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RESEARCH ARTICLE

Phytochemical-mediated silver nanoparticles synthesized from *Vachellia sieberiana* suppress redox-inflammatory signaling in 1,2-dimethylhydrazine-induced organ toxicity

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Abstract

Background: Oxidative stress and chronic inflammation are central drivers of chemically induced organ injury and colorectal carcinogenesis, particularly following exposure to 1,2-dimethylhydrazine (DMH), a potent pro-oxidant and pro-inflammatory carcinogen. Plant-mediated nanotechnology has emerged as a promising strategy to enhance the bioactivity and systemic efficacy of phytochemicals against oxido-inflammatory damage. This study investigated the protective effects of *Vachellia sieberiana*–functionalized silver nanoparticles (AgNPs) on DMH-induced oxidative stress and inflammation in male Wistar rats.

Methods: Silver nanoparticles were synthesized using an aqueous leaf extract of *V. sieberiana* and characterized by UV–visible spectroscopy, confirming nanoparticle formation with a surface plasmon resonance peak at 437 nm. Thirty-five male Wistar rats were assigned to 7 groups of n=5. Group 1 served as the normal control, Group 2 DMH only (25 mg/kg). Groups 3, 4, and 5 received DMH + 100, 200, 400 mg/kg b.w of *V. sieberiana*-AgNPs. Group 6; DMH + Dox, and Group 7; 400 mg/kg of *V. sieberiana*-AgNPs only. Nanoparticles were administered daily via oral gavage for six weeks, concurrent with weekly DMH administration. Oxidative and inflammatory stress was induced by weekly subcutaneous administration of DMH (25 mg/kg) for six weeks, alongside oral treatment with *V. sieberiana*–AgNPs (100, 200, and 400 mg/kg). Antioxidant enzymes (GPx, GST, SOD), reduced glutathione (GSH), and malondialdehyde (MDA) were quantified in the colon, liver, and kidney, while colonic IL-6, IL-1β, and TNF-α were measured using ELISA. Data were analyzed using one-way ANOVA with Tukey's post hoc test.

Results: DMH markedly suppressed colonic GPx (61.43%, p=0.0017), GST (68.72%, p=0.0009), GSH (57.07%, p=0.0012), and SOD (42.45%,), with a concomitant increase in MDA levels (39.55%). Hepatic and renal tissues showed similar antioxidant depletion, including reductions in hepatic GPx (73.53%, p=0.0003) and renal GSH (65.51%, p<0.0001), alongside significant elevations in MDA (up to 73.25%). *V. sieberiana*–AgNPs dose-dependently restored antioxidant defenses and reduced lipid peroxidation, achieving MDA reductions of 54.38% (p=0.0016) in the colon and 47.15% (p=0.0002) in the kidney at 400 mg/kg. DMH-induced increases in colonic IL-6 (23.42%, p=0.0110), IL-1β (43.30%, p<0.0001), and TNF-α (18.37%, p=0.0278). These were significantly attenuated following AgNPs treatment.

Conclusion: *Vachellia sieberiana*–functionalized silver nanoparticles confer significant protection against DMH-induced oxidative stress and inflammatory injury, highlighting their potential as phytochemical-based nanotherapeutics for managing chemically induced tissue damage.

Keywords: 1,2-Dimethylhydrazine; antioxidant defense; inflammation; oxidative stress; silver nanoparticles; *Vachellia sieberiana*.

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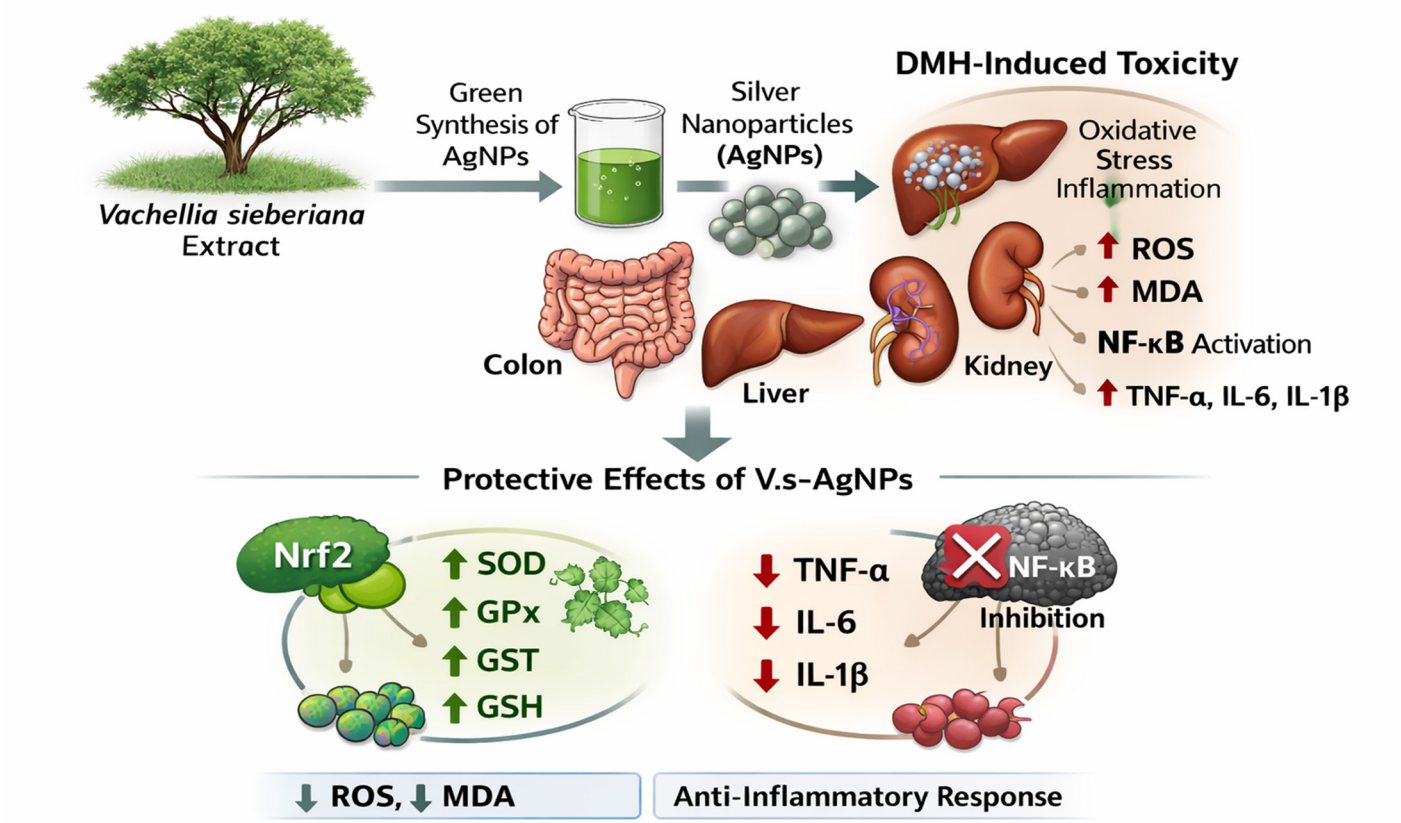

Graphical Abstract

Introduction

Oxidative stress and inflammation are closely interconnected biological processes that contribute to tissue injury and disease progression in chemically induced toxicity models. An imbalance favoring reactive oxygen species (ROS) over endogenous antioxidant defences such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione S-transferase (GST) promotes lipid peroxidation, macromolecular damage, and activation of pro-inflammatory signaling pathways [1]. Persistent oxidative stress stimulates nuclear factor-κB (NF-κB), a transcription factor that regulates the expression of pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), thereby sustaining inflammatory injury [2].

The chemical carcinogen 1,2-dimethylhydrazine (DMH) is widely employed to model oxidative stress– and inflammation-mediated colon toxicity, reproducing early molecular events associated with colorectal carcinogenesis. Following metabolic activation, DMH generates reactive intermediates that elevate ROS production, suppress antioxidant enzyme activities, increase malondialdehyde (MDA) levels, and trigger inflammatory responses in colonic and hepatic tissues [3,4]. Experimental studies consistently demonstrate depletion of SOD, GPx, GST, and reduced glutathione (GSH) alongside increased lipid peroxidation and pro-inflammatory cytokines, indicating disruption of redox homeostasis [3].

