu, D. (2020). Codonopsis lanceolata polysaccharide CLPS alleviates high fat/high sucrose diet-induced insulin resistance via anti-oxidative stress. *Int. J. Biol. Macromol.* 145, 944–949. doi:10.1016/j.ijbiomac.2019.09.185

Zhang, Z., Zhang, L., and Xu, H. (2019b). Effect of Astragalus polysaccharide in treatment of diabetes mellitus: A narrative review. *J. Tradit. Chin. Med.* 39, 133–138.

Zhao, C., Liao, Z., Wu, X., Liu, Y., Liu, X., Lin, Z., et al. (2014). Isolation, purification, and structural features of a polysaccharide from Phellinus linteus and its hypoglycemic effect in alloxan-induced diabetic mice. *J. Food. Sci.* 79, H1002–H1010. doi:10.1111/1750-3841.12464

Zhao, H., Lai, Q., Zhang, J., Huang, C., and Jia, L. (2018). Antioxidant and hypoglycemic effects of acidic-extractable polysaccharides from cordyceps militaris on type 2 diabetes mice. *Oxid. Med. Cell. Longev.* 2018, 9150807. doi:10.1155/2018/9150807

Zheng, Y., Bai, L., Zhou, Y., Tong, R., Zeng, M., Li, X., et al. (2019). Polysaccharides from Chinese herbal medicine for anti-diabetes recent advances. *Int. J. Biol. Macromol.* 121, 1240–1253. doi:10.1016/j.ijbiomac.2018.10.072

Zhou, J., Xu, G., Yan, J., Li, K., Bai, Z., Cheng, W., et al. (2015a). Rehmannia glutinosa (Gaertn) DC. polysaccharide ameliorates hyperglycemia, hyperlipemia and vascular inflammation in streptozotocin-induced diabetic mice. *J. Ethnopharmacol.* 164, 229–238. doi:10.1016/j.jep.2015.02.026

Zhou, J., Yan, J., Bai, Z., Li, K., and Huang, K. (2015b). Hypoglycemic activity and potential mechanism of a polysaccharide from the loach in streptozotocin-induced diabetic mice. *Carbohydr. Polym.* 121, 199–206. doi:10.1016/j.carbpol.2014.12.037

Zhu, J., Sun, H., Kang, X., Zhu, H., and Yan, X. (2022). Acidic polysaccharides from Buddleja officinalis inhibit angiogenesis via the Nrf2/ARE pathway to attenuate diabetic retinopathy. *Food & Funct.* 13, 9021–9031. doi:10.1039/d2fo01075e

Glossary

ACC	1-aminocyclopropane-1-carboxylic acid
AGEs	advanced glycosylation end products
AKT	Phosphorylated protein kinase B
ALB	albumin
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATP	adenosine triphosphate
BAX	Bcl-2-associated X protein
BUN	blood urea nitrogen
CAT	catalase
CREA	Creatinine
eNOS	endothelial nitric oxide synthase
ERK	extracellular signal-regulated kinase
FOXO1	forkhead box-1
G6PC	glucose-6-phosphatase
GLUT	glucose transporters
GSH	glutathione
GSH-Px	glutathione peroxidase
GSK3β	glycogen synthase kinase 3 beta
H2O2	hydrogen peroxide
HDL	High-density lipoprotein
HO-1	heme oxygenase-1
INOS	inducible nitric oxide synthase
JNK	Jun N-terminal kinases
LDL	low-density lipoprotein
MDA	malondialdehyde
mTOR	mechanistic target of rapamycin
NF-κB	nuclear transcription factor-kappa B
NO	nitric oxide
Nox4	NADPH oxidase 4
NADPH	quinone oxidoreductase 1
Nrf2	nuclear factor erythroid-2
PEPCK	phosphoenolpyruvate carboxylase
PI3K	phosphatidylinositol 3-k; inase
PI3K	phosphoinositide 3-kinase
PKC	protein kinase C
PPARa	Peroxisome proliferator-activated receptor alpha
ROS	reactive oxygen species
SOD	superoxide dismutase
SREBP-1	Sterol regulatory element binding protein-1
TG	triglyceride
TC	total cholesterol
UA	uric acid; Urea, carbamide

frontiers | Frontiers in Pharmacology

TYPE Systematic Review
PUBLISHED 08 September 2023
DOI 10.3389/fphar.2023.1179705

OPEN ACCESS

EDITED BY
Adolfo Andrade-Cetto,
National Autonomous University of Mexico, Mexico

REVIEWED BY
Mengmeng Zhang,
Shaanxi University of Chinese Medicine, China
Juei-Tang Cheng,
Chang Jung Christian University, Taiwan

*CORRESPONDENCE
Qiu Chen,
chenqiu1005@cdutcm.edu.cn

RECEIVED 04 March 2023
ACCEPTED 02 August 2023
PUBLISHED 08 September 2023

CITATION
Xie Q, Zhang X, Zhou Q, Xu Y, Sun L, Wen Q, Wang W and Chen Q (2023), Antioxidant and anti-inflammatory properties of ginsenoside Rg1 for hyperglycemia in type 2 diabetes mellitus: systematic reviews and meta-analyses of animal studies.
Front. Pharmacol. 14:1179705.
doi: 10.3389/fphar.2023.1179705

Antioxidant and anti-inflammatory properties of ginsenoside Rg1 for hyperglycemia in type 2 diabetes mellitus: systematic reviews and meta-analyses of animal studies

Qian Xie[1], Xiaoran Zhang[2], Qian Zhou[1], Yumei Xu[1], Lisha Sun[1], Qing Wen[1], Wei Wang[3] and Qiu Chen[1]*

[1]Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]School of Clinical Medicine, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [3]School of Biomedical Sciences, Mianyang Normal University, Mianyang, China

Background: According to existing laboratory data, ginsenoside Rg1 may help cure diabetes and its complications by reducing oxidative stress (OS) and managing inflammation. However, this conclusion lacks reliability and is unclear. As a result, the purpose of this systematic review and meta-analysis was to evaluate the antioxidant and anti-inflammatory effects of ginsenoside Rg1 in the treatment of diabetes and its complications.

Methods: We searched for relevant studies published through December 2022, including electronic bibliographic databases such as PubMed, EMBASE, Web of Science, CNKI, and Wanfang. The SYstematic Review Center for Laboratory Animal Experimentation Risk of Bias (SYRCLE RoB) tool was used to conduct a meta-analysis to assess the methodological quality of animal research. The meta-analysis was conducted using RevMan5.4 software, following the Cochrane Handbook for Systematic Reviews of Interventions. This study is registered in the International Systems Review Prospective Registry (PROSPERO) as CRD42023386830.

Results: Eighteen eligible studies involving 401 animals were included. Ginsenoside Rg1 was significantly correlated with blood glucose (BG), insulin levels, body weight, superoxide dismutase (SOD), malondialdehyde (MDA), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) levels. In addition, according to subgroup analysis, the hypoglycemic, anti-inflammatory, and antioxidant effects of ginsenoside Rg1 in type 2 diabetic animals were not affected by experimental species, modeling, experimental drug dosage, or course of treatment.

Conclusion: This meta-analysis presents a summary of the hypoglycemic effects of ginsenoside Rg1, which are achieved through anti-inflammatory and antioxidant mechanisms. These findings provide evidence-based support for the medical efficacy of ginsenoside Rg1. Specifically, ginsenoside Rg1 reduced MDA levels and restored SOD activity to exert its antioxidant activity. It had a positive effect on the reduction of IL-6 and TNF-α levels. However, the inclusion of studies with low methodological quality and the presence of publication bias may undermine the validity of the results. Further investigation with a more rigorous

experimental design and comprehensive studies is necessary to fully understand the specific glycemic mechanisms of ginsenosides.

Systematic Review Registration: https://www.crd.york.ac.uk/PROSPERO/, identifier https://CRD42023386830.

KEYWORDS

antioxidant and anti-inflammatory properties, ginsenoside Rg1, hyperglycemia, type 2 diabetes mellitus, systematic reviews and meta-analyses, animal studies

1 Introduction

Ginseng, a perennial herb of the Acanthopanaceae family, has been documented in the ancient Shennong Herbal Classic for over 5,000 years (Baeg and So, 2013). A variety of diseases are treated with it in China due to its use in treating qi deficiency (Park et al., 2012). The main bioactive component of ginseng is ginsenosides (Yun, 2001). Ginsenosides are classified into three groups based on their chemical structures: protodiol (PPD), including Rb1, Rb2, Rb3, Rc, Rd, and Rg3; protriols (PPT), including Rg1, Re, Rf, and Rg2; and ginsenoside Ro (Song et al., 2017; Kim et al., 2018). A significant component of ginseng, ginsenoside Rg1, is relatively safe and has a low level of toxicity (Xie et al., 2015). Laboratory studies have shown that ginsenoside Rg1 has systemic effects and is therapeutic for a wide range of diseases. Ginsenoside Rg1 produces antioxidant and hepatoprotective effects by inducing the Keap1-Nrf2-ARE signaling pathway (Gao et al., 2017). Rg1 significantly attenuated multiple inflammatory responses in dextran sodium sulfate (DSS)-induced colitis in mice (Zhu et al., 2017). Meanwhile, ginsenoside Rg1 can treat H_2O_2-induced lens clouding through an anti-oxidative stress (OS) mechanism (Zhang et al., 2021). It acts in neurological disorders through multiple signaling pathways and related molecular mechanisms (Sun et al., 2022). In addition, rg1 reduces plasma cholesterol and triglyceride levels and inhibits the formation of aortic atherosclerosis, resulting in important benefits for cardiovascular disease (Xue et al., 2021). This article focuses on the role of ginsenoside Rg1 in T2DM. Rg1 restores glucose homeostasis and insulin sensitivity and attenuates obesity and insulin resistance. Rg1 further inhibits hepatic gluconeogenesis by preserving glucagon-impaired Akt activation while promoting Akt binding to FoxO1 and inactivating FoxO1 by phosphorylation (Liu et al., 2017). In addition, ginsenoside rg1 improved STZ-mediated diabetes in animals by reducing inflammatory cytokines (Alolga et al., 2020). At the same time, modern pharmacological studies have shown that ginsenoside rg1 has neuroprotective effects and can effectively alleviate diabetic peripheral neuropathy (Sun et al., 2022). These findings suggest that ginsenoside rg1 has beneficial effects on diabetes in terms of its antioxidant and anti-inflammatory properties.

