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Green synthesis of silver and zinc oxide nanoparticles with *Thespesia populnea* extract and investigation of their antioxidant potential against mouse mastitis model

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Introduction: Bovine mastitis in dairy cattle is often complicated by antibiotic-resistant bacteria such as *Staphylococcus aureus*. Metal-based nanoparticles, especially plant-mediated nanoparticles have emerged as promising therapeutic tools for treating *S. aureus*-associated mastitis through the intramammary route. In this study, we synthesized, characterized, and assessed the antioxidant activity of *Thespesia populnea* nano silver particles (TPNS) and *Thespesia populnea* nano zinc oxide particles (TPNZ) derived from *Thespesia populnea* leaf extract (TPE). Silver nitrate and zinc acetate were reduced using TPE to synthesize TPNS and TPNZ, which were characterized by Scanning Electron Microscopy (SEM), UV–Visible Spectroscopy, Dynamic Light Scattering (DLS), and Zeta Potential analysis. The antioxidant activity of green-synthesized nanoparticles was evaluated in mastitis-induced mice.

Methods: Forty-eight female Swiss albino mice, 10–15 days of lactation, were divided into six groups (number of mice in each group-8). Group I served as the control, while mastitis was induced in groups II, III, IV, V and VI. Group III received *T. populnea* methanolic leaf extract (TPE); groups IV and V were treated with TPNS and TPNZ respectively; and group VI received Ceftriaxone.

Results: UV–Visible Spectroscopy confirmed the successful reduction of the metal ions to nanoparticles. SEM and DLS analysis revealed agglomerated morphologies with minimal variations in particle size. TPNS had a higher zeta potential than TPNZ, indicating a greater stability in the suspension. Mastitis-induced group showed significantly increased thiobarbituric acid reacting substances (TBARS) levels ($p < 0.01$) and significantly decreased Superoxide dismutase (SOD), Glutathione- S- transferase (GST), catalase (CAT), reduced glutathione (GSH), and glutathione peroxidase (GPx) activities ($p < 0.01$) compared to group I. Improvements were observed in groups IV, VI, V, and III.

Conclusion: The TPNS-treated group (IV) showed the highest restoration of antioxidant activity, followed by the ceftriaxone (VI), TPNZ (V), and TPE-treated

groups (III). These findings suggest that phytogenic nanoparticles exhibit higher antioxidant activity than TPE extract alone.

KEYWORDS

Thespesia populnea, TPNS, TPNZ, antioxidant parameters, green synthesis, mice mastitis model

1 Introduction

Bovine mastitis is a destructive disease of cattle that causes significant economic losses in the dairy industry (1). *Staphylococcus aureus* is a common cause of bovine mastitis. The disease is linked to oxidative stress from bacterial invasion, as indicated by changes in the oxidative stress parameters in the blood (2, 3). During inflammation, phagocytes produce reactive oxygen species (ROS) that destroy the bacteria (4). Excessive ROS production can overwhelm the antioxidant system and adversely affect the immune system of cows (5). ROS can oxidize macromolecules, such as proteins, lipids, and deoxyribose nucleic acid (DNA), causing oxidative cell damage and altering metabolic pathways (6). Oxidative stress can enhance the adherence of active neutrophils to mammary endothelial cells, worsening inflammation (7). Clinical and subclinical mastitis leads to the release of free radicals and a reduction in the total antioxidant capacity (8). Severe mastitis results in antioxidant imbalance due to excessive peroxynitrite production (9). Evaluating peroxidative damage products (TBARS) and antioxidants, such as glutathione and enzymes (SOD, GPx, and catalase), may serve as markers of oxidative stress and antioxidant status (10). Mastitis alters redox potential, increases oxidative free radicals, and decreases protective antioxidant enzymes (10). In addition to oxidative stress, bacterial infections in mastitis are difficult to combat because of the ability of bacteria to evade the host immune response through biofilms, exotoxins, proteases and bacterial superantigens, and by adhering to mammary epithelial cells (11). *Staphylococcus aureus* induced mastitis poses a significant challenge in the dairy industry because of the ability of bacteria to survive in phagocytes and epithelial cells, rendering antibiotic treatment ineffective (12). Therefore, alternative treatment options are needed. Studies indicate that adequate antioxidant intake in dairy cows enhances immunological functions such as phagocytosis, bacterial killing, and neutrophil oxidative metabolism (13). Recent studies have highlighted that inorganic nanoparticles effectively scavenge reactive oxygen species (14, 15).