Phytochemical-based interventions highlight the pivotal role of oxidative stress and inflammation in DMH toxicity. Flavonoids such as hesperetin restore antioxidant enzyme activities and suppress inflammatory mediators in DMH-treated animals, while lycoperoside H and tannic acid attenuate ROS generation and cytokine expression across affected tissues, underscoring the therapeutic relevance of plant-derived compounds [3,4,5]. The nuclear factor erythroid 2–related factor 2 (Nrf2) pathway regulates antioxidant defence by inducing phase II detoxifying enzymes, while NF-κB governs inflammatory gene expression in response to oxidative cues [1,2]. Evidence of functional cross-talk between Nrf2 and NF-κB suggests that enhancing antioxidant signaling while suppressing inflammatory activation may offer protection against chemical-induced organ injury [6,7].

Medicinal plants are rich in redox-active phytochemicals, but limitations in bioavailability and stability restrict their in vivo efficacy. Green nanotechnology addresses these challenges by integrating phytochemicals into nanostructures with improved biological activity[8]. Plant-mediated synthesis of silver nanoparticles (AgNPs) utilizes phytochemicals as reducing and stabilizing agents, producing biocompatible nanoparticles capable of

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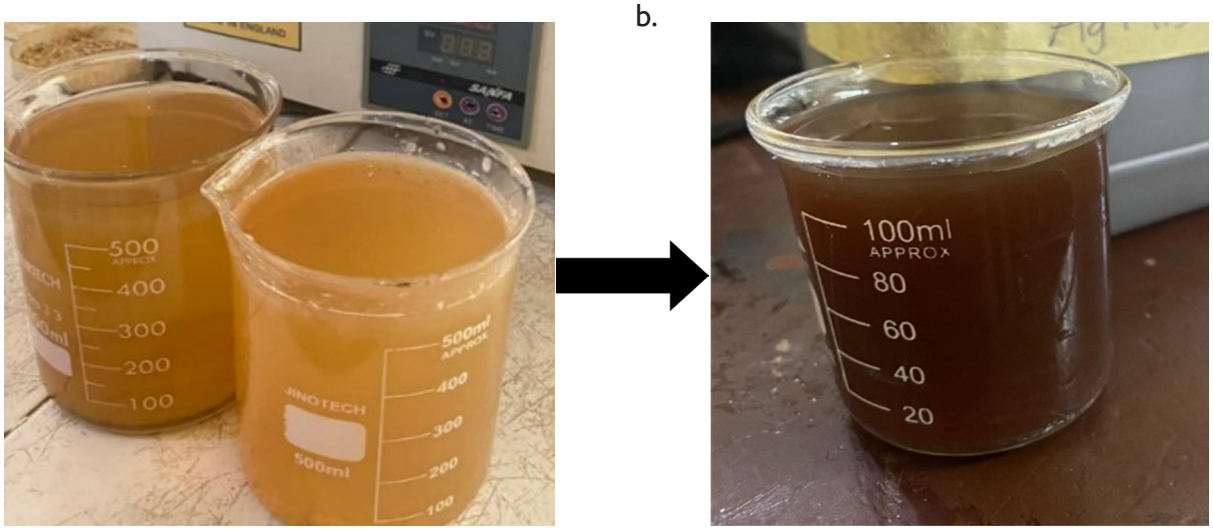

Figure 1: Synthesis of *Vachellia sieberiana*-AgNPs. (A) *Vachellia sieberiana* aqueous extract. (B) Synthesised *Vachellia sieberiana*-AgNPs

Table 1: Experimental protocol using *Vachellia sieberiana*-AgNPs.

S/N	Groups	Treatments
1	GROUP 1	Normal Control
2	GROUP 2	Positive Control (DMH; 25 mg/kg)
3	GROUP 3	DMH + 100 mg/kg *Vachellia sieberiana*-AgNPs
4	GROUP 4	DMH + 200 mg/kg *Vachellia sieberiana*-AgNPs
5	GROUP 5	DMH + 400 mg/kg *Vachellia sieberiana*-AgNPs
6	GROUP 6	DMH + Doxorubicin (2 mg/kg)
7	GROUP 7	400 mg/kg *Vachellia sieberiana*-AgNPs

modulating oxidative and inflammatory pathways [8,9,10]. Green AgNPs exhibit antioxidant and anti-inflammatory properties, including suppression of NF-κB activation and cytokine production, though their role in systemic chemical-induced toxicity remains underexplored [11,12,13].

The therapeutic efficacy of herbal medicines is often attributed to the synergistic interactions of their bioactive constituents; however, their practical application is limited by poor solubility, low bioavailability, rapid systemic clearance, and instability under physiological conditions, which can reduce their overall effectiveness [3,4]. To overcome these challenges, nanotechnology-based delivery systems have been developed to enhance the pharmacological performance of plant-derived compounds by improving solubility, stability, controlled release, and tissue targeting [9,11]. In particular, green synthesis of metallic nanoparticles, especially silver nanoparticles (AgNPs), has emerged as a promising strategy that integrates the advantages of nanotechnology with the intrinsic bioactivity of phytochemicals [9,10,11]. This approach employs plant metabolites as both reducing and stabilizing agents, resulting in biocompatible nanoparticles with enhanced antioxidant and anti-inflammatory properties and improved interaction with cellular redox systems [10,12]. Despite these advances, there remains limited evidence regarding the effectiveness of plant-mediated nanoparticles in models of chemically induced systemic toxicity, especially in relation to their ability to modulate interconnected oxidative stress and inflammatory pathways across multiple organs and regulate key signalling mechanisms such as Nrf2 and NF-κB [13].

Vachellia sieberiana is traditionally valued for its antioxidant and anti-inflammatory properties due to its rich phytochemical composition. The selection of *Vachellia sieberiana*-functionalized silver nanoparticles is based on both biological relevance and functional nanomaterial design. *Vachellia sieberiana* is widely used in African traditional medicine and has demonstrated antioxidant and anti-inflammatory activities through modulation of oxidative stress and inflammatory pathways. These properties are directly relevant to colorectal carcinogenesis, which is driven by chronic inflammation and redox imbalance. Incorporating these bioactive compounds into AgNPs may enhance their therapeutic potential as they have been established to modulate oxidative stress and inflammatory.

Despite increasing reports on plant-mediated silver nanoparticles, most studies have focused on single-organ models or isolated antioxidant effects, with limited evaluation of systemic redox–inflammatory interactions across multiple tissues in chemically induced carcinogenesis. In addition, there remains insufficient understanding of how phytochemical-functionalized nanoparticles influence the interplay between oxidative stress and inflammatory signaling in vivo.

