



Histopathological Evaluation of Liver Tissue Exposed to Biogenic Ag+ Nanoparticles Synthesized via *Rosa damascena* in Male Albino Rats

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Abstract

Recently, silver nanoparticles have been integrated as either a primary or auxiliary element in numerous effective drug formulations aimed at specific disorders. This research assessed the toxicity of silver nanoparticles (AgNPs) that were synthesized through the green method utilizing *Rosa damascena* extract. Histological alterations were identified through hematoxylin-eosin and silver differential staining, alongside serological assessments aimed at evaluating liver function. A range of concentrations (100, 200, 300, and 400 ppm/day over 30 days) was utilized to ascertain the optimal dosage and its effects, which were analyzed across five groups consisting of five rats each. UV-Vis spectroscopy indicated the presence of silver nanoparticles with crystalline sizes exceeding 30 nm. Serum tests for liver function indicated that the concentrations of 100 and 200 ppm were the most appropriate concerning permissible damage. In contrast, elevated levels prompted a pathological response marked by degeneration and hemorrhage in various regions of the liver tissue, a conclusion corroborated by serological evaluations.

The accumulation sites of silver particles were identified through specialized silver staining, which manifests as dark brown ink stains in proximity to hepatic sinusoids and areas of ion exchange between cells and blood vessels. The uniqueness of this research resides in the application of moderate concentrations (100 and 200 ppm) of silver nanoparticles over a duration of one month, levels that are comparatively safe for liver tissue when incorporated into a treatment regimen. This finding is uncommon in earlier studies, underscoring its promise for safe utilization in biomedical applications.

Keywords: AgNPs, silver staining, hepatocyte, *Rosa damascena*, therapeutic protocol, UV-Vis.

Introduction

The environmentally conscious synthesis of nanomaterials, notably silver nanoparticles (AgNPs), has surfaced as a novel and sustainable strategy within the domain of bio-nanotechnology, particularly for applications in biomedicine and therapy. Due to their distinctive physicochemical characteristics, silver nanoparticles demonstrate significant biological activities, such as antimicrobial, antioxidant, and anti-inflammatory properties. Additionally, their diminutive size and extensive surface area enhance cellular uptake and engagement with intracellular elements, thereby affecting cellular redox balance and numerous biochemical pathways (Gomes-Junior, et al., 2025).

Notwithstanding these benefits, an increasing body of evidence indicates that silver nanoparticles could induce toxic effects, particularly at elevated concentrations, thereby requiring comprehensive investigations at both the cellular and tissue levels. Consequently, it is imperative to evaluate their possible effects on critical organs, including the liver, through histopathological and biochemical assessments to guarantee their safe application (Sati, et al., 2026).

The environmentally friendly and sustainable approach to nanobiotechnology, particularly through the green synthesis of nanomaterials like silver nanoparticles (AgNPs), has garnered significant interest. This technique employs biological resources, including plant extracts, microorganisms, and natural compounds, as agents for reduction and stabilization, effectively reducing the reliance on hazardous chemicals typically linked to traditional synthesis methods. Among the diverse range of nanomaterials, AgNPs stand out as highly promising candidates for biomedical and therapeutic applications, attributed to their distinctive physicochemical properties (Shahzadi, et al., 2025).

Silver nanoparticles demonstrate a diverse array of biological functions, encompassing antimicrobial, antioxidant, and anti-inflammatory properties. Their nanoscale dimensions and elevated surface area allow for effective engagement with cellular membranes and intracellular elements, thereby promoting cellular absorption and affecting redox balance along with various biochemical pathways. Nonetheless, in light of their advantageous characteristics, mounting evidence suggests that AgNPs may induce toxic effects contingent upon factors such as size, concentration, and exposure duration, highlighting the need for thorough assessment of their biosafety (Nair, et al., 2026).

Recently, there has been an increasing interest in the plant-mediated synthesis of silver nanoparticles (AgNPs), attributed to the presence of bioactive phytochemicals that may enhance the stability of nanoparticles and potentially mitigate toxicity. The medicinal plant *Rosa damascena*, known as Damask rose, is abundant in phenolic compounds, flavonoids, and essential oils, all of which exhibit potent antioxidant and anti-inflammatory properties. These phytoconstituents render it a highly suitable candidate for the environmentally friendly synthesis of biogenic nanoparticles characterized by improved biological compatibility (Sati, et al., 2025).