Nanomedicine is an emerging field that involves the fabrication of nanoparticles for therapeutic applications (16, 17). Nanoparticles exhibit unique physicochemical properties (17). Various materials, including metals, metal oxides, and silicates, have been used to create nanoparticles (18). Noble metals like copper (Cu), silver (Ag), gold (Au), and titanium (Ti) are commonly used for nanoparticle fabrication (19).

While AgNPs can induce oxidative stress in disease-causing organisms, which indirectly reduces free radical generation, their free radical scavenging activity is attributed to the functional groups present on their surfaces (20). Zinc oxide nanoparticles have demonstrated antioxidant properties in both intracellular and extracellular environments (21). By activating antioxidant enzymes, ZnO nanoparticles reduce the quantity of free radicals intracellularly, whereas their use of electron transfer reduces free radicals in the extracellular environment to perform their free radical scavenging action (22). However, green-synthesized nanoparticles have been found to have higher antioxidant properties, which is attributed to the capping and stabilizing properties of various phytochemicals involved in their production (23).

The production of large quantities of nanoparticles often involves physical techniques that can yield highly pure nanoparticles; however, these techniques typically require expensive equipment, high pressures and temperatures (24, 25), as well as a significant amount of energy. Alternatively, chemical processes such as chemical reduction and electrochemical and sol–gel processes can also be used to create nanoparticles, but these methods may produce hazardous or polluting waste due to the inclusion of toxic reagents or solvents (26, 27).

The synthesis of nanoparticles using green methods primarily involves the incorporation of cell extracts, such as those derived from plants, microorganisms, algae, and fungi, into a substrate without the use of harmful chemicals. The aerial parts of plants, such as the leaves and flowers, are frequently utilized in green synthesis. Numerous researchers have found that proteins and secondary metabolites present in plant extracts serve as reducing and capping agents that promote the production of nanoparticles (28, 29). Phytochemicals, such as vitamins, amino acids, polysaccharides, terpenoids, alkaloids, and other compounds extracted from plants, help in the effective bio-reduction of metal ions during the synthesis of nanoparticles, which exhibit stability and variability in their structure and dimension. Plant components, ranging from leaves to roots, are widely used to produce metal oxide nanoparticles.

Thespesia populnea of the Malvaceae family, commonly known as the Indian tulip tree, is widely distributed in the southeastern and coastal forests of India. The bark, blossoms, and leaves of this tree, also known as the portia tree, possess medicinal benefits that can be used to treat skin infections (30). Research has shown that *T. populnea* leaves contain flavonoids, tannins, saponins, terpenoids, polyphenols, glycosides, alkaloids, quercetin, phytosterols, lupeol, and rutin (31, 32). The phytochemicals found in *T. populnea* have been shown to possess anti-inflammatory, anti-diarrheal, antibacterial, antifungal, and haemostatic properties so it is used in traditional medicinal systems like Sidha and Ayurveda especially the bark and leaves are often used in decoctions or poultices, while the fruits and seeds are be used in oil preparations (33, 34). However, studies examining the effectiveness of *T. populnea* herbal extract in eliminating oxidative stress related to bacterial mastitis using metal nanoparticles are limited. Considering this, the current study aimed to investigate the green synthesis and characterization of Ag and ZnO nanoparticles from *T. populnea* leaf extract, as well as the antioxidant activity of these nanoparticles in the treatment of mastitis in a murine model.

This study focuses on the synthesis and characterization of silver (AgNPs), ZnO nanoparticles derived from *Thespesia populnea* extract using green synthesis approach. The main objective is to evaluate the antimicrobial activity of the nanoparticles against *Staphylococcus*

aureus induced mouse mastitis model, assess their antioxidant properties *in vivo*, and investigate their potential for reducing oxidative stress in mastitis model. The study did not include long-term toxicity assessments and the plant extract was sourced during a single season, which may limit the seasonal variability of its bioactive compounds.

2 Materials and methods

The experiment was conducted at the Department of Veterinary Biochemistry, College of Veterinary Science, Rajendranagar, Telangana, India, using *T. populnea* leaves collected from Andhra Pradesh, India which were harvested during the flowering season (February to March). Higher concentrations of bioactive compounds were found during this period in *Thespesia populnea*.

2.1 Preparation of *T. populnea* methanolic leaf extract

Hundred gram of dried, coarsely powdered *T. populnea* leaves was soaked in 95% methanol for 72 h with intermittent mixing. The concentrated filtrate was air-dried and the percentage yield was calculated after weighing.