Therefore, this study provides a multi-organ assessment (colon, liver, and kidney) of oxidative stress and inflammatory responses in a DMH-induced model, offering

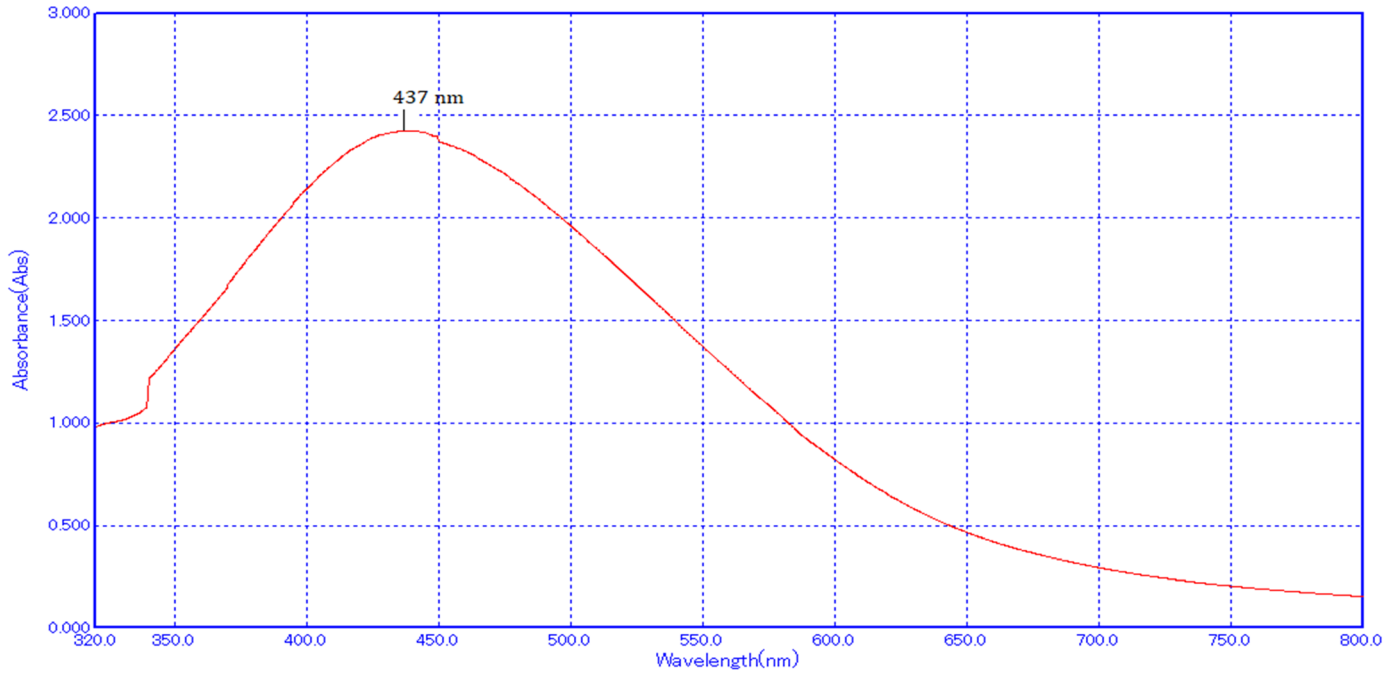

Figure 2: UV-vis Characterization of *Vachellia sieberiana*-AgNPs with an absorbance peak of 437 nm.

integrated insight into the redox–inflammation axis and the potential systemic effects of *Vachellia sieberiana*-functionalized AgNPs.

Methods

Plant-Mediated Nanoparticles Synthesis Strategy

Plant-mediated nanoparticles synthesis offers a biologically relevant approach for generating redox-active nanomaterials through the intrinsic reducing and stabilizing capacity of plant phytochemicals. *Vachellia sieberiana*, a medicinal tree species of the *Fabaceae* family, was selected as the biogenic reducer for silver nanoparticles (AgNPs) synthesis due to its abundance of redox-active constituents, including flavonoids, tannins, saponins, and phenolic compounds. These phytochemicals possess hydrogen- and electron-donating properties that enable effective reduction of metal ions while conferring antioxidant functionality to the resulting nanoparticles.

Aqueous extraction was deliberately employed to preserve polar antioxidant compounds and minimize chemical modification of phytoconstituents, thereby favouring biological redox activity and compatibility. This strategy enables the generation of phytochemical-functionalized silver nanoparticles with enhanced relevance for oxidative stress and inflammation modulation.

Collection, Authentication, and Processing of *Vachellia sieberiana*

Fresh leaves of *Vachellia sieberiana* were collected from Ogbomoso in Oyo state, Nigeria during the dry season (July, 2025). Botanical authentication was performed at the Herbarium Unit, Department of Pure and Applied Biology, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria, and a voucher specimen was deposited under the number LHO 806. To preserve phytochemical integrity and redox activity, the leaves were air-dried under ambient laboratory conditions away from direct sunlight and excessive heat prior to extraction.

Chemicals and Reagents-

All chemicals and reagents used were of analytical grade (≥99% purity). 1,2-Dimethylhydrazine (DMH) was procured from Shanghai Macklin Biochemical Co. Ltd., China. Doxorubicin was procured from a registered pharmaceutical store in Ogbomoso, Oyo State, Nigeria. The ELISA kits were acquired from Shanghai Ideal Medical Co. Ltd, China. All other chemicals and reagents used in this study were of analytical grade.

Other reagents include; methanol, Aluminum chloride, Sodium Acetate, phosphate buffer, Ethylene diamine tetra acetic acid (EDTA), Hydrogen peroxide (H2O2), Glacial acetic acid, Thiobarbituric acid (TBA), Eggyolk, sodium hydroxide, Hydrochloric acid (HCl), Sodium carbonate (Na2CO3), Folin, Trichloroacetic Acid (TCA), Tris Buffer, Sodium hydrogen phosphate (Na2HPO4), Potassium's dihydrogen phosphate (KH2PO4),

Preparation of Aqueous Extract for Nanoparticle Functionalization

The air-dried leaves were pulverized into a fine powder using an electric blender. Aqueous extraction was conducted at a concentration of 0.1 g/mL using double-

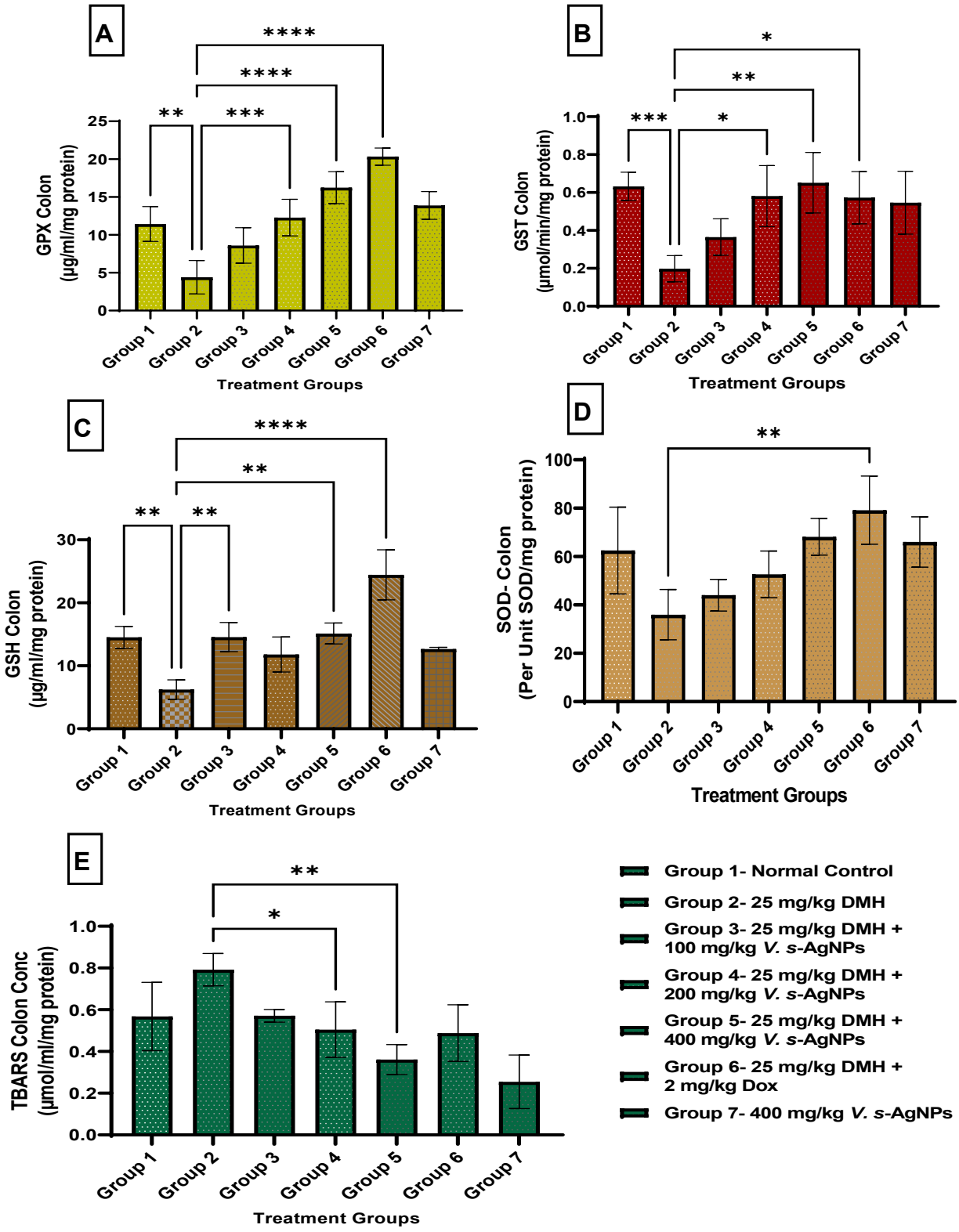

Figure 3: Oxidative stress markers in the colon of male Wistar rats with DMH-induced toxicity. (A) Colonic GPx activities. (B) Colonic GST activities. (C) Colonic reduced GSH levels. (D) Colonic SOD activities. (E) Colonic MDA concentrations.

