Pharmacogn. Res., 2026; 18(4):1284-1293.
https://www.phcogres.com

Original Article

Hepatoprotective Effect and Antioxidant Activity of Silver Nanoparticles Green Synthesis from *Moringa oleifera*

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ABSTRACT

Background: Green synthesis of Silver Nanoparticles (AgNPs) offers an environmentally friendly, safe and non-toxic alternative to traditional physical and chemical approaches. Several studies suggest that AgNPs may exhibit greater therapeutic effects than their corresponding plant extracts. **Objectives:** The objective was to synthesize AgNPs using *Moringa oleifera* leaf extract and to assess their antioxidant and hepatoprotective effect in a paracetamol-induced liver injury model. **Materials and Methods:** The UV-visible spectroscopy, X-ray diffraction, Fourier Transform Infrared Spectroscopy, and Scanning Electron Microscopy with Energy Dispersive X-ray analysis were employed to determine the physicochemical characteristics of AgNPs produced. The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. An acute oral toxicity test was conducted in rats up to 2000 mg/kg Body Weight (BW). *In vivo* hepatoprotection was assessed in rats with paracetamol-induced liver damage, measuring liver enzyme levels following treatment with low doses of the AgNPs. **Results:** The results showed that the AgNPs were spherical in shape and of crystalline nature. AgNPs displayed a strong antioxidant scavenging effect with an IC_{50} of 20.53 µg/mL. No mortality or overt toxic signs were observed up to 2000 mg/kg BW in the toxicity test. In the hepatoprotection study, administration of low doses of AgNPs significantly restored liver enzyme values toward normal levels in intoxicated rats. **Conclusion:** The *Moringa oleifera*-mediated green-synthesized AgNPs are safe, show potent antioxidant capacity, and deliver significant hepatoprotective activity in a paracetamol-induced liver damage model. These findings support the potential of these nanoparticles as a promising therapeutic agent for liver disorders.

Keywords: Antioxidant activity, Green synthesis, Hepatoprotective activity, *Moringa oleifera*, Paracetamol induced liver injury model, Silver nanoparticles.

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Received: 19-03-2026;
Revised: 04-05-2026;
Accepted: 23-06-2026.

INTRODUCTION

Liver illnesses are one of the major worldwide health issues brought on by the adoption of contemporary eating habits, exposure to numerous environmental contaminants, and excessive use of certain medications and other numerous reasons, including infections, parasites, malnutrition, inborn defects, toxic substances and cancer can result in liver problems (Praneetha, *et al*., 2018). Both synthetic and natural medications can be used to treat liver issues. synthetic drugs commonly used for liver diseases and the undesirable side effects they may bring. So, utilizing natural products that are high in bioactive compounds is becoming more popular as demand for plants with a variety of antioxidant qualities and bioactive compounds that may combat free radicals (Araibi *et al*., 2022). "Several research studies have shown that plant extracts rich in antioxidant activity can protect the liver from paracetamol-induced injury by inhibiting lipid peroxidation and boosting the activity of antioxidant enzymes" (Akram *et al*., 2012).

The drumstick tree, or *Moringa oleifera* Lam, is a native of northwest India but is extensively regarded as a crop in a number of other nations, including South Africa, Ethiopia, Sudan, and the Philippines. It belongs the genus *Moringa* and the family Moringaceae. For generations, *Moringa oleifera*, a perennial softwood tree with low-quality lumber, has been employed for both traditional industrial and medicinal reasons. It is appreciated for its edible leaves, blooms, and delicate pods (Dixit *et al*., 2019). According to certain descriptions, moringa leaves offer a favorable nutritional balance, including vitamins, minerals, fatty acids, and amino acids. Furthermore, numerous antioxidant components, such as carotenoids, flavonoids, phenolics, and ascorbic acid, have been reported to be present in the leaves. So far, studies have demonstrated that leaf extracts have strong natural antioxidant qualities. According to reports, the plant leaves contain anticancer, cardio protective, hypotensive, wound and eye healing qualities (Stohs *et al*., 2015; Islam *et al*., 2019).

DOI: 10.5530/pres.20260244

Numerous pharmacological actions have also been documented. The traditional medical system has made use of nearly every aspect of the plant. *M. oleifera* was characterized in numerous papers as having strong anti-inflammatory, hepatoprotective, antihypertensive, and anti-tumor effects. Strong *in vitro* antioxidant and radical scavenging activity has been noticed for alcoholic and aqueous extracts of *Moringa oleifera* leaves and roots (Islam *et al*., 2019).

Antibacterial agents are among the significant biological uses for AgNPs made from *Moringa oleifera*. When AgNPs adhere to the microorganism's cell membrane, they significantly impair normal processes like respiration and permeability (Morones *et al*., 2005). The creation of biological processes for producing silver nanoparticles (AgNPs) is becoming a significant area of nanotechnology and research. AgNPs can be synthesized biologically using microorganisms and plant extracts, which have been proposed as possible environmentally harmless substitutes for physical and chemical methods (Heydari *et al*., 2017; Shanker *et al*., 2017). These days, one of the most crucial research areas is the creation of environmentally friendly metallic nanoparticle production and their applications. The manufacture of AgNPs using plant extracts is one of the most straight forward and economical biological processes. Whatever the secondary metabolites found in plants provide the simplest means of reducing metal nanoparticles (Kumar *et al*., 2016). The reduced silver particles (Ag^+) induce changes in the secondary structure and facilitate the formation of silver nuclei by trapping Ag^+ on the protein surface due to electrostatic interactions. As the reduction process continues, the silver nuclei grow, leading to their accumulation and ultimately resulting in the production of AgNPs. The produced particles are also stable and the pharmacokinetics of AgNPs indicate that their modest size allows for effective interaction with the biological system. AgNPs exhibit a wide range of biological functions such as antimicrobial (Yugandhar *et al*., 2015), antilarvicidic (Sundaravadivelan *et al*., 2013), anthelmintic (Kumara Swamy *et al*., 2014), anticancer (K. Vasanth *et al*., 2014), antioxidant & anti-inflammatory (Rafie *et al*., 2014), hepatoprotective (Bhuvaneswari *et al*., 2014) and wound healing activities (Kaler *et al*., 2014).