Diabetes mellitus has become a worldwide public health problem in the 21st century due to the dramatic increase in the number of patients (Kitada et al., 2019). Globally, such diseases have spread due to population explosions, aging, urbanization, overweight, and lifestyle choices (Fernandez-Twinn et al., 2019). The main manifestation of diabetes is the development of long-term chronic hyperglycemia (Aryangat and Gerich, 2010). Studies have shown that chronic hyperglycemia greatly increases the risk of microvascular and macrovascular complications in diabetes, as well as mortality from cardiovascular disease (Nowaczewska et al., 2019). Glycated hemoglobin is a useful indicator of long-term blood sugar in the body. Therefore, actively controlling hyperglycemia and keeping glycated hemoglobin within the normal range is an important therapeutic tool for the effective treatment of diabetes and the prevention of its complications. The cause of type 2 diabetes mellitus is closely related to inflammation because bad diet and living habits lead to the accumulation of fat cells, causing a series of inflammatory reactions in the body, resulting in insulin resistance (Zozulinska and Wierusz-Wysocka, 2006; Alolga et al., 2020). According to the literature data, OS and inflammation may cause direct harm to the blood vessels of streptozotocin-induced diabetic animals, which could result in various complications (Zhao et al., 2014; Wronka et al., 2022). Most of the current drugs for the management of type 2 diabetes achieve the hypoglycemic effect by enhancing insulin sensitivity, promoting insulin secretion, inhibiting the reabsorption of terminal circulation, and supplementing exogenous insulin. Although blood glucose (BG) can be effectively controlled, the risk of hypoglycemia is high, and its complications remain inevitable. An increasing number of studies have shown that many hypoglycemic agents have antioxidant and anti-inflammatory properties. For example, metformin achieves its antioxidant and anti-inflammatory effects through the mechanism of AMPK activation (Dehkordi et al., 2019). In addition, we found that thiazolidinediones, sulfonylureas, α-glucosidase inhibitors, and glucagon-like peptide-1 receptor agonists have varying degrees of anti-inflammatory potential (Mathews et al., 2016). The study of traditional plant herbal extracts revealed that extracts, such as berberine and quercetin, have hypoglycemic, antioxidant, and anti-inflammatory properties (Li et al., 2015; Azeem et al., 2023). Therefore, the development of new drugs from antioxidant and anti-inflammatory mechanisms can effectively lower glucose hyperglycemia and prevent adverse events and complications.

There is currently no meta-analysis based on preclinical studies to summarize favorable evidence for ginsenoside rg1 in the treatment of type 2 diabetes. Furthermore, the results from animal trials are frequently influenced by several factors, which include small modeling sample numbers, modeling methodologies (Skovso, 2014), and intervention time. An umbrella review is a statistical analysis based on the review, analysis, sorting, and synthesis of the original literature, which is a method of comprehensive analysis of the results of previous similar studies. It integrates the results of previous studies in a standardized and quantitative manner, making evidence-based medical conclusions more reliable. Hence, we conducted a scientific review of these trial data to assess the antioxidant and anti-inflammatory potential of ginsenoside rg1 in the therapy of type 2 diabetes, which will help bridge the gap between animal research and clinical application and provide evidence support for future clinical work.

2 Materials and methods

This systematic review was conceived and is presented in the following paragraphs, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al., 2021). The protocol concerning this study is obtainable at PROSPERO, CRD42023386830.

2.1 Search strategies

The electronic bibliographic information databases, comprising PubMed, EMBASE, Web of Science, Zhiwang, and Wanfang, were used for pertinent research published through December 2022. There are no language restrictions on retrieval. The medical subject terms (MeSH) and free terms used for database searches are [(" diabetes" or "type 2 diabetes") and (" ginsenoside" or "ginsenoside rg1") and ("animal" or "animal model" or "rat" or "mouse")].

2.2 Inclusion criteria

1) Model: for all animal models with diabetes, blood sugar (BG) ≥ 11.1 mmol/L was considered as the criteria for successful modeling; 2) intervention: ginsenoside rg1 was given at any dose and duration; 3) control: the control group was given equal dose volume of non-functional sterile liquid (e.g., water and normal saline) or no treatment; 4) outcome: BG, insulin level, OS index, and inflammatory cytokines were observed; and 5) study design: it encompassed control study and separate control group.

2.3 Exclusion criteria

The exclusion criteria were as follows: 1) clinical trials or *in vivo* experiments; 2) the treatment group not treated with ginsenoside rg1; 3) control: other ginsenoside rg1 preparations (some medicinal preparations or supplements containing ginsenoside rg1); 4) study design: case study, crossover study, and study without a separate control group; 5) not original or incomplete research papers; 6) repeated release; and 7) no full-text study available.

2.4 Research selection and data extraction

All searched articles are entered into EndNote X9, and duplicate articles are deleted. Two investigators independently conducted literature collection according to the inclusion and exclusion criteria. Initially, titles and abstracts were selected to preclude extraneous articles. After the initial screening, potentially eligible articles underwent full-text screening for final determination.

Two evaluators abstracted the following messages from the enrolled studies: 1) basic data: initial author's surname, name, and year of publication; 2) features of the experimental animals, which included animal type, sex, sample size, age, and weight; 3) modeling approach and criteria for successful modeling; 4) treatment information: administration method, source, duration, and dose of intervention drugs; 5) and outcome indicators: BG, insulin levels, TNF-α, IL-6, MDA, and SOD. Analyses of indicators, such as IL-1β, ROS, and GSH, were abandoned due to the inadequacy of the included experiments. All resultant measures were continuous data; therefore, means and standard deviations were drawn for each intervening group. We built a database and hand-pulled data from the collected papers. If the results were presented only in the form of graphs, we attempted to contact the authors for more details. If there was no response, graphical data were quantified using WebPlotDigitizer4.5 software (https://automeris.io/WebPlotDigitizer).

If results were presented at more than one time point, data were retrieved from the final time point. If the drug involved more than one dose in the treatment group, we extracted only the data for the highest dose. At each stage, two evaluators independently assessed and extracted each study. Disagreements between the two investigators about whether the study should be integrated and the data extracted were addressed through discussions with a third evaluator.

2.5 Bias risk assessment

We evaluated the methodological quality of the enrolled research using the SYstematic Review Center for Laboratory Animal Experimentation Risk of Bias (SYRCLE RoB) tool (Hooijmans et al., 2014). The SYRCLE RoB tool for animal research comprises 10 programs based on six different types of biases. The maximum score for individual studies was 10 points. Any discrepancies that arose during the quality assessment process were ultimately resolved through negotiation with the appropriate authors.

2.6 Statistical analysis

The meta-analysis was performed using RevMan 5.4 software. All outcome indicators were continuous variables (e.g., BG, serum, and insulin levels). Therefore, the combined total effect sizes of the results were expressed using standardized mean differences (SMD) and 95% confidence intervals (CI), and p-values <0.05 were deemed statistically meaningful. Heterogeneity between studies was evaluated using I-squared (I^2), with a fixed-effects model combining effect sizes for $I^2 \leq 50\%$. $I^2 > 50\%$ was considered to represent substantial heterogeneity, and a random-effects model was utilized to combine effect sizes. If sufficient studies were available, subgroup analyses were performed to identify sources of heterogeneity based on the following variables: species (rats, mice), STZ dose (≤50 and ≥50 mg/kg), drug dose (<40 mg/kg/day, ≥40 mg/kg/day), and intervention duration (<8 weeks, ≥8 weeks). A sensitivity analysis was conducted to evaluate whether separate studies would impact the total effect size by excluding one study at each stage to appraise the stability of the overall outcome. If there were at least 10 studies per outcome, potential publishing bias was evaluated using funnel plots and Egger tests (Egger et al., 1997). In addition, trimming and padding methods were conducted in the presence of publication bias.

3 Results

3.1 Research inclusion

A systematic evaluation and meta-analysis of the search database resulted in the establishment of 437 animal studies.

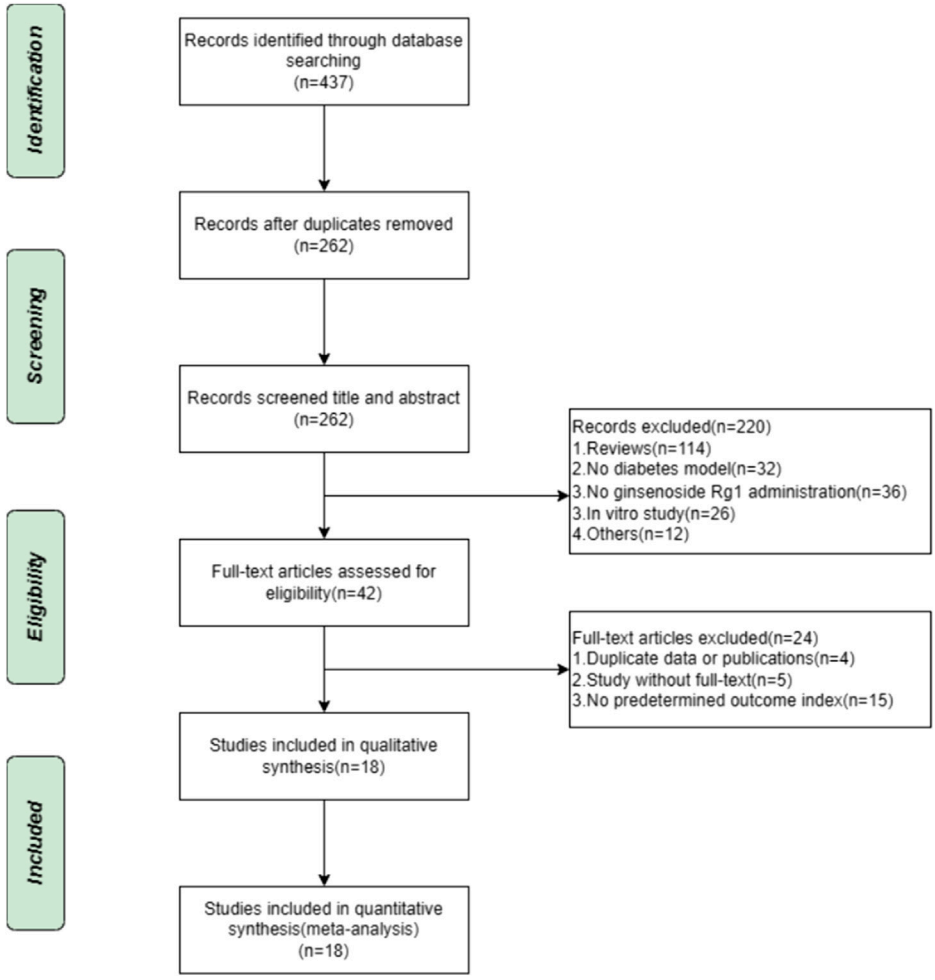


FIGURE 1
Flowchart for inclusion of studies.