distilled water at 60 °C for 2 h. Harsh extraction conditions and organic solvents were deliberately avoided to prevent degradation of hydrogen-donating and redox-sensitive phytochemicals. The extract was allowed to cool, centrifuged at 3000 rpm for 10 min, and filtered through Whatman No. 1 filter paper to obtain a clear aqueous extract, which was subsequently used for nanoparticles synthesis.

Phytochemical-Guided Synthesis of Silver Nanoparticles (AgNPs)

Phytochemical-functionalized silver nanoparticles were

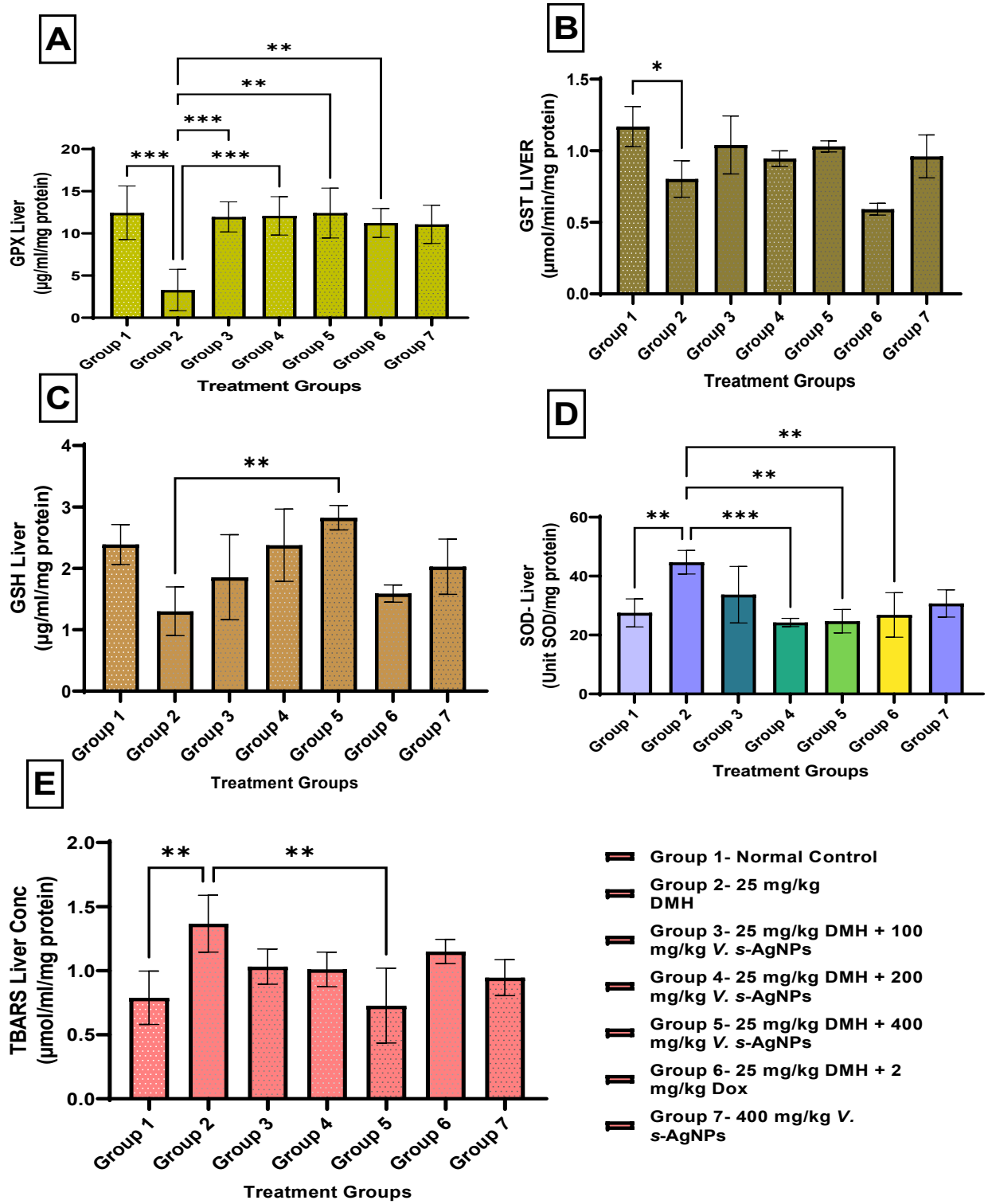

Figure 4: Oxidative stress markers in the liver of male Wistar rats with DMH-induced toxicity. (A) Hepatic GPx activities. (B) Hepatic GST activities. (C) Hepatic reduced GSH levels. (D) Hepatic SOD activities. (E) Hepatic MDA concentrations.

generated using *V. sieberiana* aqueous extract as a reducing, capping, and stabilizing agent. Briefly, 25 mL of the aqueous extract was added to 1000 mL of 1 mM silver nitrate ($AgNO_3$) solution and continuously stirred under light exposure for 30 min, as previously reported Badmus et al. [14].

Reduction of Ag^+ ions was visually monitored through the progressive color change from pale yellow to dark brown, attributable to surface plasmon resonance (SPR), indicating the formation of silver nanoparticles stabilized by plant-derived phytochemicals.