While green-synthesized nanoparticles offer numerous benefits, their interactions with biological systems—particularly at the tissue level—constitute a significant field of study. The liver, as the primary organ tasked with detoxification and metabolism, is particularly vulnerable to the accumulation of nanoparticles and the potential harm they may cause. Consequently, evaluating the hepatotoxic effects of biogenic AgNPs through histopathological analysis yields important insights into their safety profile (Sati, et al., 2025). Thus, the current study seeks to explore the histopathological impacts of biogenic silver

nanoparticles synthesized from *Rosa damascena* extract on liver tissue in male albino rats, aiming to assess their potential hepatotoxicity and aid in the safe advancement of nanomaterials for biomedical purposes..

Experimental procedure

Rosa damascena Sample Collection:

Rosa damascena petals were purchased from a local market in Najaf, Iraq. The botanical identification of the samples was confirmed by Prof. Dr. Ahmed Abees, Professor of Plant Taxonomy, Department of Biology, University of Kufa. The authentication record was documented for reference.

Preparation of Plant Extract

A measured 4 gm of the powdered petals mixed with distilled water and heated under controlled conditions to extract bioactive compounds. The mixture was filtered, and the filtrate was used as the plant extract for nanoparticle synthesis.

Nanomaterial Synthesis

The plant extract served as a reducing and stabilizing agent and was mixed with the corresponding precursor salt solution under controlled reaction conditions. The resulting nanoparticles were collected, purified, and prepared for further characterization.

Synthesize nanoparticles (AgNPs)

4 grams of *Rosa damascena* extract was stirred with heating at 45°C in 400 ml of deionized water for 15 minutes. 1.69 grams of silver nitrate (1mM) dissolved in 1000 ml of deionized water. After filtering the plant extract using filter paper. The nitrate solution was mixed with plant extract by dropper to prepare: 100, 200, 300, and 400 ppm concentrations. The mixture was carried out at 60°C for 30 minutes, until the color of the mixture was changed (H AL-SAIDI, 2025).

UV-Vis Spectrophotometer was used to determine the energy bandgap for each concentration before being administered orally ((Lasmi, *et al.*, 2025). A SCINCO Mega-2100 UV-Vis spectrophotometer was used to study the spectral properties and confirm the formation of silver nanoparticles in solution. The results showed a characteristic absorption band within the wavelength range of 400–450 nm, attributed to the surface plasmon resonance (SPR) phenomenon of the silver nanoparticles. The instrument employs a double-beam system, providing high accuracy and stability in absorbance measurements, making it suitable for spectroscopic analyses in chemical and research applications.

Animals

White *Rattus rattus* (30 male) weighing 180-220 g, were classified into 5 groups. Nanoparticles were administered orally via a feeding tube once daily for 30 days (100, 200, 300, and 400 ppm)

Histological Preparation and Staining

After being collected, liver samples were cleaned with regular saline and preserved for 24 to 48 hours in 10% neutral buffered formalin. After that, the tissues were cleaned in xylene, dehydrated in graded ethanol, and embedded in paraffin wax. Using a microtome, sections with a thickness of 4 to 5 µm were cut and placed on glass slides. The slices were deparaffinized, rehydrated, and stained with hematoxylin for nuclear coloring and eosin for cytoplasmic contrast to perform Hematoxylin and Eosin (H&E) staining. Slides were cleared and dehydrated, then mounted under a light microscope to check for histopathological changes such as inflammation, congestion, degeneration, and necrosis (Hadi, *et al.*, 2025).

For histological staining, a silver staining solution was prepared using the following components: silver nitrate (AgNO₃) as the primary silver source, a reducing agent (commonly formalin or hydroquinone) to

facilitate the reduction of silver ions to metallic silver, and a stabilizing buffer solution to maintain appropriate pH and ionic strength. The prepared solution was used to treat fixed liver tissue sections, enabling selective deposition of metallic silver at specific cellular and extracellular sites. This resulted in the visualization of tissue structures as brown spots under light microscopy, highlighting hepatocyte morphology and sinusoidal architecture for further analysis of nanoparticle-induced effects.