After the elimination of duplicates, 262 publications were left. Of the titles and abstracts screened, 220 publications were rejected for the following factors: 1) review articles, 2) not diabetic animal model, 3) no interventions using ginsenoside rg1, 4) *in vitro* studies, and 5) others. The remaining 42 animal studies were then screened for full text. A total of 24 studies were found to be non-compliant for the following reasons: 1) duplicate data or publications (n = 4), 2) studies without full text (n = 5), and 3) the absence of predetermined outcome indicators (n = 15). Ultimately, 18 eligible projects were included in this systemic evaluation. The selection process is depicted in Figure 1.

3.2 Research characteristics

Eighteen studies published between 2010 and 2022 were accepted: nine in English (Yang et al., 2012; Yu et al., 2016; Tian et al., 2017; Yin and Wang, 2017; Yuan et al., 2019; Gao et al., 2020; Liu et al., 2021; Ma et al., 2022; Peng et al., 2022) and nine in Chinese (Jie, 2010; Xin, 2015; Jianchao, 2016; Zheng Yongren, 2016; Chen Xianren, 2020; Yang Jing, 2020; Eshu, 2021; Wu Lina, 2021; Jie, 2022). A total of 401 animals were involved, of which the test group comprised 203 animals and the control group comprised 198 animals. The animal species consisted of rats and mice, with six studies (33.3%) using mice and 12 studies (66.7%) using rats. In all studies, body weights were 180–280 g for rats and 20–26 g for mice. Four studies did not report animal weights. Multiple levels of ginsenoside rg1 dosing ranging from 1 to 100 mg/kg/day were implemented in these studies, and two studies did not report ginsenoside rg1 dosing. The control group consisted mainly of the same solvent, such as saline. Intervention durations were <8 weeks and ≥8 weeks, with durations ranging from 4 to 12 weeks. The intervention duration was <8 weeks in five studies (27.8%) and ≥8 weeks in 11 studies (61.1%), and two studies did not report the intervention duration. Table 1 describes the basic features of the 18 studies. Furthermore, a summary table depicting ginsenoside rg1 is displayed in Supplementary Table S2.

3.3 Research quality

We evaluated the quality of the included studies. The overall quality of each study was low, with a range of three–five points.

TABLE 1 Characteristics of the included studies.

Study (year)	Species (sex, *n* = treatment/control group, age, and weight)	Modeling method and standard	Ginsenoside Rg1 intervention (administration, drug dose, and duration)	Outcomes
Feng Jie (2010)	ICR mice (male, 10/10, 22 ± 3 g)	Tail vein injection of alloxan (85 mg/kg) BG > 11.1 mmol/L	By gavage, 10/20/40 mg/kg/d, 4 weeks	1. BG 2. Weight 3. SOD
Zhen et al. (2016)	SD rats (male, 12/12, aged 8 weeks, 200 ± 20 g)	Intraperitoneal injection of STZ (55 mg/kg) + HSFD	By gavage, 20/40/80 mg/kg/d, 6 weeks	1. BG 2. Insulin
Yao Jianchao (2016)	SD rats (male, 12/12)	HSFD FBG ≥ 7.8 mmol /L or RBG≥ 11.1 mmol /L	By gavage, 2/4/8 mg/kg/d, 8 weeks	1. BG 2. TNF-α 3. IL-6 4. SOD 5. MDA
Ruan et al. (2021)	SD rats (male, 6/6, 180~200 g)	Intraperitoneal injection of STZ (35 mg/kg) + HSFD	By gavage, 25/50/100 mg/kg/d, 4 weeks	1. TNF-α 2. IL-6
Yang Jing (2020)	SD rats (male, 20/20, 200 ± 20 g)	Intraperitoneal injection of STZ (55 mg/kg) BG > 16.7 mmol/L	By gavage, 21 mg/kg/d, 12 weeks	1. SOD 2. MDA
Li et al. (2015)	SD rats (10/10, 200~220 g)	Intraperitoneal injection of STZ (30 mg/kg) + HSFD FBG ≥ 7.8 mmol/L or RBG ≥ 11.1 mmol/L	By gavage, 2/4/8 mg/kg/d, 8 weeks	1. BG 2. TNF-α 3. IL-6 4. SOD 5. MDA
Wu Lina (2021)	SD rats (male, 10/10, 180~200 g)	Intraperitoneal injection of STZ (40 mg/kg) + HSFD FBG ≥ 11.1 mmol/L	By gavage, 10/30 mg/kg/d, 8 weeks	1. TNF-α 2. IL-6
Li et al. (2022)	C57BL/6J mice (male, 8/8, aged 8 weeks, 20~26 g)	Intraperitoneal injection of STZ (110 mg/kg) + HFD BG > 16.7 mmol/L	By gavage, 1/5/10 mg/kg/d, 8 weeks	1. BG
Chen Xianren (2020)	SD rats (male, 15/15, aged 3 weeks, 160~200 g)	Intraperitoneal injection of STZ (30 mg/kg) + HSFD BG ≥ 16.7 mmol/L	By gavage, 10 mg/kg/d, 8 weeks	1. BG 2. SOD 3. MDA
Ma et al. (2022)	C57BL/6J mice (10/10, aged 4 weeks)	Intraperitoneal injection of STZ (60 mg/kg) BG > 16.7 mmol/L	By gavage	1. TNF-α 2. IL-6
Yang et al. (2012)	C57BL/6J mice (male, 23/23, aged 7 weeks, 20–25 g)	Intraperitoneal injection of STZ (50 mg/kg) FBG > 11.1 mmol/L	By gavage, 10 mg/kg/d, 4 weeks	1. BG 2. IL-6
Gao et al. (2019)	40 db/db mice and 10 wild-type mice (male, 10/10, aged 22 weeks)	Spontaneous diabetic model	By gavage, 25/50 mg/kg/d, 8 weeks	1. BG 2. SOD
Yuan et al. (2019)	6/6	Intraperitoneal injection of STZ (35 mg/kg) BG > 16.7 mmol/L	By gavage	1. BG
Liu et al. (2021)	SD rats (male, 8/8, aged 8 weeks, 180–200 g)	Intraperitoneal injection of STZ (50 mg/kg)	By intraperitoneal injection, 50 mg/kg/d, 8 weeks	1. TNF-α 2. IL-6 3. SOD 4. MDA

(Continued on following page)

TABLE 1 (*Continued*) Characteristics of the included studies.

Study (year)	Species (sex, *n* = treatment/control group, age, and weight)	Modeling method and standard	Ginsenoside Rg1 intervention (administration, drug dose, and duration)	Outcomes
Yaoyao Yin and Junxia Wang, 2017	SD rats (male, 10/10, 210–280 g)	Intraperitoneal injection of STZ (65 mg/kg) BG > 16.7 mmol/L	By gavage, 50 mg/kg/d, 8 weeks	1. BG
				2. TNF-α
Tian et al. (2017)	SD rats (male, 10/10, aged 4 weeks, 200 ± 20 g)	Intraperitoneal injection of STZ (30 mg/kg) + HFD BG > 16.7 mmol/L	By gavage, 25/50 mg/kg/d, 8 weeks	1. BG
				2. Insulin
				3. TNF-α
				4. IL-6
Yu et al. (2015)	Wistar rats (male, 15/10, aged 4 weeks, 200±20 g)	Intraperitoneal injection of STZ (40 mg/kg) + HSFD BG > 16.7 mmol/L	By intraperitoneal injection, 10/15/20 mg/kg/d, 12 weeks	1. BG
Peng et al. (2022)	SD rats (male, 8/8, adult, 180–200 g)	Intraperitoneal injection of STZ (35 mg/kg) + HFDB G > 16.7 mmol/L	By gavage, 25/100 mg/kg/d, 4 weeks	1. BG
				2. Insulin
				3. TNF-α
				4. IL-6
				5. SOD
				6. MDA

Abbreviations: BG, blood glucose; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; SOD, superoxide dismutase; MDA, malondialdehyde; STZ, streptozocin; Scr, serum creatinine; HFD, high-fat diet; HSFD, high-sugar and high-fat diet.

TABLE 2 Risk of bias of included studies.

Study (year)	A	B	C	D	E	F	G	H	I	J	Total
Feng Jie, 2010	–	+	?	–	–	–	–	+	+	+	4
Zhen et al., 2016	–	–	?	+	–	–	–	+	+	+	4
Yao Jianchao, 2016	–	–	–	–	–	–	–	+	+	+	3
Ruan et al., 2021	–	+	–	–	–	–	–	+	+	+	4
Yang Jing (2020)	–	–	–	–	–	–	–	+	+	+	3
Li et al. (2015)	–	–	+	?	–	–	–	+	+	+	4
Wu Lina (2021)	–	–	?	+	–	–	–	+	+	+	4
Li et al., 2022	–	–	–	–	–	–	–	+	+	+	3
Chen Xianren (2020)	–	–	+	–	–	–	–	+	+	+	4
Ma et al. (2022)	–	–	–	?	–	–	–	+	+	+	3
Yang et al. (2012)	–	+	–	–	–	–	–	+	+	+	4
Gao et al., 2019	–	–	+	–	–	–	–	+	+	+	4
Yuan et al. (2019)	–	–	+	+	–	–	–	+	+	+	5
Liu et al. (2021)	+	+	?	?	–	–	–	+	+	+	5
Yaoyao Yin and Junxia Wang, 2017	–	+	–	–	–	–	–	+	+	+	4
Tian et al. (2017)	–	+	+	+	–	–	–	+	+	+	6
Yu et al., 2015	+	+	?	+	–	–	–	+	+	+	6
Peng et al. (2022)	–	+	+	?	–	–	–	+	+	+	5

A, sequence generation; B, baseline characteristics; C, allocation concealment; D, random housing; E, blinding of experimentalists; F, random for outcome assessment; G, blinding of outcome assessors; H, incomplete outcome data; I, selective outcome reporting; J, other biases; +, low risk of bias; –, high risk of bias; ?, unclear risk of bias.