Nanoparticle Characterization Relevant to Biological Activity

The synthesized *V. sieberiana*-functionalized AgNPs were

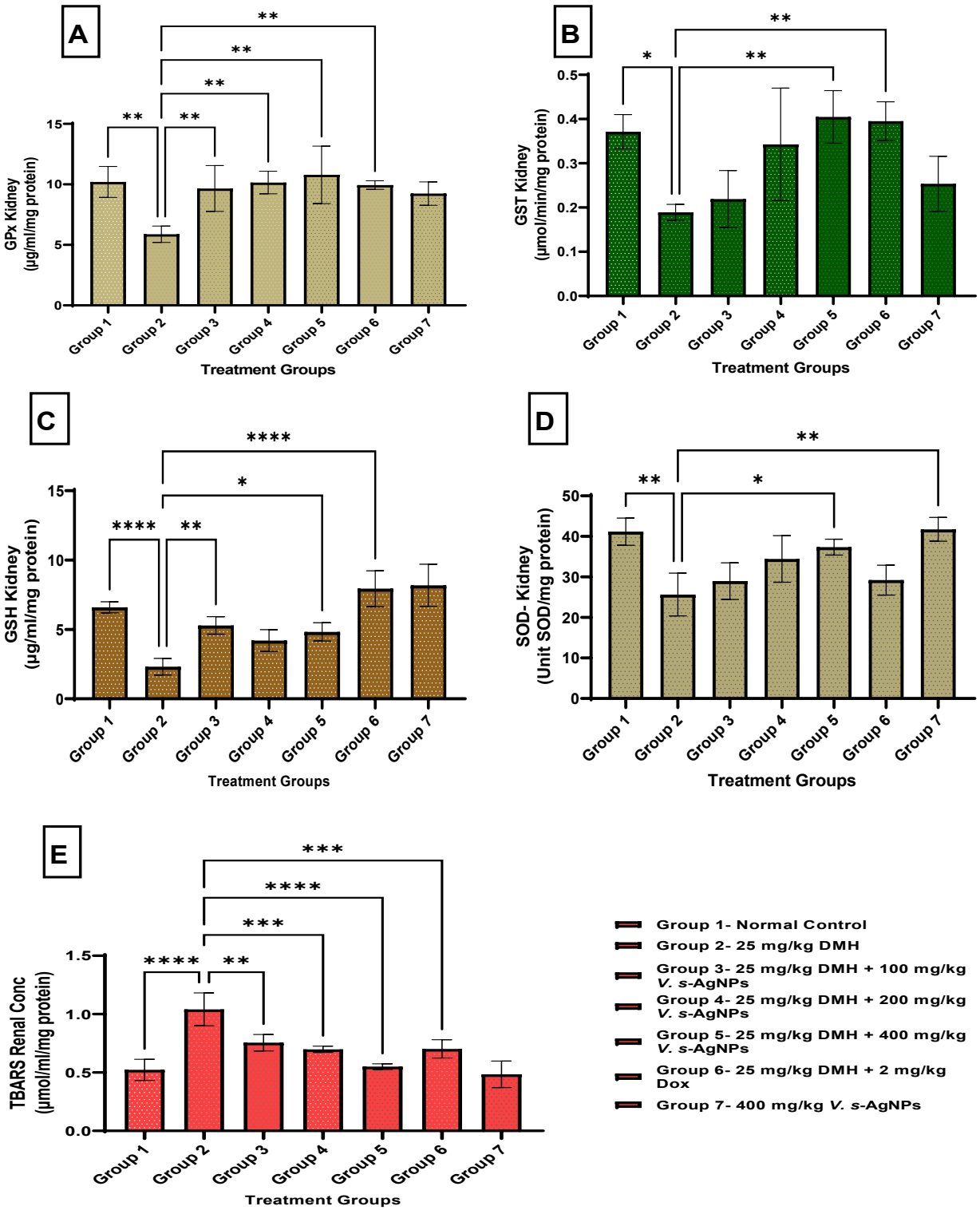

Figure 5: Oxidative stress markers in the kidney of male Wistar rats with DMH-induced toxicity. (A) Renal GPx activities. (B) Renal GST activities. (C) Renal reduced GSH levels. (D) Renal SOD activities. (E) Renal MDA concentrations.

characterized with emphasis on properties relevant to their biological activity. UV–visible spectroscopy was employed as an initial and widely accepted method to confirm nanoparticle formation based on the detection of the characteristic surface plasmon resonance (SPR) band of silver nanoparticles, typically observed within the 400–450 nm range, as reported in previous studies [15]. Absorption spectra were recorded within the 200–800 nm wavelength range using a UV–visible spectrophotometer and analyzed using UV-Vis Analyst software (version 5). The presence of the SPR peak was taken as preliminary confirmation of successful phytochemical-mediated nanoparticle synthesis.

Acute Toxicity Study

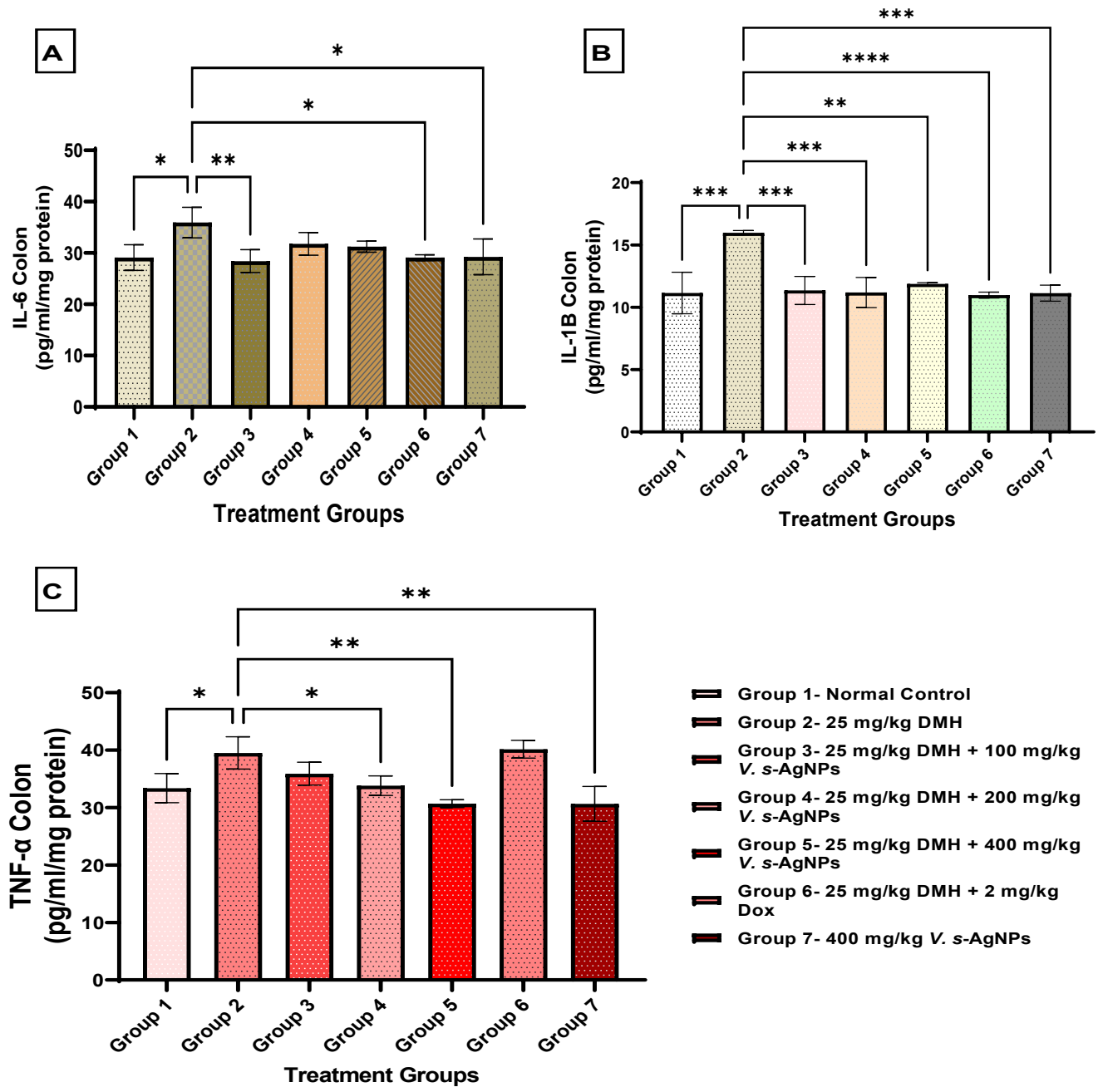

Figure 6: Colonic levels of pro-inflammatory cytokines in male Wistar rats with DMH-induced toxicity. (A) Interleukin-6 concentrations. (B) Interleukin-1β concentrations. (C) Tumor Necrosis Factor-α concentrations.

An acute oral toxicity study was conducted using Lorke's method to determine the safety profile of the synthesized silver nanoparticles. Animals were administered graded doses of the nanoparticles and observed for signs of toxicity and mortality over a 24-hour period, followed by continued monitoring. The median lethal dose (LD_{50}) was estimated to be 4000 mg/kg, indicating a relatively wide safety margin.

The selection of experimental doses (100, 200, and 400 mg/kg) was guided by the results of an acute toxicity study conducted using Lorke's method, which established the median lethal dose (LD_{50}) of the synthesized AgNPs to be 4000 mg/kg. The administered doses therefore represent 1/40, 1/20, and 1/10 of the LD_{50}, respectively, in line with standard toxicological practices for evaluating pharmacological activity within a safe margin. Notably, this LD_{50} value is consistent with previous reports indicating relatively low acute toxicity of silver nanoparticles, with values exceeding 2000 mg/kg and 5000 mg/kg in experimental models [16,17]. This consistency further supports the safety profile of the synthesized nanoparticles and justifies the selected dose range for the present study.