Results and Discussion

UV-Vis Characterization of AgNPs

Synthesis of silver nanoparticles by the reduction of an aqueous silver nitrate solution into AgNPs by consuming different concentrations of plant seed of *Coffea arabica* extract, as 100, 200, 300, and 400 mg/ml. Conversion of (Ag^+) to AgNPs was confirmed using UV-Vis spectroscopy as shown in Figure 1. UV-Vis spectrum provides information about the shape, size, and validation of AgNPs, depending on quantum confinement and Surface Plasmon Resonance (SPR) (Paramelle, *et al.*, 2014). The localized surface plasmon resonance (LSPR) of the UV-Vis spectrum is a sensitive fingerprint of metallic Ag presence and encodes particle size, shape, and its environment. The relationship between LSPR frequency and the interparticle distance, particularly in relation to particle size, becomes potentially useful for measuring nanoscale distances and its application in biological systems (Haes, A. J., & Van Duyne, R. P., 2004).

From Figure 1, we can get the energy bandgap by using Tauc plot as listed in Table 1, where it was decreased with increasing concentration compared to the negative control. The decrease in the energy bandgap indicates an increase in AgNPs size (Saadoun, *et al.*, 2024). The widening UV-Vis spectrum indicates the formation of AgNPs and also that the particles are poly-dispersed (Staszak, *et al.*, 2022).

Table 1: Energy bandgap of AgNPs with *Coffea arabica* seeds extract at different concentrations. (A) control negative, (B) 100 ppm, (C) 200 ppm, (D) 300 ppm, and (E) 400 ppm.

Sample	energy bandgap (eV)
Negative control	3.00
100 ppm	1.90
200 ppm	1.67
300 ppm	1.44
400 ppm	1.47

The spectrum was corrected using a blank in which a cuvette was filled with the extract. The widening UV-Vis spectrum indicates the formation of AgNPs and also that the particles are poly-dispersed (Staszak, *et al.*, 2022).

The green-method, which synthesized silver nanoparticles using Rosa as a catalyst, revealed a characteristic surface plasmon resonance (SPR) beam in the UV-Vis absorption spectrum of the synthesized silver nanoparticles. This confirmed the reduction of silver ions (Ag^+) and the formation of colloidal silver nanoparticles. The position and amplitude of the SPR peak determine the size of the nanoparticles, as has been widely reported in recent scientific literature. Recent studies have indicated that

smaller silver nanoparticles (less than 20–30 nm) exhibit SPR position and a direct signal in the 390–420 nm range, while increasing particle size leads to a redshift of the SPR peak towards longer wavelengths (greater than 430 nm), followed by a marked amplitude due to increased scattering and multi-size variation (Khan *et al.*, 2023; Iqbal *et al.*, 2022).

The redshift and broadening of the surface plasmon resonance beam, as observed in this study, indicate that the synthesized nanoparticles fall within a larger size range, approximately 30 nm. This is consistent with the size-dependent plasmon behavior reported in recent experimental and theoretical studies (Lakshmi *et al.*, 2024).

This trend has also been confirmed in green synthesis systems, where biomolecules act as moderate reducing and stabilizing agents, often resulting in silver nanoparticles with medium to large diameters due to the slow nucleosynthesis rates (Phu, et al., 2010). Therefore, the UV-Vis spectrum obtained in this study provides strong optical evidence that the produced silver nanoparticles are larger than 30 nm.