The Introduction establishes the context of liver illnesses, the shift toward natural products due to side effects of synthetic drugs, and the value of plants with antioxidant qualities, specifically mentioning *Moringa oleifera*. It then transitions coherently to the role of Silver Nanoparticles (AgNPs) synthesized biologically, noting their diverse biological functions (like antimicrobial and hepatoprotective effects) and setting up the rationale for using *Moringa oleifera* extract for green synthesis.

MATERIALS AND METHODS

Materials

The equipment used included mixer grinder (Bajaj Rex 500W), Digital weighing Balance (Shimadzu, India), a lyophillizer (Modulspin), a centrifuge (Heraeus, Germany), a UV-Vis spectrophotometer (Systronics, India), an FTIR spectrophotometer (Bruker Alpha), a SEM (Hitachi, S-37000N). The chemicals such as acetone, aluminium chloride, ascorbic acid (99%), chloroform, DPPH (95%), ethanol, ethyl acetate, Fehling solution, ferric chloride, gallic acid (97.5%), lead acetate, quercetin, silver nitrate (99%), silymarin and sodium hydroxide. "They were purchased from Finar chemicals, HI media and Yucca Enterprises, Mumbai. All solvents used were of analytical reagent grade.

Plant collection, Authentication and Extraction

Healthy *Moringa oleifera* leaves were gathered locally from Karimnagar and Hanamkonda districts, Telangana, India in winter season. Removed the decayed, discoloured, or infected parts manually. To remove soil and debris of leaves rinse thoroughly with tap water. Then, wash 2-3 times with deionized water. Cleaned leaves were shade dried on clean surface. For species confirmation, the *Moringa oleifera* leaves were sent to the Department of Botany, Kakatiya University, Warangal, Telangana and Identified the scientific name by botanist Dr. MD. Mustafa. A voucher specimen was deposited therein in the Herbarium (KUW).

To obtain extractable powder, the gathered materials were carefully cleaned with Double Deionized (DDI) water, divided into smaller pieces, and then shade dried for a week at 35 to 40°C before being ground into a powder in an electric grinder. The powder was extracted via maceration using 96% ethanol, with a ratio of powder to solvent of 1:10. Container was stored in dark place for 7 days. Each macerated plant material was stirred occasionally to ensure proper extraction. Following solvent washing, the powder's contents were gathered in a container and dried using a rotary evaporator at lower pressure to produce a viscous product. A solid mass was eventually achieved and stored in a refrigerator.

Phytochemical Screening

Chemical tests were performed on the ethanolic extract of *Moringa* to discover a variety of phytoconstituents, including alkaloids, flavonoids and their glycosides, phenolics, saponins, steroid/triterpenoidal substances, and tannins (Kokate *et al*., 1997).

Fractionation

The crude ethanolic extract was suspended in 100 mL of deionized water and subsequently fractionated with ethyl acetate (300 mL). A rotary evaporator was used to concentrate the ethyl acetate

fraction to dryness in a vacuum at 40°C and it was then freeze dried (Abubakar & Mainull *et al*., 2020).

Determination of total phenolic content and total flavonoid content

The ethanol fraction's total phenolic content was determined using the Folin-Ciocalteu reagents. Gallic acid was produced as a stock solution with a concentration of 1 mg/mL. This led to the preparation of gallic acid solutions at various concentrations (10-100 µg/mL) for the phenolic content calibration curve. A 25 mL volumetric vial was filled with 1 mL of the extract (1 mg/mL), followed by 1 mL of Folin-Ciocalteus phenol reagent (1:10 v/v diluted with deionized water) before shaken. 10 mL of 7% aqueous sodium carbonate was incorporated to the mixture after 5 min. The solution was diluted to 25 mL with deionized water and mixed. A reagent blank was prepared using deionized water instead of sample. After incubation for 90 min. at room temperature, development of blue colour was observed. Finally, the absorption against prepared reagent blank was determined at λ_{max} 760 nm using UV-Visible spectrophotometer. Gallic Acid Equivalents (GAEs) in mg per gram of plant extract were calculated in relation to the standard gallic acid (Praneetha *et al*., 2014; Das *et al*., 2019).

The total flavonoid concentration in ethyl acetate fraction was determined using the aluminium chloride assay, with measurements performed in triplicate. Results were expressed as Quercetin (QUEs) (mg QUE per gram of plant extract). A 1mg/mL Quercetin stock solution was prepared, from which standard solution ranging from 10 to 100 µg/mL were made to establish a calibration curve. A 10 mL volumetric vial containing 4 mL of deionized water was filled with 1 mL of the plant extract (1 mg/mL) for the purpose to prepare the sample. The vessel was filled with 0.3 mL of a 5% sodium nitrite solution. After 5 min, 0.3 mL of a 10% aluminum chloride solution was injected and further 2 mL of 1M NaOH were added and the volume was adjusted to 10 mL, using de-ionized water. Absorption was measured at λ_{max} 510 nm against an established blank of reagents after 15 min of incubation (Praneetha *et al*., 2014; Das *et al*., 2019).