The selected doses (100, 200, and 400 mg/kg) represent conservative fractions (1/40, 1/20, and 1/10) of the LD_{50} (4000 mg/kg), ensuring a wide safety margin. Furthermore, no overt signs of toxicity or additional oxidative damage were observed in hepatic and renal tissues, supporting the tolerability of these doses within the experimental duration.

Nevertheless, long-term toxicity and nanoparticle accumulation remain important considerations for future investigation.

Experimental Animals and Ethical Considerations

Thirty-five apparently healthy male Wistar rats (average body weight ≈100 g) were obtained from the Animal House, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Animals were housed under standard laboratory conditions (12-h light/dark cycle, controlled temperature and humidity) and allowed to acclimatize for one week with free access to standard pellet diet and water. All experimental procedures complied with established guidelines for the care and use of laboratory animals as approved by the Research Ethical Committee of the Faculty of Basic Medical Sciences, LAUTECH (REC/FBMS No. 019/0257) that is in agreement with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health.

Induction of Oxidative and Inflammatory Stress Using DMH

Oxidative and inflammatory stress was induced using 1,2-dimethylhydrazine (DMH), a well-established pro-oxidant and pro-inflammatory agent known to generate reactive oxygen species (ROS) and trigger inflammatory signaling cascades. DMH was procured from Shanghai Macklin Biochemical Co. Ltd. and dissolved in 0.9% NaCl prior to administration. Rats received subcutaneous injections of DMH at a dose of 25 mg/kg body weight once weekly for six weeks. Administration of *V. sieberiana*-functionalized AgNPs was carried out concurrently during this period to evaluate their modulatory effects on DMH-induced oxidative and inflammatory responses.

Treatment Protocol with *V. sieberiana*-Functionalized AgNPs

The experimental design is presented in Table 2.1. Group 1 served as the normal control, while Group 2 received DMH only (25 mg/kg body weight). Groups 3, 4, and 5 received DMH alongside graded doses (100, 200, and 400 mg/kg body weight) of *V. sieberiana*-functionalized AgNPs to assess dose-dependent redox and anti-inflammatory modulation. Group 6 received DMH in combination with doxorubicin (2 mg/kg body weight) as a reference treatment, while Group 7 received *V. sieberiana*-AgNPs alone (400 mg/kg body weight). Nanoparticles were administered daily via oral gavage for six weeks, concurrent with weekly DMH administration.

Assessment of Oxidative Stress and Antioxidant Defense

Redox status was evaluated by assessing key antioxidant defense biomarkers. Colonic, hepatic and renal activities of GST[18], GPx [19], SOD [20], and levels of GSH [21] were evaluated using standard spectrophotometric method. Lipid peroxidation was quantified by measuring thiobarbituric reactive substances (TBARS) in the tissues, a measure of malondialdehyde (MDA), were evaluated according to Okhawa *et al.* [22] using a spectrophotometric (532 nm) method. All reagents utilized were of analytical grade.

Evaluation of Inflammatory Cytokines

Colonic concentrations of pro-inflammatory cytokines; Interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumour necrosis factor-alpha (TNF-α), were quantified using enzyme-linked immunosorbent assay (ELISA) assay kit according to the manufacturer's protocols (Shanghai Ideal Medical Technology Co. Ltd., China).

Histological Evaluation

For the investigation of histological changes, colon and liver tissues were carefully excised and immediately preserved in 10% formalin and sectioned for H&E staining following the method described by Feldman and Wolfe. This staining technique highlights the cellular and tissue structures, enabling detailed examination under a microscope.

Statistical analysis

Data were expressed as mean ± standard deviation and analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test. Statistical analyses were performed using GraphPad Prism software (version 9; GraphPad Software Inc., California, USA), with statistical significance set at $p < 0.05$.

Results

Characterization of *Vachellia sieberiana*-Silver Nanoparticles (AgNPs) by UV-Visible Spectroscopy.

The relatively sharp SPR peak observed at 437 nm further suggests the formation of stable and well-dispersed nanoparticles. This absorbance profile is consistent with previously reported plant-mediated silver nanoparticles, including *Annona muricata*-AgNPs with a reported peak at 420 nm (*Badmus et al.*[14]), supporting successful phytochemical-mediated nanoparticles synthesis.

Results of Oxidative Stress and Antioxidant

Defense Activities

Oxidative stress status was assessed by evaluating antioxidant defense systems;glutathione peroxidase (GPx), glutathione-S-transferase (GST), reduced glutathione (GSH), and superoxide dismutase (SOD), alongside lipid peroxidation indexed by malondialdehyde (MDA) levels in the colon, liver, and kidney.

Colonic Oxidative Stress Markers

Administration of DMH alone (Group 2) resulted in marked suppression of colonic antioxidant defense systems relative to the normal control (Group 1). Specifically, significant reductions were observed in GPx (61.43%; p = 0.0017), GST (68.72%; p = 0.0009), reduced GSH (57.07%; p = 0.0012), and SOD (42.45%; p = 0.1117).

Co-treatment with *V.s*-AgNPs produced a dose-dependent restoration of antioxidant enzyme activities, with the highest dose (400 mg/kg; Group 5) showing the most pronounced increases across all measured parameters.

Colonic lipid peroxidation was significantly elevated following DMH administration, with MDA levels increased by 39.55% relative to the normal control. Treatment with *V. sieberiana*-AgNPs significantly attenuated MDA accumulation in a concentration-dependent manner, with significant reductions observed at 200 mg/kg (36.33%; p = 0.0296) and 400 mg/kg (54.38%; p = 0.0016).

Hepatic Oxidative Stress Markers

In the liver, DMH administration significantly depleted antioxidant defenses, as evidenced by reductions in GPx (73.53%; p = 0.0003), GST (31.35%; p = 0.0155), and reduced GSH (45.56%; p = 0.0829) relative to the normal control. Co-treatment with *V. sieberiana*-AgNPs (Groups 3–5) markedly restored hepatic antioxidant enzyme activities, returning most parameters toward normal levels.