2.2 Synthesis of TPNS

Ninety milliliter of 0.1 M silver nitrate solution was added to 10 mL of 2% *T. populnea* methanolic leaf extract at 95°C with vigorous stirring. The color change of the solution from pale yellow to brown indicates the formation of TPE-mediated AgNPs.

2.3 Synthesis of TPNZ

Four milliliter of TPE was added dropwise to 0.5% zinc acetate, and the solution was mixed using a magnetic stirrer for 10 min. The pH was adjusted to 12 using 2 M NaOH, resulting in a white crystalline ZnO precipitate, which was repeatedly washed, filtered, and dried at 60°C to obtain ZnO nanoparticles.

2.4 Scanning electron microscopy

Morphology of TPNS and TPNZ nanoparticles was determined by SEM machine (JEOL JSM—5,600, Japan) operating in high vacuum mode with an acceleration voltage of 15 kV.

2.5 Dynamic light scattering analysis

The particle velocity distribution was assessed by measuring the dynamic fluctuations in the light-scattering intensity, and the Stokes-Einstein equation was used to determine the hydrodynamic radius or diameter, with measurements conducted using a Nanopartica SZ-100 instrument (Horiba, Japan).

2.6 Zeta potential

Zeta potential provides the net surface charge of the nanoparticles, as determined by Kim et al. (35).

2.7 Animals

Female albino mice (25–35 g) were sourced from M/s. Jeeva Life Sciences, Hyderabad, Telangana, India and were approved by the Institutional Animal Ethics Committee (I/2018-3/IAEC/C.V.Sc., Hyd).

2.8 Experimental design

Forty-eight lactating female *Swiss Albino* mice (10–15 days postpartum) weighing 35–40 g were randomly divided into six groups ($n = 8$). Group I was the control group. After anaesthesia using a mixture of ketamine and xylazine at the rate of 87 and 13 mg/kg of body weight, respectively, mastitis was induced in groups II to VI via intramammary inoculation of 20 µL of *S. aureus* (4.0×10^4 C.F.U.) isolated from a field strain isolated from bovine mastitis in Left 4th teat (36) with a 33-gauge hamilton blunt needle after exposing the teat canal by cutting the end of the teat under a binocular microscope. The antibiotic susceptibility profile of the *S. aureus* strain was determined prior to the study using the disc diffusion method. The strain was tested for sensitivity to ceftriaxone using a ceftriaxone disc (30 µg), and the zone of inhibition was measured. The highest zone of inhibition was found to be against ceftriaxone followed by tetracycline, gentamycin, O floxacin and streptomycin.

Later, the mice were administered butorphenol at a rate of 3–5 mg/kg body weight to prevent post-inoculation trauma. The CPCSEA guidelines were followed during the procedure. Six hours post-inoculation, Group I received PBS; Groups III, IV, V, and VI were intramammary administered 20 µL each of TPE (in 1% aqueous DMSO), TPNS, TPNZ, and Ceftriaxone (Intacef-4, INTAS Pharmaceuticals Limited, INDIA) into L4. The induction of mastitis in the mice was confirmed by observing characteristic signs of inflammation (swelling, redness, and discharge) at the site of infection within first 24 h. After 48 h of inocculation, the signs were more pronounced and the mice were anesthetized with ketamine and euthanized using Co_2 chamber. Blood collected via cardiac puncture was stored in heparin-coated tubes for oxidative stress and antioxidant analysis. To evaluate oxidative stress and antioxidant parameters, whole blood was used to estimate GSH (37), and hemolysate was prepared to assess TBARS (38), SOD (39), CAT (40), GPx (41), and GST (42).

The green synthesized nanoparticles were characterized using SEM, DLS, and UV–Vis spectroscopy. *In vitro* antimicrobial testing was performed against *Staphylococcus aureus*, while *in vivo* antioxidant effects were assessed in murine mastitis model. No seasonal variation of the plant extract was considered, and the study did not include investigations into chronic toxicity.

2.9 Statistical analysis

The data obtained from the experimental animals of different treatment groups were tabulated and analyzed to determine the significance among the experimental groups according to the

procedures of Snedecor and Cochran (43) using a statistical package for social sciences (SPSS – 20 software, IBM, United States). Statistical significance was analyzed using one-way factorial analysis of variance (ANOVA) and evaluated using Duncan's multiple comparison test. The significance level was set at $p < 0.01$. Data are expressed as mean ± standard error (SE).

3 Results

Synthesis and Characterization of Nanoparticles UV–VIS Analysis: Figures 1A,B display the UV–visible absorption spectra of the TPNS and TPNZ particles, respectively. TPNS particles exhibited a maximum absorbance peak at 421 nm, confirming the bioreduction of Ag^{+} to Ag (0). The absorption spectrum of the TPNZ particles, recorded between 200 and 800 nm, showed a peak at 260 nm, indicating the formation and stability of ZnO nanoparticles.