Green synthesis of *Moringa oleifera* silver nanoparticles (MO -AgNPs)

45mL of 1mM aqueous $AgNO_3$ was mixed with an aliquot (5 mL) of the ethyl acetate fraction of plant extract and the alkaline pH (8-9) was adjusted using sodium hydroxide. The reaction mixtures were exposed to direct sunshine to promote the production of nanoparticles. The formation of nanoparticles, which is indicated by a dark brown color, was determined by monitoring the color change of the reaction mixtures. To stop the nanoparticles from aggregating together, the vessels were taken out of the sun and kept at room temperature in the dark when the color intensities of the liquids reached. To remove contaminants from AgNPs, the dark brown solution was centrifuged (Heraeus, speed (rpm)-300-11,500, capacity-4×180 mL, refrigerated- 0-40°C) for 30 min at 4000 RPM. The resulting nanoparticle pellet was then collected and cleaned three times using DDI water. Allowed to air dry then lyophilized (Module Spin-Hanil, vacuum-15 pounds per square inch, temperature: -80°C). The collected nano pellet was utilized for further characterization. Silver nitrate solution alone was used as negative control and observed the solution colour was not changed. It confirmed that no AgNPs had formed (Moodley *et al*., 2018; Shanker *et al*., 2015).

Characterization technique of silver nanoparticles

Particle size of AgNPs was measured by Particle size analyzer (Mohammed *et al*., 2022; Dixit *et al*., 2019).

Ultraviolet-visible (UV-vis) spectra: UV-visible spectroscopy (Systronics Elico SL-159) was used to detect the bio reduction of silver ions in the 300-700 nm region.

X-ray diffraction (XRD) analysis: Crystalline nature of the AgNPs was confirmed by XRD (Bruker AXS D8) analysis.

Scanning Electron Microscope (SEM) analysis: Morphology of the AgNPs was determined by using SEM (HITACHI, S-3700N).

Energy Dispersive X-ray (EDX) analysis: Elemental composition was determined by EDX (HITACHI, S-3700N) analysis. The analysis indicated that silver was present in the test sample.

FTIR: The functional groups involved in the reduction of silver were detected by recording an infrared spectrum (Bruker Alpha).

Antioxidant Activity of MO AgNPs

The antioxidant activity of AgNPs was find out by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. 1 mL of AgNPs and Ascorbic acid (standard) at various concentrations (50 - 500 µg/mL) and 1 mL of ethanolic solution (0.1 mM) of DPPH were included in the test reaction mixture. For control solution, mix 1 mL DPPH and 1 mL ethanol and blank solution contain only ethanol. Which were then incubated for half an hour in dark condition (a condition where no light is allowed) at room temperature. Absorbance readings were taken at λ_{max} 517 nm using UV-visible spectrophotometer. The antioxidant activity of MO AgNPs was calculated using the following formula.

$$\text{DPPH scavenging activity } (\%) = (A_0 - A_1)/A_0 \times 100$$

Where A_0 is the absorbance of the control and A_1 is the sample absorbance. The assay was performed in three times (Mohammed *et al*., 2022; Omede *et al*., 2016).

In vivo Animal Studies

The study employed albino rats weighing approximately 175-200 g. They were kept in a conventional laboratory environment with a 12-hr dark-light cycle, ambient temperature of 25±2°C, and relative humidity of 50±15%.""The animals were given a

commercial pellet nourishment and an unlimited supply of water. "The experiment was carried out with prior consent from Animal Ethics Committee of UCPSc, Kakatiya University.

Ethical Review

The study was carried out with prior consent from the Animal Ethics Committee of UCPSc, Kakatiya University, and the Acute oral toxicity experiment followed OECD-423 principles with Institutional Animal Ethics Committee approval (Approval no: 12/IAEC/UCPSc/KU/2022).

Acute Toxicity Study

The acute oral toxicity study was performed in accordance with OECD guideline 423, using the minimum number of animals. Silver nanoparticles (AgNPs) were administered orally at dose levels of 5, 50, 300, and 2000 mg/kg body weight. Animals were observed for 14 days for clinical signs of toxicity, behavioral changes, and mortality. No mortality was observed at any dose up to 2000 mg/kg. At the end of the observation period, the rats were euthanized by cervical dislocation, and tissues were collected, fixed in 10% formalin, and processed for histopathological analysis. (Shanker *et al*., 2015; Rakesh. R *et al*., 2020).

Hepatoprotective activity

Experimental procedure

Six groups of six animals in each group were randomly selected from among the animals.

Group I: received water once daily for 7 days, and served as normal control.

Group II: received water once daily for 7 days and served as paracetamol control.

Group III: received standard drug silymarin (100mg/ kg). Once daily for 7 days, serving as standard.

Group IV: received *Moringa oleifera* leaf extract (200 mg/kg) once daily for 7 days.

Group V and VI: received *Moringa oleifera* silver nanoparticles (200 and 100 mg/kg respectively) once daily for 7 days.

Except for group I, all groups were administered a single oral dose of paracetamol 3 g/kg body weight on the eighth day. Then, 36 hours after paracetamol was administered, blood and liver samples were taken from the animals in each group for histological investigations and the measurement of various biochemical parameters, respectively. Centrifugation was used to separate the serum. "Using standard enzymatic colorimetric methods, serum samples were tested for liver function by estimating the enzymes such as Glutamate Pyruvate Transaminase (GPT/ALT), Glutamate Oxaloacetate Transaminase (GOT/AST), Alkaline Phosphatase (ALP), total bilirubin, and total protein (Islam *et al*., 2019; Abhay *et al*., 2023; Aubert *et al*., 2012).