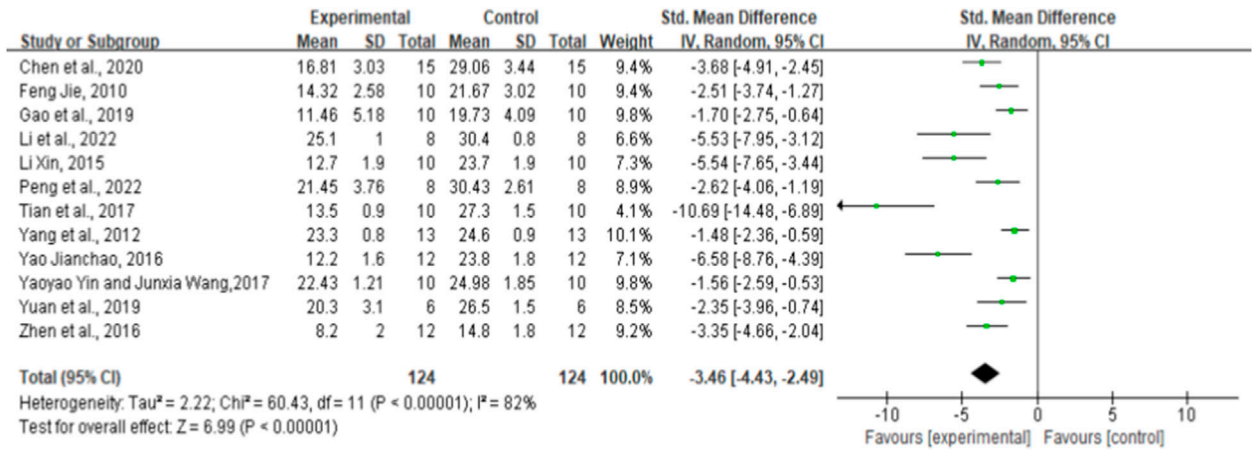


FIGURE 2
Forest plot: effect of ginsenoside Rg1 on blood glucose.

Two studies obtained six points, three studies obtained five points, nine studies obtained four points, and four studies obtained three points. Of the 18 included studies, two studies (11.1%) mentioned random sequence generation, eight studies (44.4%) reported baseline characteristics in full, six studies (33.3%) reported allocation concealment, and five studies (27.8%) described randomized captivity. All studies did not report blinded allocation, randomization of outcome evaluation, or blinding of outcomes. All of these studies have comprehensive outcome-based data and published intended results. Concerning other sources of bias, all studies showed no conflict of interest between authors. Methodologically relevant qualities regarding the incorporated studies are summarized in Table 2.

3.4 Effect of ginsenoside Rg1 on blood glucose

Twelve randomized controlled trials showed the effect of ginsenoside rg1 on BG. The aggregated results show that ginsenoside rg1 significantly reduced BG levels compared to controls [n = 248, SMD = −3.46, 95% CI (−4.43, −2.49), p < 0.00001; heterogeneity: X^2 = 60.43, p < 0.00001; I^2 = 82%, Figure 2]. Subgroup analysis was performed based on the type of animal model, intervention duration, drug dose, and STZ dose. Additional beneficial effects were noted when rats (p < 0.001), STZ doses <50 mg/kg (p < 0.001), intervention durations ≥8 weeks (p < 0.001), and ginsenoside rg1 doses <40 mg/kg (p < 0.001) were studied (Supplementary Table S3). For the BG subgroup, analyses did not reveal sources of heterogeneity between studies, and significant heterogeneity remained. In addition, visual inspection of funnel plots revealed asymmetric effects of ginsenoside rg1 on BG (Supplementary Figure S1), whereas the outcome of the Egger test was statistically significant [intercept: −7.90, 95% CI (−8.44, −4.73); p = 0.000] (Supplementary Figure S1).

3.5 Effect of ginseng Rg1 on insulin levels

By combining the effect sizes of the three pairwise comparisons, a significant reduction in insulin levels was observed after ginseng rg1 administration compared with the control group [n = 60, SMD = −3.18, 95% CI (−4.00, −2.35), p < 0.00001; heterogeneity: X^2 = 3.71, p = 0.16; I^2 = 46%, Figure 3]. In addition, no publication bias was applied to insulin levels because fewer than 10 studies were included.

3.6 Effect of ginsenoside Rg1 on TNF-α

By combining the effect sizes of the eight pairwise comparisons, TNF-α levels were significantly reduced after ginsenoside rg1 administration compared to control [n = 156, SMD = −6.49, 95% CI (−8.34, 4.64), p < 0.00001; heterogeneity: X^2 = 33.73, p < 0.0001; I^2 = 79%, Figure 4]. Subgroup analysis was performed based on the animal model type, intervention duration, drug dose, and STZ dose. Additional beneficial effects were noted when mice (p < 0.001), STZ doses ≥50 mg/kg (p = 0.02), intervention durations ≥8 weeks (p < 0.001), and ginsenoside rg1 doses ≥40 mg/kg (p < 0.001) were studied (Supplementary Table S3). The results of the subgroup analysis indicate that drug and STZ doses may be the source of TNF-α heterogeneity. Moreover, no publication bias was applied to TNF-α because fewer than 10 studies were included.

3.7 Effect of ginsenoside Rg1 on IL-6

Regarding the effect on IL-6, seven randomized controlled trials showed the effect of ginsenoside rg1 on this outcome. The aggregated results showed that ginsenoside rg1 significantly decreased IL-6 levels compared to controls [n = 136,

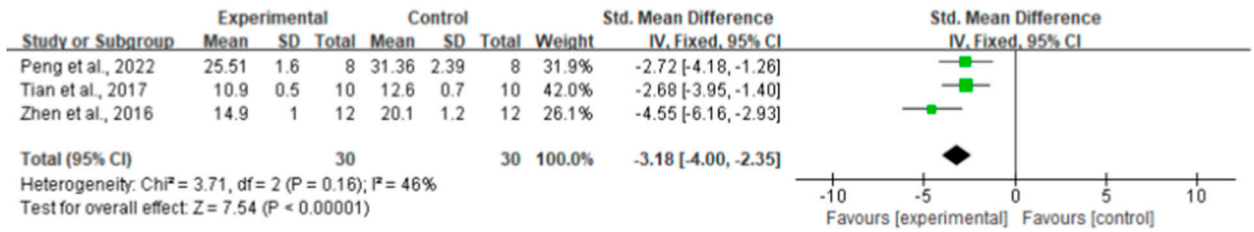


FIGURE 3
Forest plot: effect of ginseng Rg1 on insulin levels.

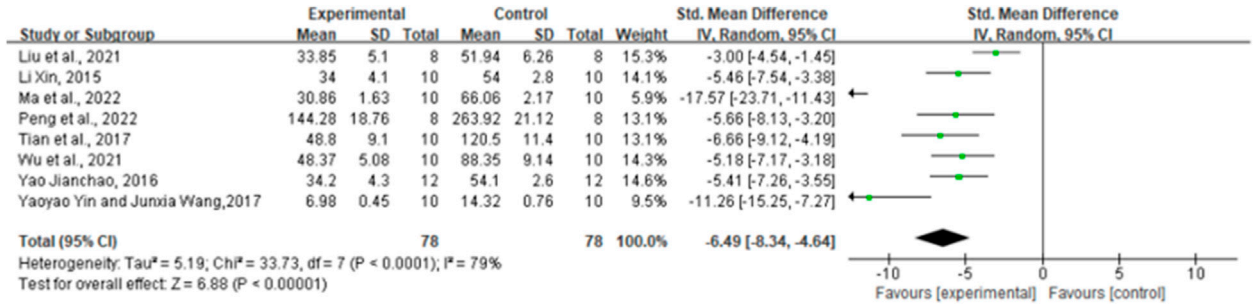


FIGURE 4
Forest plot: effect of ginseng Rg1 on TNF-α.

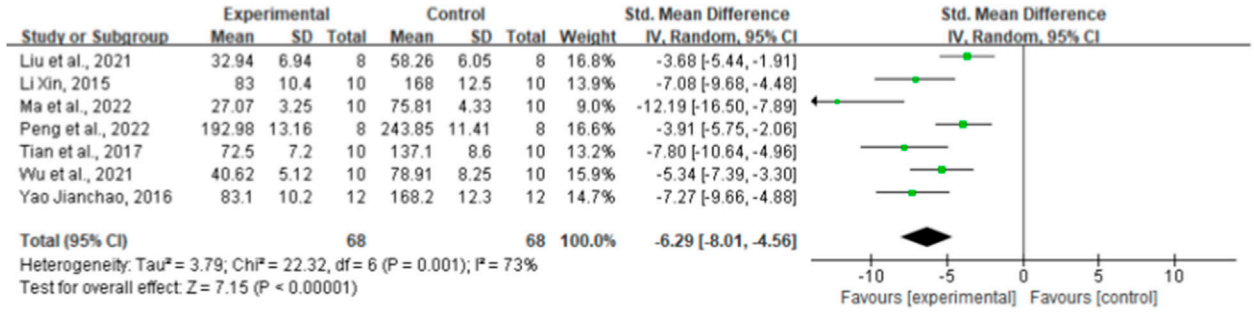


FIGURE 5
Forest plot: effect of ginseng Rg1 on IL-6.

SMD = −6.29, 95% CI (−8.01, 4.56), $p < 0.00001$; heterogeneity: $X^2 = 22.32$, $p = 0.001$; $I^2 = 73\%$, Figure 5]. Subgroup analysis was performed based on animal model type, intervention duration, drug dose, and STZ dose. Additional beneficial effects were noted when mice ($p < 0.001$), STZ doses ≥50 mg/kg ($p < 0.001$), intervention durations ≥8 weeks ($p < 0.001$), and ginsenoside rg1 doses <40 mg/kg ($p < 0.001$) were studied (Supplementary Table S3). The results of the subgroup analysis indicate that drug dose may be a source of IL-6 heterogeneity. In addition, no publication bias was applied to IL-6 because fewer than 10 studies were included.