3.1 Characterization of *T. populnea* methanolic extract mediated nanoparticles using UV–visible spectroscopy

The reduction of pure nano ions was monitored by measuring the UV–visible spectrum of the reaction medium after 5 h, with the sample diluted in distilled water, using a UV–Visible Spectrophotometer (Spectrophotometer UV–VIS spectrophotometer UV-2450, Shimadzu, Japan).

3.2 Scanning electron microscopy analysis

SEM analysis of the TPNS particles, depicted in Figure 2A, shows electron-dense and elliptical-to-spherical nanoparticles arranged in clusters. The SEM analysis of the TPNZ particles (Figure 2B) showed that the spherical particles were uniformly distributed.

3.3 DLS technique

DLS technique was used to determine the hydrodynamic diameter of the nanoparticles. The measurements revealed that the TPNS particles (Figure 3A) had a size of 99 nm, while the TPNZ particles (Figure 3B) exhibited a size of 87.7 nm.

3.4 Zeta potential

The zeta potential for *T. populnea*-mediated nano-silver nanoparticles was measured as 90.5 mV (Figure 4A) with an electrophoretic mobility (mean) of −0.000700 cm^2 /Vs. The zeta potential and electrophoretic mobility (Figure 4B) of *T. populena* mediated nano ZnO particles were found to be 48.5 mv and 000376 cm^2 /*Vs*, respectively.

3.5 Evaluation of oxidative stress and antioxidant parameters

Oxidative stress marker assays confirmed the antioxidant efficacy of the synthesized TPNS and TPNZ particles. Group II exhibited significantly elevated TBARS levels and reduced SOD, CAT, GSH, GPx, and GST activities ($p < 0.01$) compared with the other groups. No significant differences were observed in TBARS and GSH activities between groups IV and I. Similarly, SOD and GST activities did not differ significantly between groups III and V. Table 1 shows no significant difference in GPx activity between Groups V and VI. TBARS levels in Groups VI, V, and III were significantly lower ($p < 0.01$) than those in Group II. The activities of SOD, CAT, GSH,

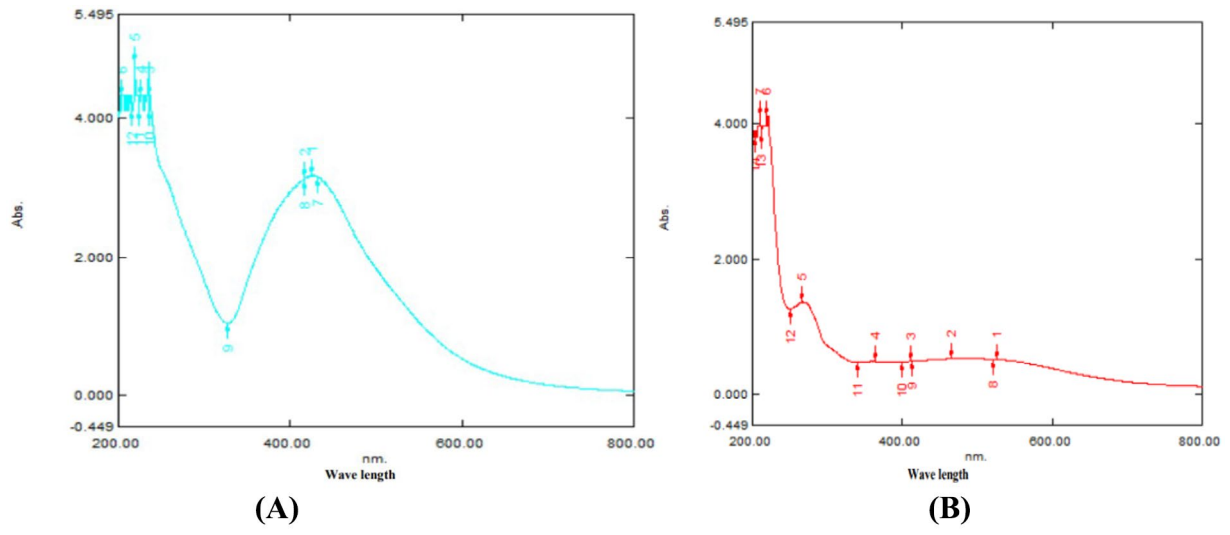

FIGURE 1
(A) UV-Visible spectrum of TPNS. **(B)** TPNZ nanoparticles.