Statistical Analysis

Results were expressed as mean±S.E.M. For statistical analysis of the data group, the mean was compared with a one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test and Dunnett's multiple comparison test. $p<0.05$ was considered to be statistically significant.

RESULTS

Crude Ethanolic Extract and Yield

The percentage yield of the crude ethanolic extract of plant material is 5.88±0.08.

Phytochemical screening

The phytochemical analysis revealed that the major chemical constituents of the leaves of *Moringa oliefera* are flavonoid compounds and their glycosides, phenolic compounds, saponins, steroid/triterpenoidal compounds and tannins.

Total phenolic content and total flavonoids content

The crude extract and ethyl acetate fraction of *Moringa oleifera* exhibited total phenolic contents of 0.66±0.04 and 0.99±0.08 mg Gallic Acid Equivalents (GAEs)/g dry weight, respectively. The total flavonoid content, expressed as Quercetin Equivalents (QUEs) per gram of plant extract, was found to be 1.14±0.31 and 1.7±0.1 mg QUEs/g dry weight for the crude extract and ethyl acetate fraction, respectively (Table 1).

Green synthesis of *Moringa oleifera* Silver Nanoparticles (MO AgNPs)

The results showed that the ethyl acetate fraction possessed a significant concentration of phenolic and flavonoid compounds. Therefore, in the green synthesis of *Moringa oleifera*-mediated Silver Nanoparticles (MO-AgNPs), the ethyl acetate fraction was utilized as both a reducing and stabilizing agent for nanoparticle formation. The synthesis of AgNPs was visually confirmed by the appearance of a brown coloration, which is attributed to the excitation of surface plasmon vibrations in silver nanoparticles (Figure 1A).

Characterization technique of silver nanoparticles

The absorbance spectrum of the synthesized AgNPs was recorded in the wavelength range of 300-700 nm. A distinct absorbance peak was observed around 470 nm, which corresponds to the Surface Plasmon Resonance (SPR) of AgNPs (Figure 1B).

The SEM image of the synthesized AgNPs revealed that the nanoparticles were predominantly spherical in shape with no significant agglomeration (Figure 1C). The EDX spectrum exhibited a characteristic peak at approximately 2.125 keV, confirming the presence of elemental silver in the prepared AgNPs (Figure 1D). FTIR analysis was performed to identify

the biomolecules responsible for stabilizing and capping the AgNPs. The FTIR spectrum of *Moringa oleifera*-derived AgNPs displayed prominent peaks at 3341.32 cm^{-1}, 1636.39 cm^{-1}, and 508.24 cm^{-1} (Figure 1E). These peaks are attributed to hydroxyl or amino groups (O-H/N-H), amide groups (C=O), and aliphatic chain vibrations, respectively.

The crystalline structure of the synthesized Silver Nanoparticles (AgNPs) was characterized by X-ray Diffraction (XRD) analysis. The XRD pattern (Figure 1F) exhibited distinct diffraction peaks at 21.44º, 38.05º, 44.31º, 64.50º, and 77.46º, which correspond to the (100), (111), (200), (220), and (311) planes, respectively. These reflections are consistent with the standard data of Face-Centered Cubic (FCC) silver (JCPDS file no. 04-0783). The sharp diffraction peaks confirm the nanocrystalline nature of the biosynthesized AgNPs. Dynamic Light Scattering (DLS) analysis was performed to determine the particle size distribution of the synthesized AgNPs. The results revealed that the nanoparticles exhibited a size range of 80-100 nm with a high intensity distribution, indicating the formation of uniformly dispersed nanoparticles.

Antioxidant activity

The antioxidant potential of *Moringa oleifera*-mediated AgNPs was evaluated using the DPPH radical scavenging assay. The nanoparticles exhibited dose-dependent scavenging activity (Table 2). The highest scavenging percentage (66.1±0.36%) was recorded at a concentration of 500 µg/mL, followed by 400 µg/mL (56.2±0.37%), 300 µg/mL (50.83±0.76%), 200 µg/mL (33.09±0.36%), and 100 µg/mL (20.66±0.76%), whereas the lowest scavenging capacity (12.03±0.75%) was observed at 50 µg/mL

Acute Oral Toxicity

The absence of mortality or organ toxicity in the acute oral toxicity test indicates that the synthesized MO-AgNPs are safe and non-toxic at the tested dose of 2000 mg/kg. The normal histoarchitecture of vital organs such as the liver, heart, and kidneys supports their biocompatibility and systemic safety (Figure 2).

Table 1: Total phenolic and flavonoid contents in *Moringa oleifera*.

Constituents	Crude extract	Ethyl acetate fraction
Total phenolic content (mg/g)	0.66±0.04	0.99±0.08
Total flavonoid content (mg/g)	1.14±0.31	1.7±0.1

Data expressed as Mean±SD, *n*=3. SD: Standard deviation.