Notably, rats treated with *V. sieberiana*-AgNPs exhibited

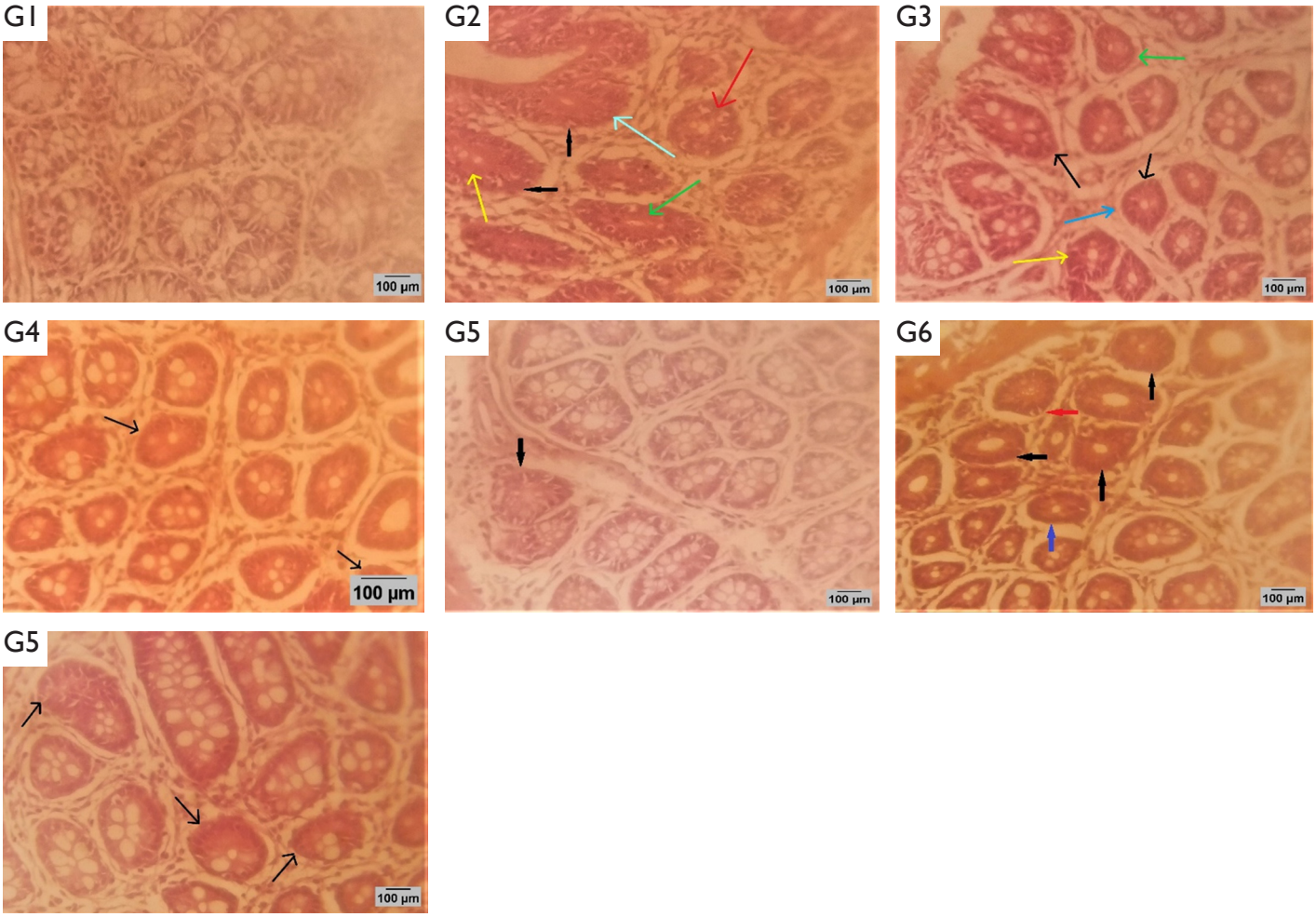

Plate 1: Photomicrographs of *V. sieberiana*-AgNPs effects on colon tissue architecture in DMH induced premalignant colon cancer in male Wistar rats. G1 (Normal control). G2 (Positive control; 25 mg/kg DMH). G3 (DMH plus 100 mg/kg AgNPs). G4 (DMH plus 200 mg/kg AgNPs). G5 (DMH plus 400 mg/kg AgNPs). G6 (DMH plus 2 mg/kg Dox). G7 (400 mg/kg AgNPs only). (H &E) x400. Black arrow indicates dysplastic cells, red arrow indicate inflammation, blue arrow indicates hyper-chromatic nucleus, yellow arrow indicates pseudo-stratified nucleus, and green arrow indicate loss of nucleus polarity.

higher preservation of antioxidant enzymes compared with the doxorubicin-treated group (Group 6).

In contrast to other antioxidant enzymes, hepatic SOD activity was significantly elevated following DMH exposure (62.46%; p = 0.0033). Co-administration of *V. sieberiana*-AgNPs at 200 and 400 mg/kg normalized SOD activity toward control values. Similarly, doxorubicin- and nanoparticles-only–treated groups showed no significant deviation from normal control levels.

Hepatic MDA concentrations were significantly increased by DMH administration (73.25% increase vs. Group 1). Treatment with *V. sieberiana*-AgNPs significantly reduced lipid peroxidation, with the 400 mg/kg dose producing the greatest reduction (46.78%; p = 0.0041).

Renal Oxidative Stress Markers

DMH exposure significantly impaired renal antioxidant defense, resulting in decreases in GPx (42.36%; p = 0.0012), GST (49.00%; p = 0.0117), reduced GSH (65.51%; p < 0.0001), and SOD (37.69%; p = 0.0019).

Co-treatment with *V. sieberiana*-AgNPs significantly ameliorated these alterations, restoring antioxidant enzyme activities toward normal levels. GST and SOD activities demonstrated a clear dose-dependent response to nanoparticles treatment.

Renal lipid peroxidation was significantly elevated following DMH administration, with a 49.74% increase in MDA levels (p < 0.0001). *V. sieberiana*-AgNPs significantly reduced renal MDA concentrations in a concentration-dependent manner, with the most pronounced effect observed at 400 mg/kg (47.15% reduction; p < 0.0001).

Effects of *V. sieberiana*–AgNPs on Colonic Pro-inflammatory Cytokines

Colonic Interleukin-6 (IL-6)

DMH administration significantly increased colonic IL-6 concentrations by 23.42% (p = 0.0110) compared with the normal control. Co-treatment with *V. sieberiana*-AgNPs significantly attenuated this elevation, with the most pronounced reduction observed at 100 mg/kg (20.90%; p = 0.0047). Doxorubicin treatment also resulted in a significant reduction in IL-6 levels (18.99%; p = 0.0201).

Colonic Interleukin-1β (IL-1β)

Colonic IL-1β concentrations were markedly elevated following DMH exposure (43.30%; p = 0.0001). Treatment with *V. sieberiana*-AgNPs at all administered doses significantly suppressed IL-1β expression, restoring cytokine levels toward normal control values. A comparable reduction was observed in the doxorubicin-treated group.

Colonic Tumor Necrosis Factor-α (TNF-α)

DMH administration resulted in a significant increase in colonic TNF-α concentrations (18.37%; p = 0.0278). Co-treatment with *V. sieberiana*-AgNPs significantly suppressed TNF-α expression in a dose-dependent manner, with reductions of 14.43% and 22.33% observed at 200 and 400 mg/kg, respectively, compared with the positive control.

Histopathological Evaluation

Histopathological examination (H&E staining) of colon tissues revealed marked mucosal damage, inflammatory cell infiltration, and epithelial disruption in the DMH-treated group. Treatment with *V. sieberiana*-AgNPs ameliorated these alterations, as evidenced by improved mucosal architecture and reduced inflammatory infiltration.

In contrast, liver sections across experimental groups did not exhibit pronounced histopathological alterations, with general preservation of hepatic architecture observed.

Discussion

The present study demonstrates that *Vachellia sieberiana*-functionalized silver nanoparticles (*V.s*-AgNPs) significantly attenuate 1,2-dimethylhydrazine (DMH)-induced oxidative stress and inflammatory signaling in male Wistar rats. DMH is a well-established colonotropic chemical that induces reactive oxygen species (ROS) overproduction, lipid peroxidation, and depletion of endogenous antioxidant defenses (superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), and reduced glutathione (GSH)), culminating in inflammation and molecular damage in gastrointestinal tissues [23,24]. These perturbations mimic early events in colon carcinogenesis and oxidative injury, making DMH a valuable toxicology model for studying redox–inflammation interactions. It is important to note that the present study focuses on the biological evaluation of phytochemical-mediated nanoparticles, and therefore UV–visible spectroscopy was employed as a preliminary confirmation method. While additional physicochemical characterization (e.g., DLS, TEM, zeta potential) would provide more detailed structural insights, the observed biological responses across multiple organs serve as functional validation of the synthesized nanostructures.

In this study, DMH administration produced significant reductions in GPx, GST, GSH, and SOD activities across colonic, hepatic, and renal tissues, accompanied by elevated malondialdehyde (MDA), a marker of lipid peroxidation. This pattern of antioxidant depletion and oxidative injury aligns with reports that oxidative stress

markers are elevated in DMH models and that several phytochemical interventions (e.g., hesperetin, tannic acid, luteolin) can reverse these biochemical disruptions [24,25,26]. For example, hesperetin treatment restored GPx and SOD and reduced expression of pro-inflammatory proteins such as TNF-α, IL-6, iNOS, and NF-κB in the colon of DMH rats, suggesting that antioxidant supplementation can ameliorate both oxidative and inflammatory insults [24,25]. Similarly, tannic acid diminished DMH-induced TNF-α release and attenuated oxidative enzymes, further demonstrating the interdependence of redox status and inflammation [25]. These prior studies establish a strong backdrop for interpreting the mechanistic effects observed with *V.s*-AgNPs.