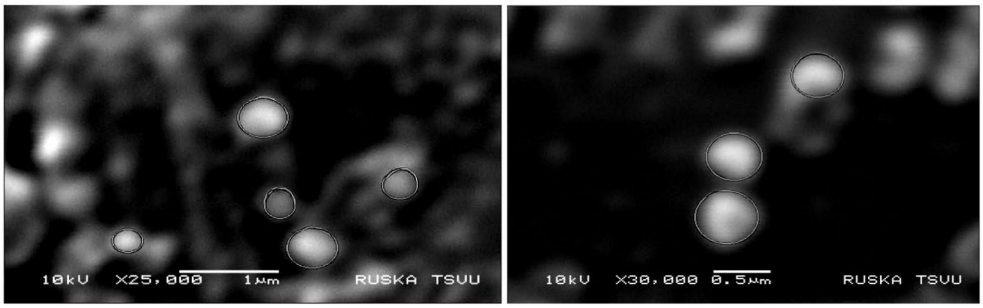

FIGURE 2
(A) SEM image analysis of TPNS particles. **(B)** TPNZ particles.

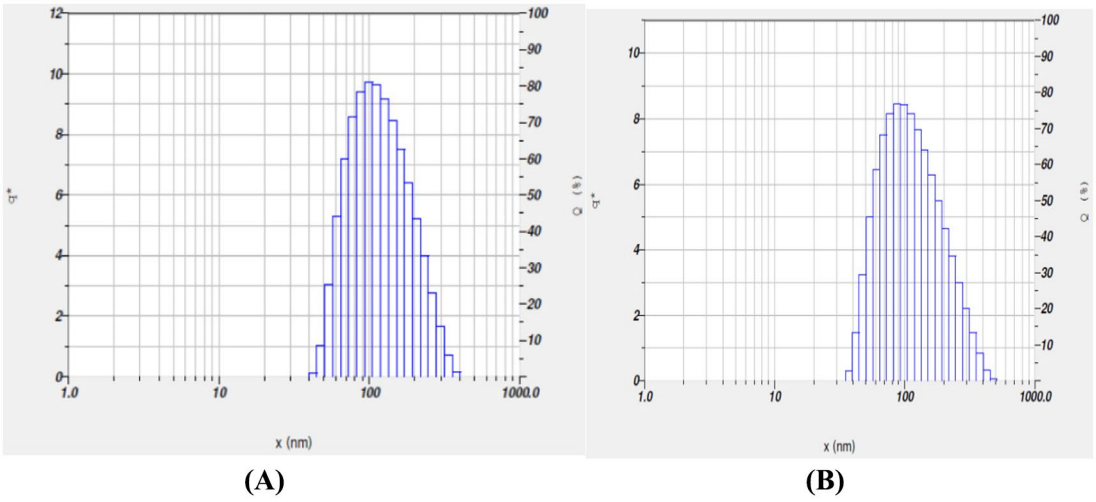

FIGURE 3
(A) Dynamic Light Scattering (DLS) analysis of TPNS nanoparticles. **(B)** Dynamic Light Scattering (DLS) analysis of TPNZ nanoparticles.

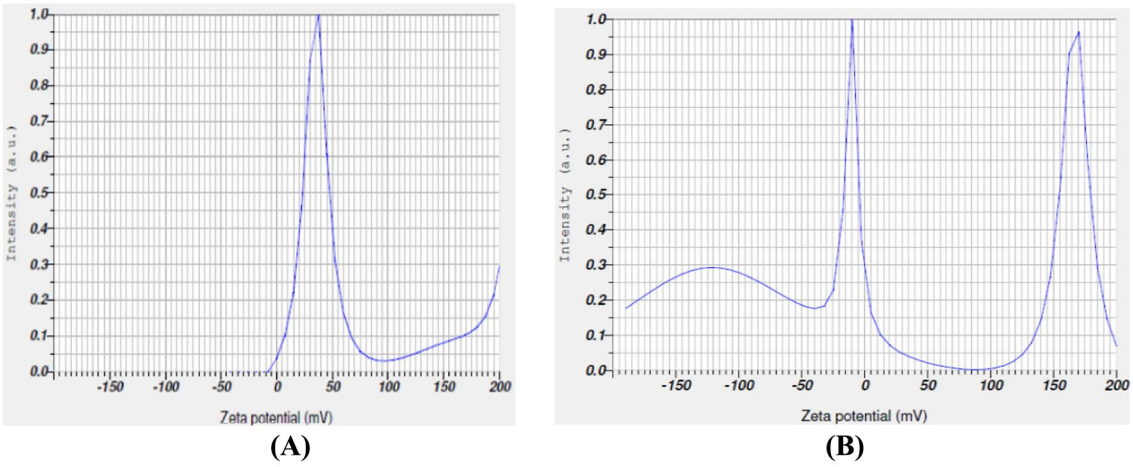

FIGURE 4
(A) Zeta potential measurement of TPNS nanoparticles. **(B)** Zeta potential measurement of TPNZ nanoparticles.