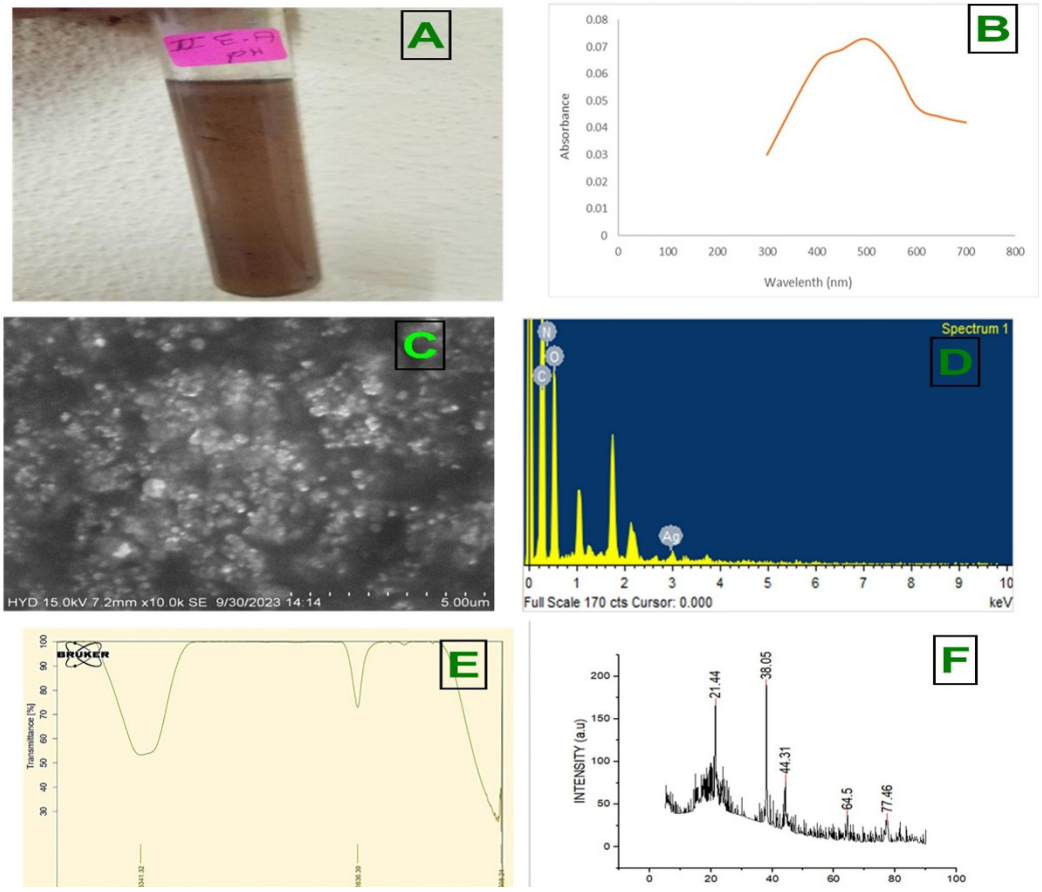

Figure 1: Characterization of MO AgNPs. A-Reddish brown colour of MO AgNPs; B-UV- Visible spectrum; C- FESEM micrograph; D- EDX graph; E- FTIR spectrum; F- XRD pattern.

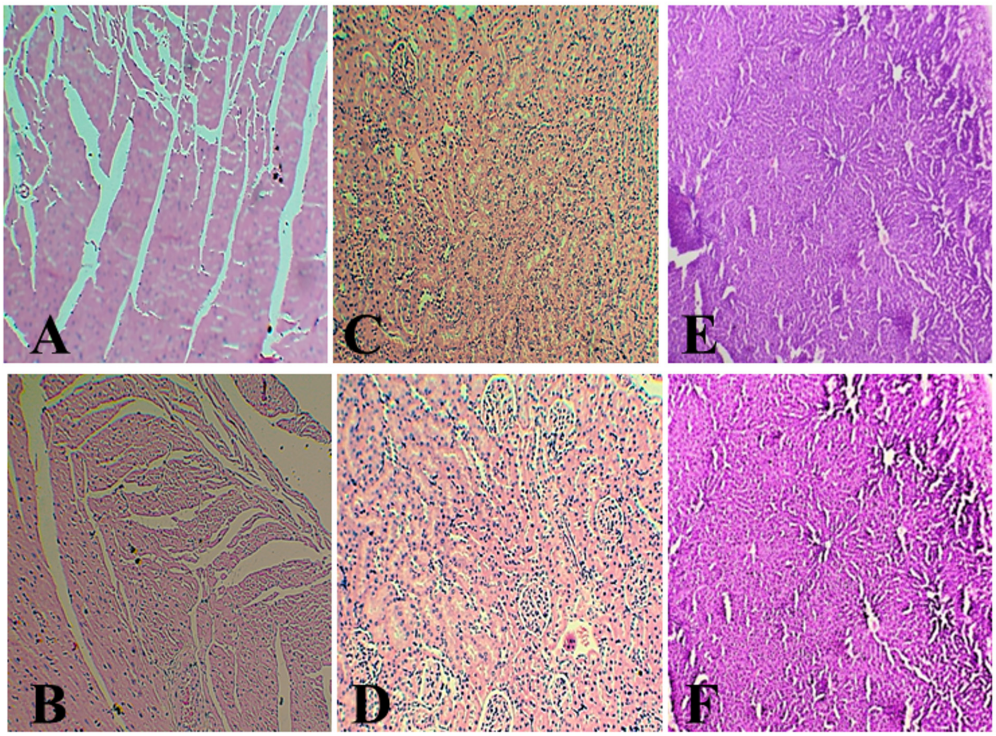

Figure 2: A-Photograph of section female Wistar rat of the heart (control) & B-MO AgNPs treated heart shows normal myocardium and pericardium; C-kidney (control) & D-MO AgNPs treated kidney shows normal glomeruli and tubules without lesions of pathological significance; E-liver (control) & F-MO AgNPs treated liver shows no sign of inflammation and hepatocytes are normal.