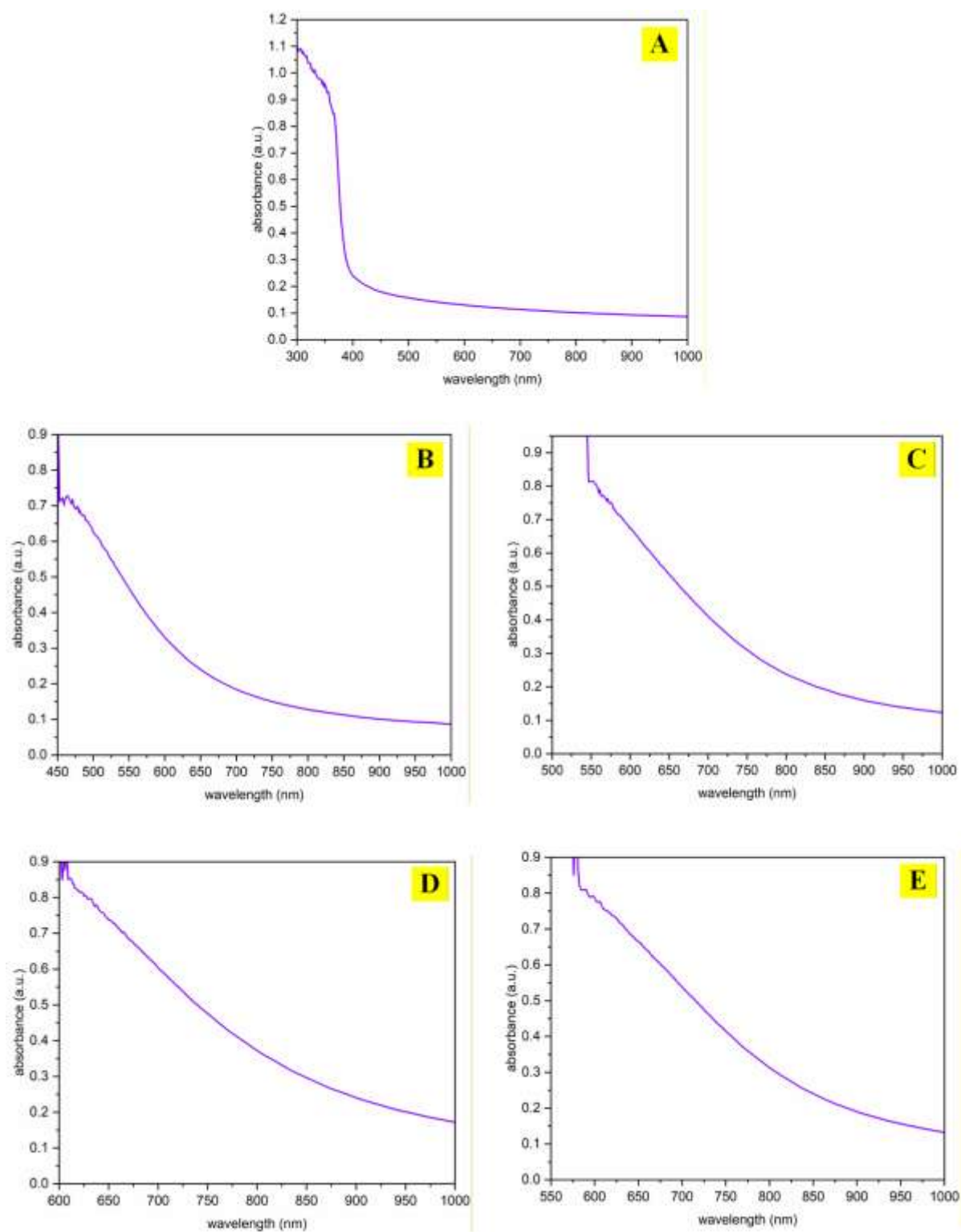


Figure 1: absorbance spectra of AgNPs with *Coffea arabica* seeds extract at different concentrations. (A) control negative, (B) 100 ppm, (C) 200 ppm, (D) 300 ppm, and (E) 400 ppm.

Histological study

Compared to the treated groups, the control group showed marked changes in the histopathological analysis of liver sections (Figure 3) from mice exposed to bioavailable silver nanoparticles. Hepatocytes and major veins were in a normal position in the livers of the control group, indicating normal liver structure. In contrast, the treated groups exhibited varying degrees of hepatocyte degeneration, characterized by the formation of cytoplasmic vacuoles and loss of typical cell boundaries. An increase in nucleus size was indicative of the onset of cellular degeneration.

Scattered hemorrhagic areas were observed within the hepatic tissue, along with marked vascular congestion in both the central and portal veins. Dilatation of the hepatic sinusoids was also evident, accompanied by moderate infiltration of inflammatory cells in certain sections. Increased nanoparticle concentrations or prolonged exposure durations led to more pronounced histopathological alterations, suggesting a dose-dependent hepatotoxic effect.

Although they are derived through biological synthesis, green-produced silver nanoparticles demonstrated toxic effects on hepatic tissue, as indicated by cellular degeneration, vascular engorgement, and hemorrhagic manifestations. The generation of oxidative stress and reactive oxygen species can trigger hepatocyte damage by promoting lipid peroxidation and compromising the integrity of cell membranes. Additionally, damage to the sinusoidal lining and increased vascular permeability likely contribute to the observed congestion and hemorrhage, ultimately disrupting normal blood circulation and facilitating the escape of erythrocytes into adjacent tissues (Almarui & Elnaggar, 2025).