3.8 Effect of ginsenoside Rg1 on superoxide dismutase

Seven paired comparisons mentioned the effect of ginsenoside rg1 on SOD. The aggregated results show that ginsenoside rg1 significantly increased SOD levels compared to the control [$n = 156$, SMD = 3.63, 95% CI (2.33, 4.93), $p < 0.00001$; heterogeneity: $X^2 = 33.88$, $p < 0.00001$; $I^2 = 82\%$, Figure 6]. Subgroup analysis was performed based on animal model type, intervention duration, drug dose, and STZ dose. Studies showed more beneficial effects when using rats ($p <$

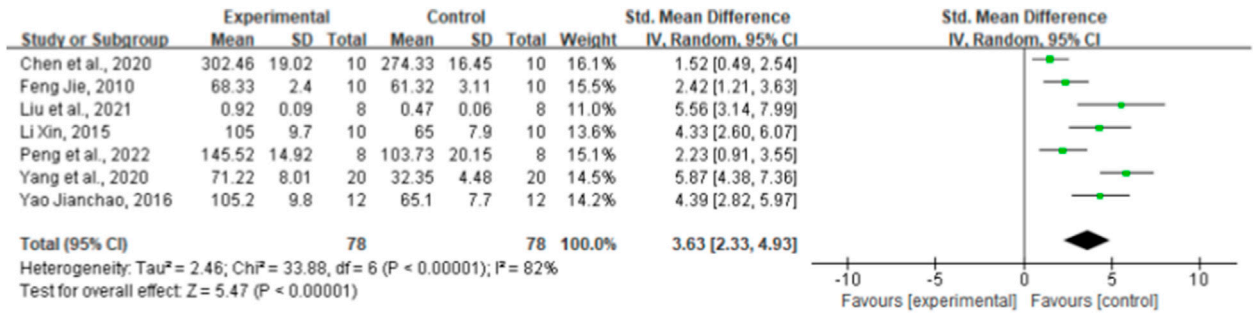


FIGURE 6
Forest plot: effect of ginseng Rg1 on SOD.

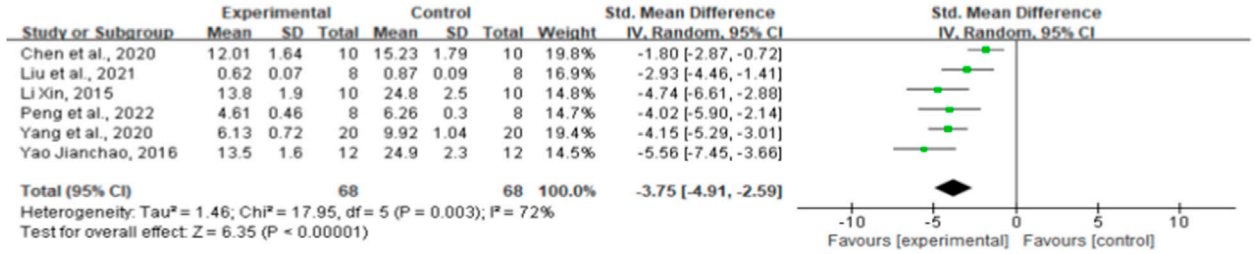


FIGURE 7
Forest plot: effect of ginseng Rg1 on MDA.

0.001), STZ doses ≥50 mg/kg ($p < 0.001$), intervention durations ≥8 weeks ($p < 0.001$), and ginsenoside rg1 doses <40 mg/kg ($p < 0.001$) (Supplementary Table S3). The results of the subgroup analysis indicate that the STZ dose and intervention duration may be a source of SOD heterogeneity. In addition, publication bias was not applied to SOD because fewer than 10 studies were included.

3.9 Effect of ginsenoside Rg1 on malondialdehyde

MDA effect sizes were calculated using data from six animal trials. Ginsenoside rg1 resulted in significantly lower MDA levels [$n = 136$, SMD = −3.75, 95% CI (−4.91, −2.59), $p < 0.00001$; heterogeneity: $X^2 = 17.95$, $p = 0.003$; $I^2 = 72\%$, Figure 7]. The included studies were stratified based on several variables, such as intervention duration, drug dose, and STZ dose. More positive effects were observed when studies used STZ doses ≥50 mg/kg ($p < 0.001$), when intervention duration was <8 weeks ($p < 0.001$), and when ginsenoside rg1 doses <40 mg/kg were used ($p < 0.001$) (Supplementary Table S3). The findings of the subgroup analysis imply that the sources of MDA heterogeneity may be STZ and medication doses. In addition, publication bias was not applied to MDA because fewer than 10 studies were included.

3.10 Sensitivity analysis

Sensitivity analyses for BG, TNF-α, IL-1β, SOD, and MDA were performed separately by deleting one study at each stage and identifying that none of the studies significantly affected the combined effect size.

4 Discussion

4.1 Research purpose

The purpose of this study was to assess the active effect of ginsenoside rg1 on T2DM model animals and its underlying mechanisms. A total of 18 studies comprising 437 animals were involved, and the results from this systematic evaluation indicate that ginsenoside rg1 significantly reduced FBG levels and improved the antioxidant and anti-inflammatory capacity of the organism. Therefore, we hypothesize that ginsenoside rg1 achieves its hypoglycemic effect on T2DM animals through an anti-inflammatory and antioxidant mechanism. We further performed subgroup analyses of the primary outcomes, including FBG, superoxide dismutase (SOD), malondialdehyde (MDA), and tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels. Findings of the subgroup analysis indicate that animal species, ginsenoside rg1 dose, treatment duration, and STZ dose may not

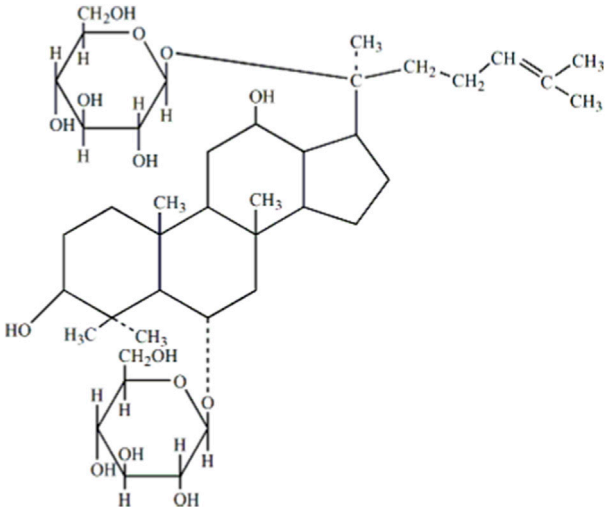


FIGURE 8
Chemical structure of ginsenoside Rg1.

be a source of study heterogeneity. Consequently, we hypothesize that heterogeneity could be caused by other differences in the research, for instance, in the design of the study protocol, criteria for successful modeling, characteristics of the samples, and the size of the experimental samples. Therefore, more studies are necessary to provide more precise evidence.

4.2 Ginseng and the anti-inflammatory and oxidative effects of ginsenosides

Chinese herbal medicines (CHM) and their bioactive components usually act on multiple targets and exhibit a pleiotropic spectrum of action. Hence, they may simultaneously affect the underlying processes of diabetes pathogenesis and achieve better efficacy in treating diabetes. Ginsenoside Rg1 (molecular formula: C42H72O14) is primarily obtained from the roots or stems of ginseng, which is derived from the hydride of dammarane, and its chemical structure is shown in Figure 8 (He et al., 2020). Ginsenosides are classified into three categories according to their chemical structures: protopanaxadiol, protriol, and ginsenoside Ro (Song et al., 2017; Kim et al., 2018). According to their different hydroxylation positions because of the core triterpene saponin structure, ginsenosides can be classified into 20(S)-protanediol (PPD) and 20(S)-protatriol (PPT). Different from PPD-type ginsenosides, which are slowly excreted into the bile, PPT-type ginsenoside rg1 is mainly eliminated through rapid hepatobiliary excretion (Gao et al., 2017). Therefore, the molecule-specific structure of Rg1 is a primary parameter in determining the plasma pharmacokinetics of Rg1 and possibly a factor in the drug interaction between Rg1 and its intended molecule. Owing to its distinctive chemical structure, ginsenoside rg1 has a wealth of pharmacological characteristics. Studies have shown that Rg1 can affect a variety of systems in the body, displaying various pharmacological activities (Ahmed et al., 2016; Zhou et al., 2019; Nakhjavani et al., 2020). Ginsenoside rg1 was effective in reducing OS in diabetic rats by participating in the AMPK/Nrf2/HO-1 pathway (Qin et al., 2019). Meanwhile, Rg1 treats T2DM by reducing insulin resistance due to dietary reasons and decreasing the inflammatory response (Fan et al., 2019; Alolga et al., 2020). Current reviews have shown that ginsenosides can be used to treat diabetes through a variety of mechanisms, and ginsenoside rg1 shows the best therapeutic promise as a potential adjuvant for type 2 diabetes (Bai et al., 2018). In this paper, the therapeutic potential of ginsenoside rg1 for diabetes mellitus is elaborated in terms of antioxidant and anti-inflammatory mechanisms.

4.3 Oxidative stress is an important mechanism in the pathogenesis of type 2 diabetes, and ginsenosides can be antioxidants

A metabolic malfunction known as OS is caused by an excessive rise in reactive oxygen species (ROS) and an unbalanced antioxidant defense mechanism in the body. Because mitochondria are a major source of ROS, the tight connection between the two organelles via mitochondria-associated membranes (MAMs) means that ROS produced by mitochondria can further contribute to ER stress (Burgos-Moron et al., 2019). OS is recognized as a key risk factor for the onset and progression of diabetes, and the mechanism of occurrence is often multifactorial, covering many cellular signaling pathways (Singh et al., 2022). Under the condition of hyperglycemia, ROS activates many signaling pathways, such as nuclear factor-κβ (NF-κβ) and protein kinase C (PKC), all of which may be related to the dysfunction of the insulin signaling pathway and lead to insulin resistance (Zhang et al., 2020).

OS is the primary factor of β-cell dysfunction and death caused by glucose toxicity and insulin resistance in T2DM (Dinić et al., 2022). Therefore, preserving and restoring functional pancreatic beta cells is a daunting challenge in treating diabetes, regardless of its type (Galicia-Garcia et al., 2020; Batista et al., 2021). In contrast, ginsenoside Rg1, which has free-radical scavenging properties, may play a positive role in surpassing the effects of weakened islet function. Ginseng Rg1 has been demonstrated to reduce D-galactose-induced oxidative damage in the ovary by enhancing T-SOD and GSH-Px activity, decreasing MDA levels, removing free radicals, and activating antioxidant enzymes (He et al., 2017). The growing body of research reveals that ginseng Rg1 has considerable antioxidant potential. Ginseng Rg1 significantly inhibited apoptosis and cystatin-3 activation and reduced ROS and MDA production (Li Q. et al., 2017; Ning et al., 2018). It inhibits NF-κB and inflammatory factor expression, further triggering PI3K/AKT activation and FOXO3 phosphorylation to inhibit apoptosis and reduce diabetes-induced inflammation and OS (Yang et al., 2012; Liu et al., 2021). The liver's ability to absorb glucose can be further increased, and hyperglycemia can be decreased by activating the PI3K/AKT signaling pathway.