Table 2: DPPH radical scavenging activity of *Moringa oleifera* silver nanoparticles.

Sl. No.	Concentration of *M. oleifera* AgNPs (µg/mL)	Free radical scavenging activity (%)
1.	50	12.03±0.75
2.	100	20.66±0.76
3.	200	33.09±0.36
4.	300	50.83±0.76
5.	400	56.2±0.37
6.	500	66.1±0.36

All values are expressed as Mean±SEM (n=3).

Table 3 shows that paracetamol caused a decrease in body weight and a significant increase in liver weight**,** indicating liver damage. Treatment with silymarin, MO extract, and MO-AgNPs prevented weight loss and reduced liver weight toward normal**,** with MO-AgNPs (100 mg/kg) showing a response close to the normal control, indicating effective hepatoprotection.

Biochemical Evaluation of Hepatoprotective Effect

Paracetamol administration resulted in significant hepatic injury, as evidenced by a marked elevation (p<0.05) in serum levels of SGPT, SGOT, ALP, and total bilirubin, and a concomitant reduction in total protein, albumin, and globulin levels compared with the normal control group. Treatment with the standard hepatoprotective agent silymarin (200 mg/kg) significantly restored these biochemical parameters toward normal values. Similarly, treatment with the plant extract (200 mg/kg) exhibited hepatoprotective potential, although the effect was slightly less pronounced than that of the standard drug. Remarkably, the group treated with MO-AgNPs (100 mg/kg) demonstrated the most pronounced hepatoprotective effect, with enzyme levels and protein profiles returning close to, or even surpassing, normal control values. The lower dose (100 mg/kg) was more effective than the higher dose (200 mg/kg), suggesting an optimal therapeutic range rather than a linear dose-dependent effect (Table 4).

Histopathological Observations

Histological assessment of liver tissues provided supportive evidence of hepatoprotection. The normal control group (I) Displayed intact hepatic architecture with clearly defined hepatic cords and sinusoids (Figure 3A). The paracetamol-treated group (II) Exhibited severe hepatic damage characterized by centrilobular necrosis, sinusoidal dilation, and disorganized hepatic cords (Figure 3B). Animals treated with silymarin (III) Displayed nearly normal hepatic morphology (Figure 3C). The groups treated with MO extract (IV) and AgNPs (200 µg/kg) (V) Showed mild hepatic necrosis and partial restoration of tissue architecture (Figures 3D and 3E). The group treated with AgNPs (100 µg/kg) (VI) Exhibited substantial hepatocyte regeneration, evident by well-preserved hepatic cords, large nuclei, and a

Table 3: Effect of MO AgNPs on the body weight and liver weight in paracetamol induced hepatotoxicity in rats.

Group	Initial Body Weight (g)	Final Body Weight (g)	Liver Weight (G)
Normal Control (I)	187.5±9.8	188.5±10.5	7.36±0.38
Paracetamol Control (II)	185.8±12	171.6±2.4	9.50±0.37
Silymarin (200mg/kg) (III)	182.5±14.05	183.3±5.16	7.13±0.18*
MO extract (200 mg/kg) (IV)	175±8.9	174±3.93	8.58±0.16*
MO-AgNPs (200 mg/kg) (V)	175±6.3	178±4.08	8.05±0.21*
MO-AgNPs (100 mg/kg) (VI)	180±5.8	181.6±11	7.83±0.27*

*p<0.05 vs. Paracetamol Control. **Most normalized/closest to Normal Control.

Table 4: Effect of treatments on liver function biomarkers.

Group	SGPT(U/L)	SGOT(U/L)	ALP(U/L)	Total Bilirubin (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
I	31.4±1.3	58.8±1.01	269±1.6	0.508±0.4	7.45±0.31	3.46±0.06	2.83±0.13
II	139.2±0.6	116.4±1.08	418.8±0.74	2.57±0.1	3.57±0.07	1.88±0.08	1.59±0.08
III	68.7±0.37*	68.9±0.8*	274.1±0.78*	0.64±0.04*	6.58±1.55*	3.16±0.15*	2.65±0.04*
IV	92.08±0.62*	79.±0.89*	344.4±0.45*	0.77±0.05*	5.6±0.11*	2.74±0.14*	1.7±0.08*
V	68.6±0.40*	69.6±0.47*	284.5±0.46*	0.63±0.02*	6.06±0.05*	3.03±0.03*	2.04±0.18*
VI	64.8±0.74*	61.8±0.68*	262.3±3.64*	0.47±0.03*	6.34±0.07*	3.54±0.10*	2.56±0.04*

*Values are expressed as Mean±SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's test. p<0.05 compared with toxic control (Group II).