TABLE 1 Mean (±SE) values of oxidative stress and Anti-oxidant parameters in blood of different experimental groups.

Oxidative stress and anti-oxidant parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
TBARS (nano moles/gm of Protein)	$0.198^{e} \pm 0.004$	$0.365^{a} \pm 0.005$	$0.307^{b} \pm 0.007$	$0.202^{e} \pm 0.005$	$0.281^{c} \pm 0.008$	$0.251^{d} \pm 0.004$
SOD (units/mg of protein)	$25.16^{a} \pm 0.87$	$12.84^{e} \pm 0.36$	$18.12^{d} \pm 0.31$	$23.08^{b} \pm 0.45$	$19.13^{d} \pm 0.38$	$21.35^{c} \pm 0.29$
CAT (μ moles of H2O2 utilized /min/mg of protein)	$133.28^{a} \pm 0.43$	$107.91^{f} \pm 0.39$	$118.19^{e} \pm 0.43$	$131.73^{b} \pm 0.45$	$122.80^{d} \pm 0.38$	$126.25^{c} \pm 0.48$
GSH (μmoles/mg of protein)	$5.58^{a} \pm 0.02$	$2.33^{e} \pm 0.03$	$3.53^{d} \pm 0.02$	$5.54^{a} \pm 0.03$	$4.46^{c} \pm 0.02$	$4.76^{b} \pm 0.01$
GPx (units/gm of protein)	$29.93^{a} \pm 0.32$	$15.56^{e} \pm 0.49$	$20.28^{d} \pm 0.48$	$27.23^{b} \pm 0.51$	$22.24^{c} \pm 0.39$	$23.4^{c} \pm 0.49$
GST (μmoles of CDNB-GSH conjugate formed/min/mg of protein)	$3.11^{a} \pm 0.18$	$0.88^{e} \pm 0.02$	$1.94^{d} \pm 0.08$	$2.89^{b} \pm 0.06$	$2.05^{d} \pm 0.1$	$2.43^{c} \pm 0.05$

The different superscripts in the column are the mean values which differ significantly ($P < 0.01$).

GPx, and GST were significantly increased ($p < 0.01$) in groups IV, VI, V, and III, respectively, compared with those in group II.

4 Discussion

Green synthesis of nanoparticles, leveraging various phytochemicals in plant extracts, is biocompatible and environmentally friendly, making it efficient for large-scale biomedical applications (44). In this study silver and ZnO nanoparticles were synthesized using *T. populnea* methanolic leaf extract and characterized by UV–VIS analysis, SEM, DLS, and Zeta potential measurements. The addition of 1 mM silver nitrate and zinc acetate to *T. populnea* leaf extract resulted in a color change, confirming the production of TPNS and TPNZ (45, 46). UV–VIS spectroscopy indicated peaks at 421 nm and 260 nm for TPNS and TPNZ, respectively, suggesting bioreduction of aqueous silver ions (Ag+) upon exposure to plant extracts. Phytochemicals in *T. populnea* leaf extracts facilitate the transformation of silver ions into metallic nanoforms (47). Previous studies have shown peaks around 420 nm for *T. populnea*-synthesized silver nanoparticles (48) and 295 nm for TPNZ (49), while ZnO nanoparticles from *Deverra tortuosa* and the aqueous extract exhibited peaks in the 200–800 nm range (50), which is consistent with the findings of this studyTPNS and TPNZ particles were further characterized using SEM to examine their morphologies and structures. The SEM image analysis in this study revealed the formation of elliptical to spherical agglomerated TPNS, consistent with the findings of Bhuyar et al. (51) and Widatalla et al. (52) using *Padina* sp. and green tea leaf extracts. SEM images of TPNZ showed uniformly distributed spherical particles, aligning with results from Yedurkar et al. (53) and Muhammad et al. (54), who synthesized spherical ZnO nanoparticles using *Ixora coccinea* and *Papaver somniferum* leaf extracts, respectively. DLS, a technique for measuring particle size through laser beam analysis of Brownian motion in suspension, revealed sizes of 99 nm for TPNS and 87.7 nm for TPNZ. Similar diameters were reported for TPNS synthesized from *Rizophora apiculata* (99 nm) (55). Comparable sizes of 70 and 100 nm have reported for TPE-mediated silver nanoparticles (48) and *M. oleifera* seed extract-mediated silver nanoparticles (56). Sundrarajan et al. (57) reported a size of 100 nm for *Pongamia pinnata* leaf extract-mediated nano ZnO particles via DLS, while Shukla et al. (58) showed a size range of 76.2 to 183.8 nm for Zinc oxide nanoparticles synthesized from *Aspergillus niger*.