MDA levels may be a key sign of membrane lipid peroxidation, according to the cytotoxic effects of MDA, a byproduct of lipid oxidation. By identifying free radicals, the antioxidant enzyme

SOD slows down the aging process (Wang et al., 2018). Therefore, SOD activity and MDA levels are important indicators for assessing antioxidant capacity and responding to the degree of oxidation in the body. The results of this meta-analysis showed that ginsenoside Rg1 could significantly reduce MDA levels and restore SOD activity to exert its antioxidant activity and exert potential therapeutic effects on type 2 diabetes through the antioxidant pathway.

4.4 The inflammatory response is an important mechanism in the pathogenesis of type 2 diabetes, and ginsenosides can be anti-inflammatory

Chronic inflammation has been linked to several disorders, including diabetes and cancer (Kim et al., 2017). Scientific evidence suggests that mild chronic inflammation resulting from obesity is the core underlying disease associated with obesity-associated insulin resistance and T2DM (Lontchi-Yimagou et al., 2013). Several preclinical and clinical investigations have reported a clear causal link between mild aseptic inflammation and metabolic illnesses, such as T2DM (Akash et al., 2012; Böni-Schnetzler and Meier, 2019). Additionally, several preclinical and clinical investigations have revealed a causal link between mild aseptic inflammation and metabolic illnesses, such as T2DM. The beta-cell function of the pancreas deteriorates under hypoxic stress, leading to decreased insulin secretion and, eventually, hyperglycemia. In contrast to acute inflammation, in the chronic inflammation of metabolic diseases, pro-inflammatory cytokines and chemokines expand and infiltrate throughout the organ system. Therefore, in islets, elevated innate immune cells and pro-inflammatory mediators cause a decrease in beta cell quality and function (Donath and Halban, 2004; Donath et al., 2013). In obesity, adipose tissue macrophages transform and secrete a variety of pro-inflammatory cytokines that can impair insulin signaling and thus promote the progression of insulin resistance (Zatterale et al., 2020).

Research has shown that ginsenoside Rg1 may control the activity of inflammatory signaling pathways, such as nuclear factor-kB and activator protein-1, and prevent the generation of pro-inflammatory cytokines. The specific mechanisms include the prevention of amyloid β accumulation and microglia activation by inhibiting NF-κB, phospholipase C-γ1 pathway, and downregulation of toll-like receptor 3 and 4 expression, thereby reducing basal inflammation (Hu et al., 2011; Zhao et al., 2014; Li Y. et al., 2017). Animal models have also shown that ginsenoside Rg1 has anti-inflammatory properties. These findings indicate that ginsenoside Rg1 plays a significant role in macrophage-mediated inflammatory responses by regulating the NF-kB or Akt/mTOR signaling pathways through various mechanisms of action (Kim et al., 2017). Ginsenoside Rg1 suppresses IL-6 mRNA and protein expression by suppressing the activation of the NF-kB signaling pathway (Gao et al., 2015; Lee et al., 2015).

Current studies have shown that ginsenoside rg1 can treat various diseases by inhibiting excessive inflammatory pathways, preventing apoptosis, and modulating the immune system. Ginsenoside Rg1 can protect the liver by, among other things, blocking the toll-like receptor 4 signaling pathway, inhibiting the NF-κB signaling pathway, activating AMPK, activating the inflammasome and ER stress, and raising Nrf2 production and translocation (Alana et al., 2012). Meanwhile, the glucocorticoid-like anti-inflammatory effects and immunomodulatory, antioxidant, and anti-apoptotic properties of ginsenoside Rg1 make it a potential therapeutic agent for the treatment of sepsis (Song et al., 2013; Zou et al., 2013; Juan et al., 2015; Su et al., 2015). Because diabetes mellitus is closely associated with chronic inflammation, it is worth investigating and focusing on whether ginsenoside Rg1 may prevent β-cell death or encourage its regeneration for the treatment of diabetes mellitus by lowering inflammation.

4.5 Other hypoglycemic mechanisms of ginsenoside Rg1 that alleviate diabetic complications

Furthermore, as ginsenoside Rg1 has low oral bioavailability, it must be deglycosylated and transformed into secondary saponins before it can be absorbed and used in circulation. Existing research has indicated that the majority of ginsenoside deglycosylation occurs in the gastrointestinal tract in response to the activity of intestinal microbes (Peng et al., 2022). As a result, research has demonstrated that ginsenoside Rg1 may be utilized as a dietary supplement alongside prebiotics to treat type 2 diabetes by controlling gut flora (Peng et al., 2022). Because the original search of the literature revealed that the current randomized controlled trials on the treatment of type 2 diabetes by rg1 through the regulation of intestinal flora were insufficient for meta-analysis, we abandoned the study of this mechanism.

Various experimental data have shown that ginsenoside Rg1 is effective in not only lowering BG in type 2 diabetic animals but also alleviating diabetic complications. Ginsenoside Rg1 reduces NF-κB expression and inflammatory vesicle production and attenuates OS and apoptosis in myocardial tissue, thereby alleviating cardiac insufficiency in type 2 diabetic mice (Yu et al., 2016). In studies in diabetic animal models, ginsenoside Rg1 can effectively alleviate the effects of aldosterone-induced OS and reduce the metabolites of ROS to prevent membranous nephropathy in rats while improving the inflammatory response and pathological changes of the kidney through various anti-inflammatory mechanisms (Liu et al., 2021). By establishing an animal model of obesity induced by high-fat and high-sugar diets in mice, ginsenoside Rg1 could induce AMPK activation, inhibit adipogenesis, reduce lipid deposition of fat, play a role in protecting the liver in an anti-obesity manner, and effectively reduce aspartate aminotransferase and alanine aminotransferase indexes (Tian et al., 2017). Ginsenoside Rg1 has also been studied and reported for the prevention of DR. In db/db diabetic retinopathy mouse models, ginsenoside Rg1 intervention can activate the IRS-1/Akt/GSK3β signaling pathway in the early stages of DR, block tau protein-induced neurodegeneration at retinal ganglion cell synapses, and improve visual function (Gao et al., 2020). Meanwhile, ginsenoside Rg1 can improve the angiogenesis of endothelial cells and promote wound closure in diabetic foot ulcers (Yang et al., 2012).

4.6 Summary and limitations to the study

In this review, we conducted a meta-analysis of preclinical trials regarding ginsenoside rg1 for the treatment of T2DM. The objective was to evaluate the hypoglycemic effect and the antioxidant and anti-inflammatory properties of ginsenoside rg1 in the treatment of type 2 diabetes. Studies have shown that people with type 2 diabetes are at a significantly increased risk of developing complications of type 2 diabetes when chronic hyperglycemia is not effectively controlled. For people with diabetes who have uncontrolled hyperglycemia, the likelihood of developing Alzheimer's disease later in life is greatly increased (Nowaczewska et al., 2019). Therefore, aggressive treatment of hyperglycemia becomes a key measure in the treatment of diabetes. The findings showed that ginsenoside rg1 was apparently correlated with antioxidant factors and pro-inflammatory cytokines. It inhibited the generation of MDA, TNF-α, and IL-6 and promoted SOD production. Current evidence shows that ginsenoside rg1 can effectively control hyperglycemia due to type 2 diabetes through antioxidant and anti-inflammatory mechanisms. This meta-analysis is based on animal experiments; therefore, it cannot represent the results of clinical trials. However, it still has a certain reference value and guiding significance for future experiments.

However, there are some unavoidable limitations in this study. First, in terms of study quality, none of the studies described the methods used to conceal allocation order, blinded interventions, or randomized outcome assessments. Furthermore, some studies did not provide detailed baseline characteristics. At the same time, given the inadequate methodological quality of some studies, the results of this study should be construed with care, and more qualitative studies are necessary in the future. Second, the high heterogeneity of the results diminished their dependability. Although we attempted to explore potential sources of heterogeneity using subgroup analysis, it seems unproductive. Different study protocols and intervention procedures in animal experiments may be a potential cause of the high heterogeneity. Third, there is minimal evidence for the efficacy of ginsenoside Rg1 in the treatment of type 2 diabetes *in vitro* and *in vivo* trials. Therefore, clinical investigations are required. However, the transition from preclinical to clinical investigations has been delayed by concerns with bioavailability. Fourth, based on the studies we included, investigators have set the dose and duration of Rg1 therapy in various ways. Consequently, current reports make it difficult to obtain reliable, effective doses and appropriate treatment durations. More research in this area should address this issue. Finally, we did not conduct further meta-analyses of relevant indicators because data for certain indicators only existed in separate studies. These indicators require more attention in the future.

5 Conclusion

In this meta-analysis, the findings showed that ginsenoside rg1 was apparently correlated with antioxidant factors and pro-inflammatory cytokines. It inhibited the generation of MDA, TNF-α, and IL-6 and promoted SOD production. The findings of the present study can fully demonstrate the antioxidant and anti-inflammatory properties of ginsenoside rg1. However, the low methodological quality of the included studies and publication bias may weaken the validity of the findings. The hypoglycemic effects of ginsenosides in type 2 diabetes require more rigorous experimental design and more comprehensive studies.

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

QX and QC designed this study. QZ searched the database. QW and YX collected the data. QX and WW evaluated the study quality. QX and QW performed all analyses. QX and YX wrote the manuscript. XRZ was responsible for chart making and careful revision of articles. LSS was actively involved in the revision of the article. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by a study on remote MDT diagnosis, treatment, and management mode of type 2 diabetes mellitus with Integrated Traditional Chinese and Western Medicine (Project number: 2022IR020).