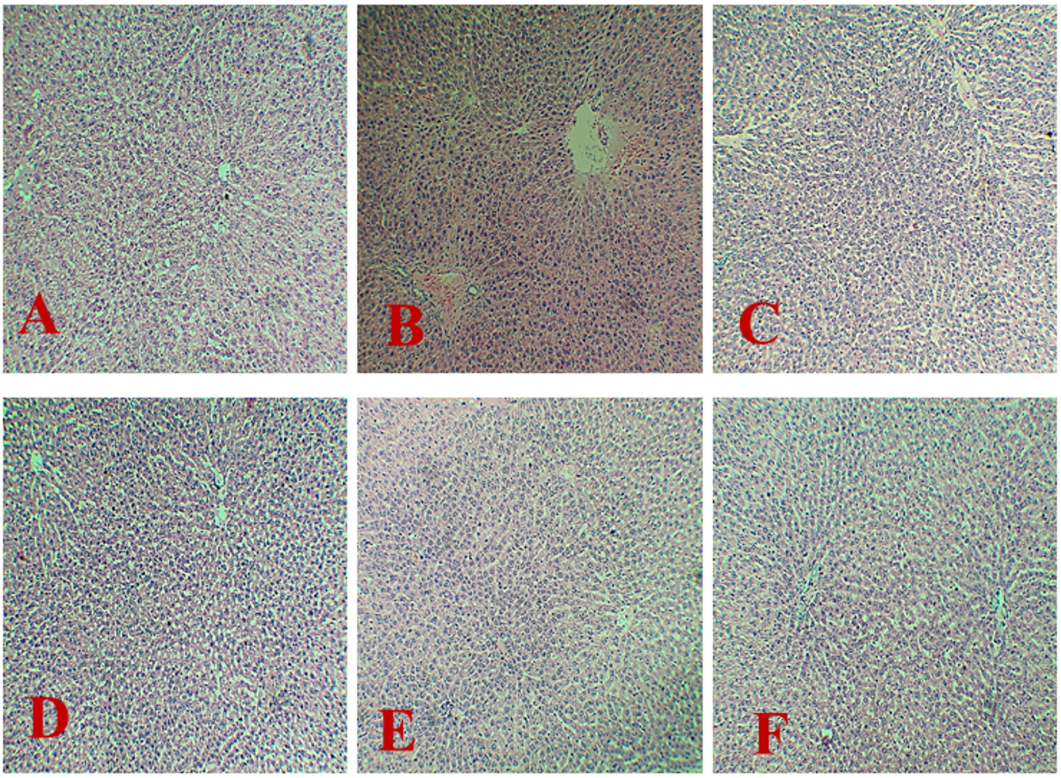

Figure 3: A-Histopathology of Wistar rats in normal control group (I); B-paracetamol treated group (II); C-silymarin treated standard group (III); D-*Moringa* extract (200 mg/kg) treated group (IV); E-AgNPs (200 mg/kg) treated group (V); F-AgNPs (100 mg/kg) treated group (VI).

distinct central vein (Figure 3F). No significant inflammatory cell infiltration was observed in this group.

DISCUSSION

This study demonstrated the hepatoprotective potential of green synthesized Silver Nanoparticles (AgNPs) derived from *Moringa oleifera* leaf extract. The ethanolic extract of *Moringa oleifera* AgNPs exhibited significant antioxidant and hepatoprotective effects against oxidative stress induced by paracetamol toxicity. The green synthesis method was rapid, reliable, eco-friendly, and cost-effective, representing a sustainable alternative to conventional chemical synthesis routes.

The ethyl acetate fraction demonstrated a higher concentration of phenolic and flavonoid compounds compared to the crude extract. This indicates that phenolic and flavonoid constituents are more soluble and concentrated in semi-polar solvents such as ethyl acetate. The enrichment of these phytoconstituents suggests that the ethyl acetate fraction may exhibit greater antioxidant and therapeutic potential.

The observed brown colour change during synthesis signifies the successful reduction of Silver Ions (Ag^+) to Metallic Silver (Ag^0). This colour transition confirms the occurrence of a redox reaction, wherein the phytochemical constituents present in the ethyl acetate fraction act as electron donors, undergoing oxidation while simultaneously reducing Ag^+ ions.

The UV-visible spectrum exhibited a strong absorbance peak at approximately 470 nm, confirming the formation of AgNPs through the excitation of surface plasmon resonance. The observed reddish-brown coloration is a visual indicator of nanoparticle formation and signifies the reduction of Ag^+ ions to Ag^0 by bioactive compounds present in the *M. oleifera* extract.

SEM analysis confirmed that the nanoparticles were spherical and well-dispersed, suggesting efficient capping and stabilization by phytochemicals. The EDX analysis verified the elemental composition of the synthesized AgNPs, with a distinct silver peak around 2.125 keV, confirming the successful reduction process.

FTIR analysis revealed the involvement of hydroxyl, amide, and aliphatic functional groups in nanoparticle synthesis and stabilization. The peaks corresponding to O-H and N-H stretching vibrations indicate the presence of polyphenols and proteins, which act as both reducing and capping agents. The C=O stretching at 1636.39 cm^{-1} suggests the contribution of protein amide linkages in the stabilization of nanoparticles. These functional groups are known to prevent aggregation and maintain nanoparticle stability. The results correlate well with previous studies by Moodley *et al*. (2018) and Abeer Mohammed *et al*. (2022) which reported similar functional group interactions during plant-mediated nanoparticle synthesis.

The XRD analysis confirmed the crystalline and nanostructured nature of the biosynthesized AgNPs. The presence of characteristic peaks at (111), (200), (220), and (311) planes correspond to the typical diffraction pattern of metallic silver with a face-centered cubic geometry. The absence of additional peaks indicates high purity and successful reduction of silver ions. The DLS analysis revealed a particle size distribution between 80 and 100 nm, which falls within the expected range for biologically synthesized AgNPs. This narrow distribution suggests effective stabilization by biomolecules present in the *M. oleifera* extract, preventing aggregation and ensuring colloidal stability.

The DPPH radical scavenging assay demonstrated that *Moringa*-based AgNPs possess significant antioxidant activity. The observed dose-dependent increase in scavenging percentage indicates that higher nanoparticle concentrations enhance free radical neutralization. This antioxidant potential can be attributed to the presence of phenolic and flavonoid compounds adsorbed on the nanoparticle surface during synthesis, which act as electron donors to reduce DPPH radicals.