Co-treatment with *V.s*-AgNPs in this study significantly restored antioxidant enzyme activities and reduced MDA accumulation in a dose-dependent fashion. These findings suggest that *V.s*-AgNPs not only scavenge ROS but may also participate in upregulating endogenous antioxidant defense systems. Plant-mediated green synthesis confers nanoparticles with a phytochemical corona possessing flavonoids, phenols, tannins, and other bioactive compounds that function as reducing, capping, and stabilizing agents during synthesis [27]. These phytochemicals are well known for their ability to donate electrons, neutralize free radicals, and regenerate intracellular antioxidants, enhancing GPx and GST activity and stabilizing GSH pools [27]. In this context, *V.s*-AgNPs combine the physicochemical properties of silver with the biological functionality of plant phytochemicals, yielding enhanced antioxidant activity relative to unmodified nanoparticles.

Mechanistically, restoration of redox balance by *V.s*-AgNPs likely involves modulation of the Nrf2/ARE signaling pathway, a master regulator of antioxidant gene expression. Under oxidative stress, Nrf2 translocates to the nucleus to activate genes encoding detoxifying enzymes, including GPx, GST, and heme oxygenase-1 (HO-1), thereby strengthening the cell's capacity to counteract ROS [28,29]. Importantly, crosstalk between Nrf2 and the pro-inflammatory transcription factor NF-κB provides a pivotal regulatory node linking oxidative stress to inflammatory responses, where ROS-mediated activation of NF-□B promotes the expression of pro-inflammatory cytokines (IL-6, IL-1β, TNF-α), while Nrf2 activation enhances antioxidant defenses and concurrently suppresses NF-κB signaling [30]. Beyond this regulatory axis, the protective effects of *V.s*-AgNPs may also extend to the preservation of mitochondrial function, a key source and target of ROS during chemical-induced toxicity. Excessive ROS generation by DMH can compromise mitochondrial membrane integrity and disrupt electron transport chain activity, thereby amplifying oxidative stress and inflammatory signaling. By attenuating ROS accumulation and restoring antioxidant capacity, *V.s*-AgNPs may help maintain mitochondrial stability, limit ROS propagation, and prevent downstream activation of inflammatory pathways. Furthermore, phytochemical constituents associated with the nanoparticles may contribute to maintaining mitochondrial redox homeostasis and protecting cellular components from oxidative damage, collectively reinforcing their protective role.

Although the observed effects are consistent with modulation of Nrf2 and NF-κB signaling pathways, these pathways were not directly measured in the present study and are therefore proposed based on established literature. Importantly, the biological activity observed is likely attributable to a synergistic interaction between the silver nanoparticle core and the phytochemical constituents acting as capping and stabilizing agents. These phytochemicals contribute intrinsic antioxidant and anti-inflammatory properties, while the nanoparticle structure may enhance cellular uptake and bioavailability, collectively amplifying the overall biological effect.

Consistent with this mechanistic model, *V.s*-AgNPs significantly suppressed colonic IL-6, IL-1β, and TNF-α levels compared with DMH controls. This anti-inflammatory effect mirrors findings from plant-derived nanoparticles studies reporting reduced pro-inflammatory mediator expression and improved tissue antioxidant status. For instance, green-synthesized silver nanoparticles using diverse plant extracts have demonstrated anti-inflammatory activities, including inhibition of NF-κB activation and downregulation of inflammatory cytokines, by virtue of their phytochemical constituents and small size facilitating cellular uptake [11,27]. These findings are congruent with *V.s*-AgNPs attenuating not only oxidative damage but also inflammatory signaling, reinforcing the critical link between redox homeostasis and inflammation in DMH toxicity [31].

Although aqueous extracts of *Vachellia sieberiana* are known to exhibit antioxidant and anti-inflammatory effects, their efficacy is often limited by poor bioavailability and instability of active constituents [27]. The nanoformulation used in this study appears to overcome some of these limitations. The small particle size and larger surface area of the *V.s*-AgNPs likely improve cellular uptake and interaction with redox-sensitive pathways, while the phytochemical coating may help preserve the activity of bioactive compounds. This could explain the more marked improvements observed in antioxidant enzymes (SOD, GPx, GST, and GSH), along with the reductions in MDA and pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α). Overall, these findings suggest that formulating *Vachellia sieberiana* into silver nanoparticles enhances its biological activity compared to the conventional aqueous extract.

Organ-specific observations provide further nuance. The

colon, being the primary site of DMH-induced insult, responded robustly to *V.s*-AgNPs with normalization of antioxidant enzymes and cytokines, reflecting restoration of epithelial resilience and mucosal integrity. In contrast, hepatic SOD activity in DMH-only animals exhibited an initial compensatory increase, a phenomenon noted in other toxicant models where early antioxidant responses may transiently upregulate to counter acute ROS surges, but are insufficient to prevent damage [32]. *V.s*-AgNPs appeared to normalize this response, suggesting improved ROS handling that precludes excessive compensatory upregulation. The kidneys, similarly affected by DMH, demonstrated restored antioxidant activity with *V.s*-AgNPs, indicating that these nanoparticles reach systemically relevant tissues and exert protective effects beyond the colon.

Given the well-documented concerns regarding the potential toxicity of silver nanoparticles, particularly in the liver and kidneys, the present findings provide important insight into their biocompatibility. In this study, *V.s*-AgNPs did not induce further oxidative damage in hepatic and renal tissues; rather, they restored antioxidant enzyme activities and reduced lipid peroxidation, suggesting a protective rather than toxic effect at the administered doses. This may be attributed to the phytochemical-mediated green synthesis approach, which enhances nanoparticle stability and reduces the likelihood of adverse biological interactions. The histopathological improvements observed in colon tissues further support the anti-inflammatory and antioxidant effects of the synthesized nanoparticles. These observations support the potential biocompatibility of *V.s*-AgNPs and highlight their promise for safe therapeutic application, although further detailed toxicological and histopathological evaluations are warranted.

Comparing *V.s*-AgNPs with conventional agents underscores their translational potential. Some chemotherapeutic or synthetic antioxidant drugs possess inherent oxidative side effects that limit their utility; plant-functionalized nanoparticles may provide a dual modality that both enhances antioxidant capacity and suppresses inflammation without the same adverse profiles. Indeed, green synthesized AgNPs from garlic peel and other botanical sources have shown liver-protective and anti-inflammatory effects by enhancing antioxidant enzymes and reducing pro-inflammatory cytokines in hepatotoxic models, suggesting broad applicability of plant-mediated nanoparticles in redox-related diseases [33].

The choice of *Vachellia sieberiana* relates critically to phytochemical composition. Rich in flavonoids, tannins, saponins, and phenolics, this plant species provides abundant hydrogen-donating and redox-active constituents that improve nanoparticles biosynthesis and biological functionality. Preserving these labile compounds through aqueous extraction likely maximized their contribution to nanoparticles capping and bioactivity, consistent with best practices in green nanotechnology [27].

Despite these promising results, certain subtleties warrant discussion. For example, maximal suppression of IL-6 occurred at intermediate *V.s*-AgNPs doses rather than strictly increasing with dose, suggesting potential hormetic or biphasic effects, a phenomenon observed in other phytochemical and nanoparticles studies wherein moderate doses elicit optimal signaling responses [27]. Such dynamics emphasize the need for comprehensive dose–response analysis in future studies.

Despite these promising findings, certain limitations should be acknowledged. While UV–visible spectroscopy provided preliminary evidence for nanoparticle formation, further physicochemical characterization, including particle size distribution, zeta potential, and morphological analysis, would be necessary to comprehensively establish nanoparticle properties, stability, and reproducibility. Future studies should incorporate these techniques to strengthen the translational applicability of the present findings.

Conclusion

In summary, this study provides compelling evidence that *V.s*-AgNPs effectively disrupt the DMH-induced oxidative stress–inflammation axis by both enhancing endogenous antioxidant defenses through Nrf2/ARE modulation and suppressing inflammatory signaling via NF-κB attenuation. The synergistic integration of plant phytochemicals with nanostructured silver confers a mechanistically distinct advantage that merits further translational exploration in oxidative stress and inflammation-driven disorders.

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