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/fphar.2023.1179705/full#supplementary-material

References

Ahmed, T., Raza, S. H., Maryam, A., Setzer, W. N., Braidy, N., Nabavi, S. F., et al. (2016). Ginsenoside Rb1 as a neuroprotective agent: A review. *Brain Res. Bull.* 125, 30–43. doi:10.1016/j.brainresbull.2016.04.002

Akash, M. S. H., Shen, Q., Rehman, K., and Chen, S. (2012). Interleukin-1 receptor antagonist: A new therapy for type 2 diabetes mellitus. *J. Pharm. Sci.* 101 (5), 1647–1658. doi:10.1002/jps.23057

Alana, G. L., Upton, J-P., Praveen, P. V. K., Ghosh, R., Nakagawa, Y., and Aeid, L. (2012). IRE1a induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. *Cell Metab.* 16, 250–264. doi:10.1016/j.cmet.2012.07.007

Alolga, R. N., Nuer-Allornuvor, G. F., Kuugbee, E. D., Yin, X., and Ma, G. (2020). Ginsenoside Rg1 and the control of inflammation implications for the therapy of type 2 diabetes: A review of scientific findings and call for further research. *Pharmacol. Res.* 152, 104630. doi:10.1016/j.phrs.2020.104630

Aryangat, A. V., and Gerich, J. E. (2010). Type 2 diabetes: Postprandial hyperglycemia and increased cardiovascular risk. *Vasc. Health Risk Manag.* 6 (default), 145–155. doi:10.2147/vhrm.s8216

Azeem, M., Hanif, M., Mahmood, K., Ameer, N., Chughtai, F. R. S., and Abid, U. (2023). An insight into anticancer, antioxidant, antimicrobial, antidiabetic and anti-inflammatory effects of quercetin: A review. *Polym. Bull.* 80 (1), 241–262. doi:10.1007/s00289-022-04091-8

Baeg, I., and So, S. (2013). The world ginseng market and the ginseng (Korea). *J. Ginseng Res.* 37 (1), 1–7. doi:10.5142/jgr.2013.37.1

Bai, L., Gao, J., Wei, F., Zhao, J., Wang, D., and Wei, J. (2018). Therapeutic potential of ginsenosides as an adjuvant treatment for diabetes. *Front. Pharmacol.* 9, 423. doi:10.3389/fphar.2018.00423

Batista, T. M., Haider, N., and Kahn, C. R. (2021). Defining the underlying defect in insulin action in type 2 diabetes. *Diabetologia* 64 (5), 994–1006. doi:10.1007/s00125-021-05415-5

Böni-Schnetzler, M., and Meier, D. T. (2019). Islet inflammation in type 2 diabetes. *Semin. Immunopathol.* 41 (4), 501–513. doi:10.1007/s00281-019-00745-4

Burgos-Moron, E., Abad-Jimenez, Z., Maranon, A. M., Iannantuoni, F., Escribano-Lopez, I., Lopez-Domenech, S., et al. (2019). Relationship between oxidative stress, ER stress, and inflammation in type 2 diabetes: The battle continues. *J. Clin. Med.* 8 (9), 1385. doi:10.3390/jcm8091385

Chen Xianren, G. Y. D. X. (2020). Effects of ginsenoside Rg1 on liver injury, oxidative stress and Toll-like receptor 4 expression in liver tissues of diabetic rats. *Chin. J. Clin. Pharmacol.*

Dehkordi, A. H., Abbaszadeh, A., Mir, S., and Hasanvand, A. (2019). Metformin and its anti-inflammatory and anti-oxidative effects; new concepts. *J. Ren. Inj. Prev.* 8 (1), 54–61. doi:10.15171/jrip.2019.11

Dinić, S., Arambašić Jovanović, J., Uskoković, A., Mihailović, M., Grdović, N., Tolić, A., et al. (2022). Oxidative stress-mediated beta cell death and dysfunction as a target for diabetes management. *Front. Endocrinol.* 13, 1006376. doi:10.3389/fendo.2022.1006376

Donath, M. Y., Dalmas, E., Sauter, N. S., and Boni-Schnetzler, M. (2013). Inflammation in obesity and diabetes: Islet dysfunction and therapeutic opportunity. *Cell Metab.* 17 (6), 860–872. doi:10.1016/j.cmet.2013.05.001

Donath, M. Y., and Halban, P. A. (2004). Decreased beta-cell mass in diabetes: Significance, mechanisms and therapeutic implications. *Diabetologia* 47 (3), 581–589. doi:10.1007/s00125-004-1336-4

Egger, M., Davey, S. G., Schneider, M., and Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315 (7109), 629–634. doi:10.1136/bmj.315.7109.629

Eshu, R. (2021). Renoprotective effect of ginsenoside Rg1 on type 2 diabetic rats. *J. Kunming Med. Univ.*

Fan, X., Zhang, C., Niu, S., Fan, B., Gu, D., Jiang, K., et al. (2019). Ginsenoside Rg1 attenuates hepatic insulin resistance induced by high-fat and high-sugar by inhibiting inflammation. *Eur. J. Pharmacol.* 854, 247–255. doi:10.1016/j.ejphar.2019.04.027

Fernandez-Twinn, D. S., Hjort, L., Novakovic, B., Ozanne, S. E., and Saffery, R. (2019). Intrauterine programming of obesity and type 2 diabetes. *Diabetologia* 62 (10), 1789–1801. doi:10.1007/s00125-019-4951-9

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., et al. (2020). Pathophysiology of type 2 diabetes mellitus. *Int. J. Mol. Sci.* 21 (17), 6275. doi:10.3390/ijms21176275

Gao, Y., Chu, S., Li, J., Li, J., Zhang, Z., Xia, C., et al. (2015). Anti-inflammatory function of ginsenoside Rg1 on alcoholic hepatitis through glucocorticoid receptor related nuclear factor-kappa B pathway. *J. Ethnopharmacol.* 173, 231–240. doi:10.1016/j.jep.2015.07.020

Gao, Y., Chu, S., Zhang, Z., and Chen, N. (2017). Hepataprotective effects of ginsenoside Rg1 - a review. *J. Ethnopharmacol.* 206, 178–183. doi:10.1016/j.jep.2017.04.012

Gao, Y., Ji, Y., Luo, Y., Sun, J., Sun, G., and Sun, X. (2020). Ginsenoside Rg1 prevents early diabetic retinopathy via reducing retinal ganglion cell layer and inner nuclear layer cell apoptosis in db/db mice. *Ann. Transl. Med.* 8 (5), 232. doi:10.21037/atm.2019.12.155

He, F., Yu, C., Liu, T., and Jia, H. (2020). Ginsenoside Rg1 as an effective regulator of mesenchymal stem cells. *Front. Pharmacol.* 10, 1565. doi:10.3389/fphar.2019.01565

He, L., Ling, L., Wei, T., Wang, Y., and Xiong, Z. (2017). Ginsenoside Rg1 improves fertility and reduces ovarian pathological damages in premature ovarian failure model of mice. *Exp. Biol. Med.* 242 (7), 683–691. doi:10.1177/1535370217693323

Hooijmans, C. R., Rovers, M. M., de Vries, R. B. M., Leenars, M., Miranda, W. M., and Langendam, M. W. (2014). SYRCLE's risk of bias tool for animal studies. *BMC Med.* 14 (43), 43. doi:10.1186/1471-2288-14-43

Hu, J., Song, X., Chu, S., Chen, J., Ji, H., Chen, X., et al. (2011). Inhibitory effect of ginsenoside Rg1 on lipopolysaccharide-induced microglial activation in mice. *Brain Res.* 1374, 8–14. doi:10.1016/j.brainres.2010.11.069

Jianchao, Y. (2016). A study on protective effect of ginsenoside Rgl on pathological live injury in rats with type 2 diabetes. *Clin. J. Chin. Med.* 8 (28).

Jie, F. (2010). A study on the hypoglycemic effect of ginsenoside Rg1 on alloxan-induced diabetes in mice. *Chin. ARCHIVES TRADITIONAL Chin. Med.* 28 (11), 2427–2429.

Jie, F. (2022). A study on the hypoglycemic effect of ginsenoside Rg1 on alloxan-induced diabetes in mice. *Chin. ARCHIVES TRADITIONAL Chin. Med.*

Juan, D., Cheng, B., Zhu, X., and Ling, C. (2015). Ginsenoside Rg1, a novel glucocorticoid receptor agonist of plant origin, maintains glucocorticoid efficacy with reduced side effects. *J. Immunol.* 187 (2), 942–950. 10.4049/jimmunol.1002579.

Kim, J., Byeon, H., Im, K., and Min, H. (2018). Effects of ginsenosides on regulatory T cell differentiation. *Food Sci. Biotechnol.* 27 (1), 227–232. doi:10.1007/s10068-017-0255-3

Kim, J. H., Yi, Y., Kim, M., and Cho, J. Y. (2017). Role of ginsenosides, the main active components of Panax ginseng, in inflammatory responses and diseases. *J. Ginseng Res.* 41 (4), 435–443. doi:10.1016/j.jgr.2016.08.004

Kitada, M., Ogura, Y., Monno, I., and Koya, D. (2019). Sirtuins and type 2 diabetes: Role in inflammation, oxidative stress, and mitochondrial function. *Front. Endocrinol.* 10, 187. doi:10.3389/fendo.2019.00187

Lee, S. Y., Jeong, J. J., Eun, S. H., and Kim, D. H. (2015). Anti-inflammatory effects of ginsenoside Rg1 and its metabolites ginsenoside Rh1 and 20(S)-protopanaxatriol in mice with TNBS-induced colitis. *Eur. J. Pharmacol.* 762, 333–343. doi:10.1016/j.ejphar.2015.06.011

Li, Q., Xiang, Y., Chen, Y., Tang, Y., and Zhang, Y. (2017a). Ginsenoside Rg1 protects cardiomyocytes against hypoxia/reoxygenation injury via activation of Nrf2/HO-1 signaling and inhibition of JNK. *Cell Physiol. Biochem.* 44 (1), 21–37. doi:10.1159/000484578

Li, Y., Wang, F., and Luo, Y. (2017b). Ginsenoside Rg1 protects against sepsis-associated encephalopathy through beclin 1–independent autophagy in mice. *J. Surg. Res.* 207, 181–189. doi:10.1016/j.jss.2016.08.080

Li, Z., Zuo, Z., Song, D., Li, Y., Jiang, J., and Kong, W. (2015). Berberine analogue Y53 has improved antioxidant and anti-inflammatory activities in diabetic C57bl/6J mice with liver steatosis. *Int. J. Pharmacol.* 12 (1), 11–18. doi:10.3923/ijp.2016.11.18

Liu, H., Chen, W., Lu, P., Ma, Y., Liang, X., and Liu, Y. (2021). Ginsenoside Rg1 attenuates the inflammation and oxidative stress induced by diabetic nephropathy through regulating the PI3K/AKT/FOXO3 pathway. *Ann. Transl. Med.* 9 (24), 1789. doi:10.21037/atm-21-6234

Liu, Q., Zhang, F., Zhang, W., Pan, A., Yang, Y., Liu, J., et al. (2017). Ginsenoside Rg1 inhibits glucagon-induced hepatic gluconeogenesis through akt-FoxO1 interaction. *Theranostics* 7 (16), 4001–4012. doi:10.7150/thno.18788

Lontchi-Yimagou, E., Sobngwi, E., Matsha, T. E., and Kengne, A. P. (2013). Diabetes mellitus and inflammation. *Curr. Diabetes Rep.* 13 (3), 435–444. doi:10.1007/s11892-013-0375-y

Ma, T., Mao, X., Meng, X., and Wang, Q. (2022). Ginsenoside Rg1 inhibits STAT3 expression by miR-15b-5p to attenuate lung injury in mice with type 2 diabetes mellitus-associated pulmonary tuberculosis. *Evid.-based Complement. Altern. Med.* 2022, 9017021–9017029. doi:10.1155/2022/9017021