The acute oral toxicity study (Table 5) revealed no mortality or observable behavioural signs of toxicity in rats treated with *Moringa oleifera*-mediated Silver Nanoparticles (MO-AgNPs) at a dose of 2000 mg/kg body weight during the 14-day observation period. Histopathological evaluation of major organs such as the heart, liver, and kidneys revealed no apparent structural or cellular damage when compared with the control group (Figures

Table 5: Acute oral toxicity results of MO-AgNPs.

Sl. No.	Identification parameters	Control	AgNPs
1.	Alertness	Normal	Normal
2.	Aggressiveness	NO	NO
3.	Tremors	NO	NO
4.	Convulsion	NO	NO
5.	Skin color	Normal	Normal
6.	Respiration	Normal	Normal
7.	Restlessness	NO	NO
8.	Pain response	Normal	Normal

2A, 2C, 2E), confirming the safety of the synthesized AgNPs at the tested dose (Figures 2B, 2D, 2F).

In the hepatoprotective study, paracetamol-induced hepatotoxicity was confirmed by elevated hepatic enzyme levels (SGPT, SGOT, ALP) and bilirubin, along with reduced total protein and albumin, signifying severe liver injury and impaired hepatic function. These findings align with the known mechanism of paracetamol toxicity, which involves the formation of N-acetyl-p-Benzoquinone Imine (NAPQI), depletion of Glutathione (GSH), and resultant oxidative stress and hepatocellular necrosis. Administration of MO-AgNPs, particularly at 100 mg/kg, effectively normalized liver enzyme levels and restored protein synthesis, demonstrating strong hepatoprotective efficacy comparable to or exceeding that of silymarin. The improved protection at a lower dose suggests an optimal dose-dependent response, possibly due to the high surface area and bioavailability of AgNPs facilitating enhanced antioxidant and anti-inflammatory activity.

Histopathological evidence corroborated these biochemical findings. The AgNPs-treated groups, especially at 100 µg/kg, exhibited regeneration of hepatocytes, restoration of hepatic cords, and absence of necrosis, confirming the structural recovery of liver tissue. These protective effects may be attributed to the antioxidant potential of phenolic and flavonoid compounds present in the *Moringa oleifera* extract, which act synergistically with AgNPs to scavenge free radicals, prevent lipid peroxidation, and restore redox balance.

The enhanced therapeutic efficacy of MO-AgNPs may be attributed to the synergistic interaction between the bioactive phytochemicals of *Moringa oleifera* and the nanoscale properties of silver. The increased surface area of AgNPs allows for improved bioavailability and cellular interaction, resulting in superior antioxidant and hepatoprotective responses compared to the crude extract.

CONCLUSION

In conclusion, the present study confirms that *Moringa oleifera*-mediated silver nanoparticles possess potent hepatoprotective and antioxidant activities with minimal toxicity. The green synthesis approach provides a simple, rapid, and economical method for nanoparticle production while enhancing biological efficacy. The hepatoprotective activity of MO-AgNPs highlights their therapeutic potential in mitigating oxidative liver damage. Further research is warranted to elucidate the molecular mechanisms underlying these protective effects and to explore the potential clinical applications of green-synthesized AgNPs in hepatoprotection and related pharmacotherapies.

ACKNOWLEDGEMENT

The author gratefully acknowledges the Pharmacy Department of Kakatiya University which is located in Warangal City, Telangana, India. We are thankful to all persons who have helped either directly or indirectly during this research work.

ABBREVIATIONS

AgNPs: Silver nanoparticles; **AgNO3:** Silver nitrate; **ALP:** Alkaline phosphatase; **ANOVA:** Analysis of variance; **BW:** Body weight; **DDI:** Double deionized; **DLS:** Dynamic Light Scattering; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **EDX:** Energy - Dispersive X-ray spectroscopy; **FCC:** Face-centered cubic; **FTIR:** Fourier Transform Infrared spectroscopy; **GAEs:** Gallic acid equivalents; **g/dL:** Grams per Deciliter; **GSH:** Glutathione; **IC_{50}:** Inhibitory concentration at 50%; **JCPDS:** Joint Committee on Powder Diffraction Standards; **mg/dL:** Milligrams per Deciliter; **MO:** *Moringa oleifera*; **NAPQI:** N-acetyl-p-benzoquinone imine; **QUEs:** Quercetin equivalents; **RPM:** Revolutions per minute; **SD:** Standard deviation; **SEM:** Scanning Electron Microscopy; **SGOT:** Serum Glutamate Oxaloacetate Transaminase; **SGPT:** Serum Glutamate Pyruvate Transaminase; **SPR:** Surface Plasmon Resonance; **U/L:** Units per Liter; **UV-vis:** Ultra violet visible spectroscopy; **XRD:** X-ray Diffraction.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed significantly to the conception and design of the study. Experimental work, data collection, data analysis and interpretation were performed by the first author. The manuscript was written by the first author and critically reviewed and approved by corresponding author prior to submission.

SUMMARY

This study demonstrates that *Moringa oleifera*-mediated silver nanoparticles exhibit strong hepatoprotective and antioxidant activities with low toxicity. The green synthesis method offers a simple, cost-effective, and efficient approach to nanoparticle production while improving biological effectiveness. These findings indicate the therapeutic potential of MO-AgNPs in protecting against oxidative liver damage, warranting further investigation into their molecular mechanisms and possible clinical applications.

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Cite this article: Sravanthi M, Shayeda. Hepatoprotective Effect and Antioxidant Activity of Silver Nanoparticles Green Synthesis from *Moringa oleifera*. Pharmacog Res. 2026;18(4):1284-93.