The zeta potential method is crucial for estimating the surface charge of nanoparticles, which is essential for their characterization and understanding the physical stability of nanosuspensions (19). Studies (59) have indicated that stable particles have a zeta potential of $\geq +30$ mV or ≤ -30 mV. A positive charge value of +37.4 mv of zeta potential for silver nanoparticles synthesized using *Morus alba* leaf extract were reported by Das et al. (60).

TPNS displays higher zeta potential than TPNZ, suggesting that TPE can effectively mediate nano-silver compared to nano ZnO particles.

Reactive oxygen species (ROS) are the natural byproducts of cellular metabolism. Oxidative stress occurs when ROS production exceeds the antioxidant defense capacity (61). In dairy cattle, both clinical and subclinical mastitis increase free radical production, increase total oxidant capacity, and reduce total antioxidant capacity (8). Lipid peroxidation products, particularly polyunsaturated fatty acids susceptible to free radical attack, are commonly used as oxidative stress markers, with TBARS being a widely recognized indicator (62). The elevated TBARS levels in Group II indicated oxidative stress. Among the treated groups, higher restoration of TBARS values was observed in group IV, followed by VI, V, and III, suggesting that TPNS had a stronger antioxidant effect than ceftriaxone, TPNZ, and TPE alone. Siddique and Al-Samman (63) observed a similar decrease in TBARS with *Delphinium denudatum* wall. Root extract-mediated AgNPs in mice with nephrotoxicity. However, aloin-mediated nano-silver and 11-α-keto boswellic acid-mediated nano-silver did not effectively exert antioxidant effects (64, 65). Kiyani et al. (66) reported a significant ($p < 0.01$) reduction in TBARS, approaching control values, in gout-affected mice treated orally with nano ZnO (Figure 5).

Endogenous antioxidants include enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reductase, play a crucial role in mitigating oxidative stress. The activities of these enzymes increase during oxidative stress to neutralize excess free radicals. During mastitis, PMNs are activated and generate reactive oxygen species (ROS) such as H_2O_2, superoxide anions, hydroxyl radicals, and halogen reactive species, partially reducing O_2 and lowering antioxidant enzyme levels (67). In this study, the activities of SOD, CAT, GSH, GPx, and GST significantly decreased ($p < 0.01$) in the blood of mastitis-induced mice, indicating oxidative stress, which is consistent with the findings of Chinchali and Kaliwal (68). The