Mathews, S. T., Kothari, V., and Galdo, J. (2016). Hypoglycemic agents and potential anti-inflammatory activity. *J. Inflamm. Res.* 27, 27–38. doi:10.2147/JIR.S86917

Nakhjavani, M., Smith, E., Townsend, A. R., Price, T. J., and Hardingham, J. E. (2020). Anti-angiogenic properties of ginsenoside Rg3. *Molecules* 25 (21), 4905. doi:10.3390/molecules25214905

Ning, C., Gao, X., Wang, C., Huo, X., Liu, Z., Sun, H., et al. (2018). Hepatoprotective effect of ginsenoside Rg1 from Panax ginseng on carbon tetrachloride-induced acute liver injury by activating Nrf2 signaling pathway in mice. *Environ. Toxicol.* 33 (10), 1050–1060. doi:10.1002/tox.22616

Nowaczewska, M., Kamińska, A., Kukulska-Pawluczuk, B., Junik, R., and Pawlak-Osińska, K. (2019). Effect of hyperglycemia on cerebral blood flow in patients with diabetes. *Diabetes. Res. Clin. Pract.* 153, 1–5. doi:10.1016/j.diabres.2019.05.024

Page, M. J., Moher, D., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021). PRISMA 2020 explanation and elaboration: Updated guidance and exemplars for reporting systematic reviews. *BMJ* 372, n160. doi:10.1136/bmj.n160

Park, H., Kim, D., Park, S., Kim, J., and Ryu, J. (2012). Ginseng in traditional herbal prescriptions. *J. Ginseng Res.* 36 (3), 225–241. doi:10.5142/jgr.2012.36.3.225

Peng, M., Wang, L., Su, H., Zhang, L., Yang, Y., Sun, L., et al. (2022). Ginsenoside Rg1 improved diabetes through regulating the intestinal microbiota in high-fat diet and streptozotocin-induced type 2 diabetes rats. *J. Food Biochem.* 46 (10), e14321. doi:10.1111/jfbc.14321

Qin, Q., Lin, N., Huang, H., Zhang, X., Cao, X., Wang, Y., et al. (2019). Ginsenoside Rg1 ameliorates cardiac oxidative stress and inflammation in streptozotocin-induced diabetic rats. *Diabetes Metab. Syndr. Obes.* 12, 1091–1103. doi:10.2147/DMSO.S208989

Singh, A., Kukreti, R., Saso, L., and Kukreti, S. (2022). Mechanistic insight into oxidative stress-triggered signaling pathways and type 2 diabetes. *Molecules* 27 (3), 950. doi:10.3390/molecules27030950

Skovso, S. (2014). Modeling type 2 diabetes in rats using high fat diet and streptozotocin. *J. Diabetes Investig.* 5 (4), 349–358. doi:10.1111/jdi.12235

Song, L., Xu, M. B., Zhou, X. L., Zhang, D. P., Zhang, S. L., and Zheng, G. Q. (2017). A preclinical systematic review of ginsenoside-rg1 in experimental Parkinson's disease. *Oxidative Med. Cell. Longev.* 2017, 2163053. doi:10.1155/2017/2163053

Song, Y., Zhao, F., Zhang, L., Du, Y., Wang, T., and Fu, F. (2013). Ginsenoside Rg1 exerts synergistic anti-inflammatory effects with low doses of glucocorticoids *in vitro*. *Fitoterapia* 91, 173–179. doi:10.1016/j.fitote.2013.09.001

Su, F., Xue, Y., Wang, Y., Zhang, L., Chen, W., and Hu, S. (2015). Protective effect of ginsenosides Rg1 and Re on lipopolysaccharide-induced sepsis by competitive binding to toll-like receptor 4. *Antimicrob. Agents. Chemother.* 59 (9), 5654–5663. doi:10.1128/AAC.01381-15

Sun, Y., Yang, Y., Liu, S., Yang, S., Chen, C., Lin, M., et al. (2022). New therapeutic approaches to and mechanisms of ginsenoside Rg1 against neurological diseases. *Cells* 11 (16), 2529. doi:10.3390/cells11162529

Tian, W., Chen, L., Zhang, L., Wang, B., Li, X. B., Fan, K. R., et al. (2017). Effects of ginsenoside Rg1 on glucose metabolism and liver injury in streptozotocin-induced type 2 diabetic rats. *Genet. Mol. Res.* 16 (1). doi:10.4238/gmr16019463

Wang, Z. L., Chen, L. B., Qiu, Z., Chen, X. B., Liu, Y., Li, J., et al. (2018). Ginsenoside Rg1 ameliorates testicular senescence changes in D-gal-induced aging mice via anti-inflammatory and antioxidative mechanisms. *Mol. Med. Rep.* 17 (5), 6269–6276. doi:10.3892/mmr.2018.8659

Wronka, M., Krzemińska, J., Młynarska, E., Rysz, J., and Franczyk, B. (2022). The influence of lifestyle and treatment on oxidative stress and inflammation in diabetes. *Int. J. Mol. Sci.* 23 (24), 15743. doi:10.3390/ijms232415743

Wu Lina, F. X. W. S. (2021). Ginsenoside Rg1 improves peripheral nerve injury in diabetic rats by regulating oxidative stress and inflammatory factor expression. *Chin. J. Immunol.* 37 (04), 486–491.

Xie, C. L., Wang, W. W., Xue, X. D., Zhang, S. F., Gan, J., and Liu, Z. G. (2015). A systematic review and meta-analysis of Ginsenoside-Rg1 (G-Rg1) in experimental ischemic stroke. *Sci. Rep.* 5, 7790. doi:10.1038/srep07790

Xin, L. (2015). Protective effect of ginsenoside Rg1 on liver injury in type 2 diabetic rats. *Chin. J. Public Health*.

Xue, Q., He, N., Wang, Z., Fu, X., Aung, L. H. H., Liu, Y., et al. (2021). Functional roles and mechanisms of ginsenosides from Panax ginseng in atherosclerosis. *J. Ginseng Res.* 45 (1), 22–31. doi:10.1016/j.jgr.2020.07.002

Yang Jing, D. Y. Y. X. (2020). Effects of ginsenoside Rg1 on serum oxidative stress indicators, inflammatory factors and renal tissue TGF-β1, MCP-1 mRNA in rats with diabetic nephropathy. *Mod. Biomed. Adv.* 20 (05), 853–856+918.

Yang, N., Chen, P., Tao, Z., Zhou, N., Gong, X., Xu, Z., et al. (2012). Beneficial effects of ginsenoside-Rg1 on ischemia-induced angiogenesis in diabetic mice. *Acta Biochim. Biophys. Sin.* 44 (12), 999–1005. doi:10.1093/abbs/gms092

Yin, Y., and Wang, J. (2017). Ginseng saponins Rg1's effect on the expression of tumor necrosis factor alpha, monocyte chemotactic factor protein-1 in diabetic nephropathy rats. *Int. J. Clin. Exp. Med.* 10 (12), 16513–16518.

Yu, H., Zhen, J., Yang, Y., Gu, J., Wu, S., and Liu, Q. (2016). Ginsenoside Rg1 ameliorates diabetic cardiomyopathy by inhibiting endoplasmic reticulum stress-induced apoptosis in a streptozotocin-induced diabetes rat model. *J. Cell. Mol. Med.* 20 (4), 623–631. doi:10.1111/jcmm.12739

Yuan, C., Wang, H., and Yuan, Z. (2019). Ginsenoside Rg1 inhibits myocardial ischaemia and reperfusion injury via HIF-1α-ERK signalling pathways in a diabetic rat model. *Pharmazie* 74 (3), 157–162. doi:10.1691/ph.2019.8858

Yun, T. K. (2001). Panax ginseng-a non-organ-specific cancer preventive? *Lancet Oncol.* 2 (1), 49–55. doi:10.1016/S1470-2045(00)00196-0

Zatterale, F., Longo, M., Naderi, J., Raciti, G. A., Desiderio, A., Miele, C., et al. (2020). Chronic adipose tissue inflammation linking obesity to insulin resistance and type 2 diabetes. *Front. Physiol.* 10, 1607. doi:10.3389/fphys.2019.01607

Zhang, G., Zhang, M., Yu, J., Kang, L., and Guan, H. (2021). Ginsenoside Rg1 prevents H2O2-induced lens opacity. *Curr. Eye Res.* 46 (8), 1159–1165. doi:10.1080/02713683.2020.1869266

Zhang, P., Li, T., Wu, X., Nice, E. C., Huang, C., and Zhang, Y. (2020). Oxidative stress and diabetes: Antioxidative strategies. *Front. Med.* 14 (5), 583–600. doi:10.1007/s11684-019-0729-1

Zhao, B., Liu, Y., Gao, X., Zhai, H., Guo, J., and Wang, X. (2014). Effects of ginsenoside Rg1 on the expression of toll-like receptor 3, 4 and their signalling transduction factors in the ng108-15 murine neuroglial cell line. *Molecules* 19 (10), 16925–16936. doi:10.3390/molecules191016925

Zheng Yongren, L. C. W. B. (2016). Effect of ginsenoside Rg1 on blood glucose regulation in type 2 diabetic rats. *China Natl. Folk Med.*

Zhou, P., Xie, W., He, S., Sun, Y., Meng, X., Sun, G., et al. (2019). Ginsenoside Rb1 as an anti-diabetic agent and its underlying mechanism analysis. *Cells* 8 (3), 204. doi:10.3390/cells8030204

Zhu, G., Wang, H., Wang, T., and Shi, F. (2017). Ginsenoside Rg1 attenuates the inflammatory response in DSS-induced mice colitis. *Int. Immunopharmacol.* 50, 1–5. doi:10.1016/j.intimp.2017.06.002

Zou, Y., Tao, T., Tian, Y., Zhu, J., Cao, L., Deng, X., et al. (2013). Ginsenoside Rg1 improves survival in a murine model of polymicrobial sepsis by suppressing the inflammatory response and apoptosis of lymphocytes. *J. Surg. Res.* 183 (2), 760–766. doi:10.1016/j.jss.2013.01.068

Zozulinska, D., and Wierusz-Wysocka, B. (2006). Type 2 diabetes mellitus as inflammatory disease. *Diabetes. Res. Clin. Pract.* 74 (2), S12–S16. doi:10.1016/j.diabres.2006.06.007

Frontiers in Pharmacology

Explores the interactions between chemicals and living beings

The most cited journal in its field, which advances access to pharmacological discoveries to prevent and treat human disease.

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