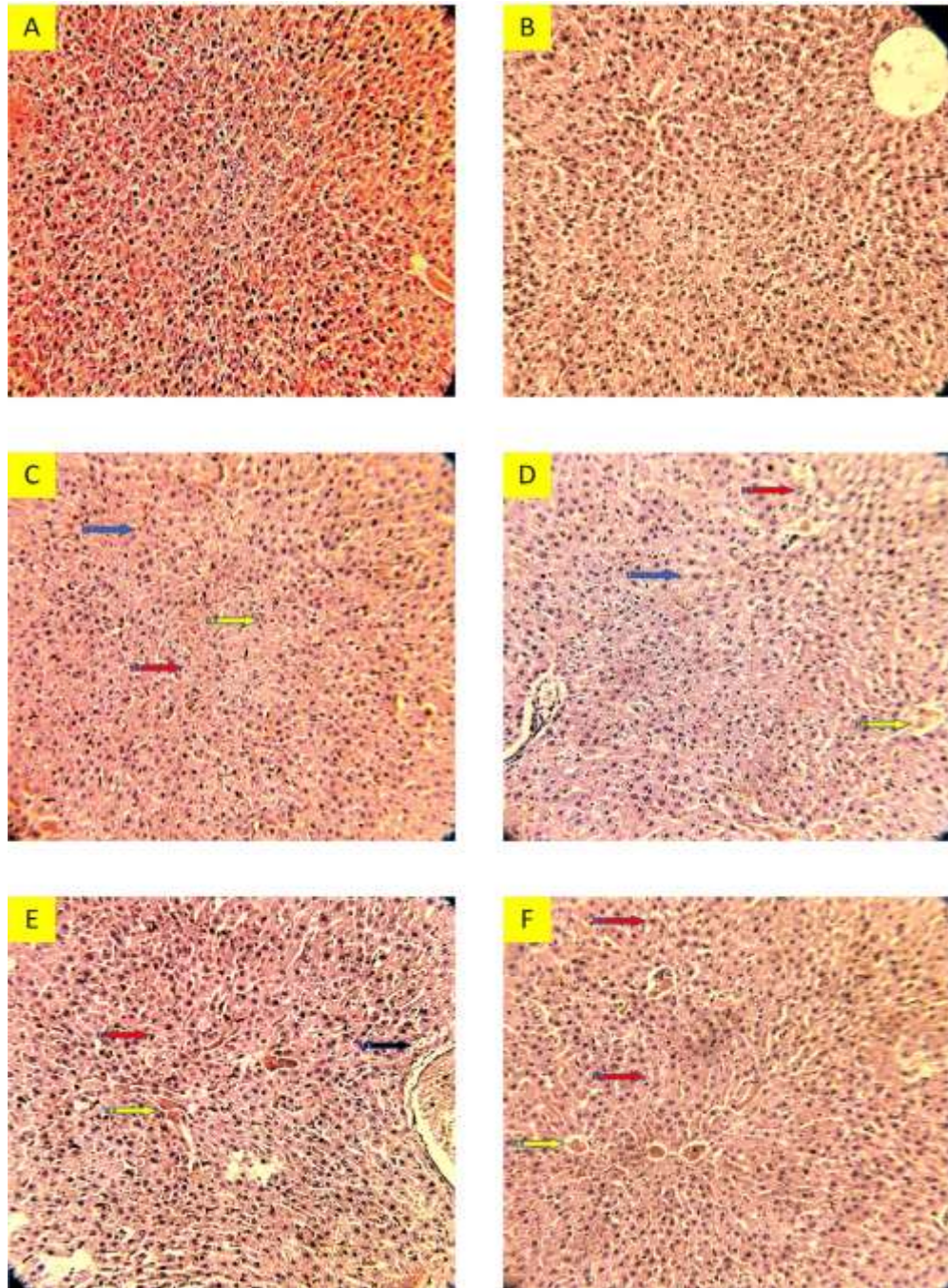


Figure 3: Hematoxylin and Eosin-stained liver sections(A) control group, (B) 100 ppm, (C) 200 ppm, (D) 300 ppm, and (E) 400 ppm. (red arrow) Subcapsular hemorrhage with severe marked expansion and congested sinusoids, (blue arrow) hepatocytes show ballooning degeneration with pyknotic apoptotic nuclei, (black arrow) the portal area shows dilated portal vein (PV) and congestion central vein, (yellow arrow) degeneration with necrosis of hepatocytes.