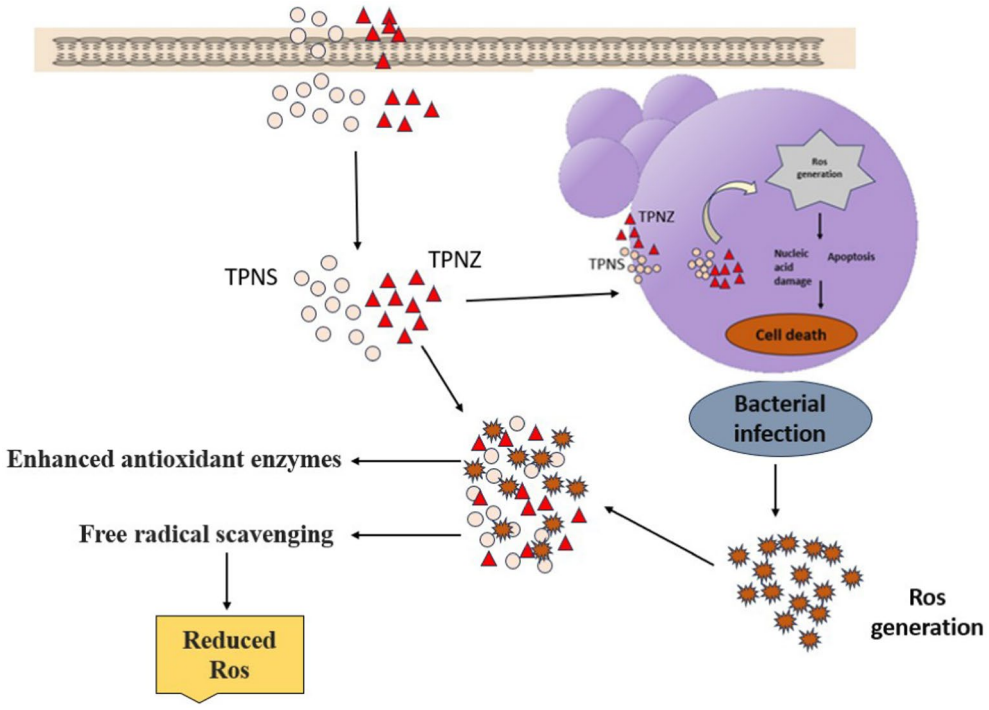

FIGURE 5
Schematic diagram representing the antioxidant activity of silver and zinc oxide nanoparticles.

reduced enzyme activities in the mastitis-affected group were normalized more effectively in group IV, followed by groups VI, V, and III, suggesting a higher antioxidant activity of TPNS, likely due to its increased free radical scavenging capacity. GPx activity was nearly restored in mice treated with *Rhizophora apiculata*-derived AgNPs in hepatotoxin-induced liver damage (69). Yadav et al. (70) reported a significant ($p < 0.001$) increase in SOD, catalase, and GPx activity in the granulation tissue of rats treated with *T. portulacastrum*-mediated nano ZnO compared to untreated rats in an induced wound model.

Suresh et al. (71) reported the antioxidant activity of *Cassia fistula*-mediated nano ZnO in *in vitro* assays. Ilavarasan et al. (72) and Pandanaboina et al. (73) demonstrated the antioxidant activity of TPE in rats with carbon tetrachloride-induced liver injury and alcohol-induced hepato-renal injury, respectively. Chaitanya et al. (64) observed improved glutathione levels with aloin-mediated silver nanoparticles in mastitis-induced mice. Jacob and Rajiv (74) showed that *Curcuma longa*-mediated nano ZnO particles possess free radical scavenging abilities through *in vitro* assays. ZnO nanoparticles enhance antioxidant enzyme activities, reduce free radical levels (OH., O_2., H_2O_2), and scavenge free radicals by electron transfer. Silver nanoparticles synthesized via plant extract phytochemicals efficiently reduce reactive oxygen species (ROS) and protecting biomolecules (75). Biosynthesized AgNPs exhibit superior antioxidant activity compared to extracts alone because of their large surface area, which enhances bioactive chemical adsorption (76). Our findings align with recent literature suggesting that AgNPs, particularly when interacting with antioxidants or phytochemicals, may offer therapeutic benefits. Specifically, studies have demonstrated that AgNPs act as catalysts in antioxidant reactions or facilitate cellular repair under controlled conditions, such as low doses or in combination with herbal compounds (77). TPE can be mediated with nano silver and nano ZnO particles, exerting more effective antioxidant effects than the methanolic extract alone.

5 Conclusion

This study explores the green synthesis and characterization of nanoparticles, specifically silver and ZnO nanoparticles, using TPE-mediated leaf extract. In the present study, it was shown that the overall antioxidant activity of TPNS was higher than that of ceftriaxone, TPNZ, and TPE indicating that biologically synthesized nanoparticles are more potent than the TPE extract alone, likely due to the combined antioxidant effect of phytochemicals and nanoparticles. Further safety studies are necessary for the upscaling and potential parental use of TPE-mediated nanoparticles as effective antioxidant agents.

5.1 Limitations of the study

The study has certain limitations, including the lack of seasonal variation analysis, as the plant extract was sourced

during a specific season, which may affect the reproducibility due to changes in bioactive compound composition. Additionally, the analysis of oxidative stress was limited to serum antioxidant parameters, and other organs such as liver and kidneys were not analyzed for oxidative stress. The *in vivo* studies were not done as it was beyond the objective of the experiment. Furthermore, the long-term stability of the nanoparticles was not assessed.

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 approved by the Institutional Animal Ethics Committee (I/2018–3/IAEC/C.V.Sc., Hyd). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

AJ: Conceptualization, Formal Analysis, Writing – original draft, Writing – review & editing. PE: Conceptualization, Supervision, Writing – review & editing. BK: Conceptualization, Supervision, Writing – review & editing. KP: Conceptualization, Supervision, Writing – review & editing. PS: Writing – original draft, Writing – review & editing. BA: Writing – review & editing. BV: Writing – review & editing.

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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.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

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