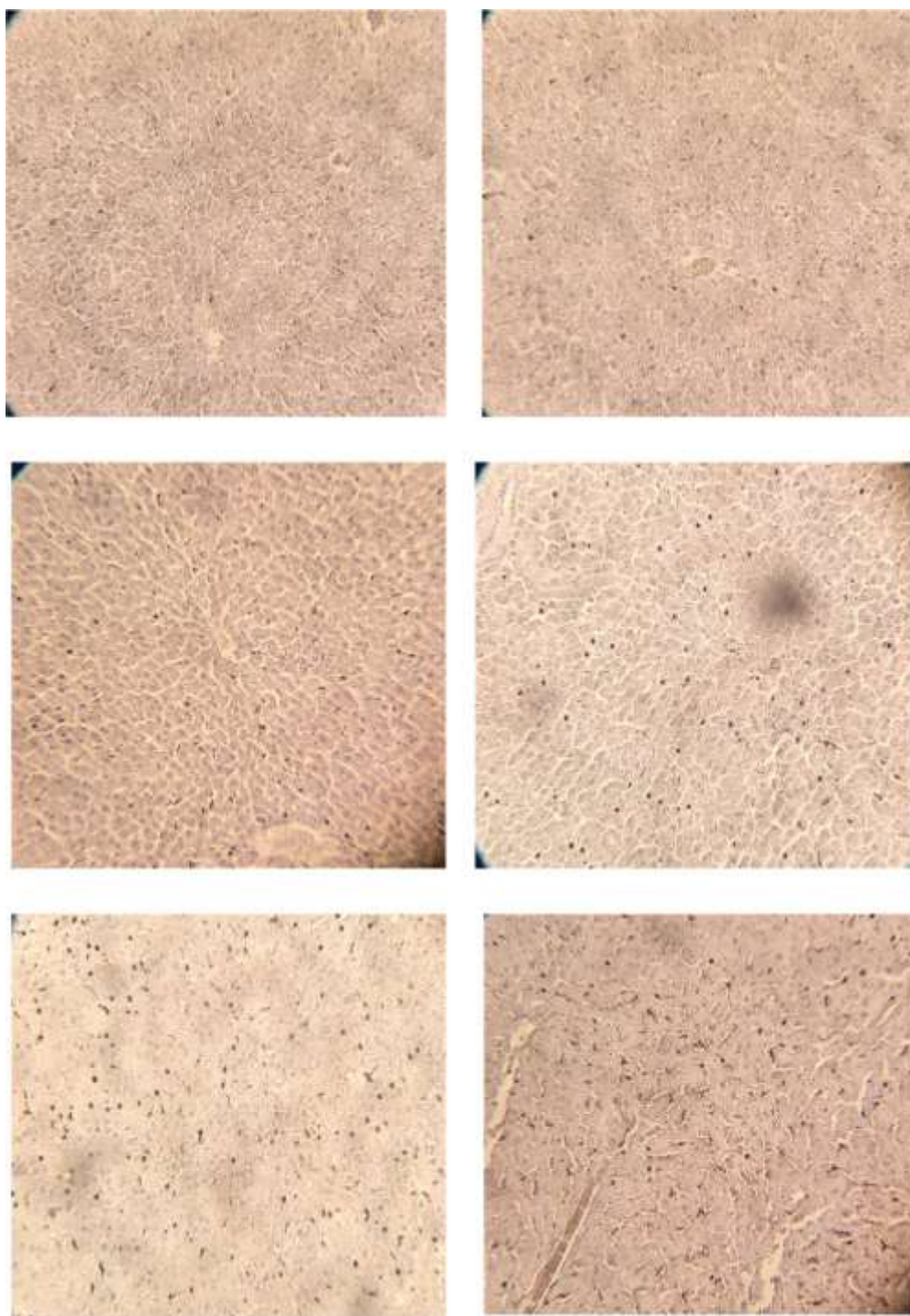


Figure 3: Silver-stained liver sections (A) control group, (B) 100 ppm, (C) 200 ppm, (D) 300 ppm, and (E) 400 ppm. Silver stain appears as dark deposits within hepatic sinusoids and hepatocytes, with additional appearance of scattered hemorrhagic spots.

These findings are consistent with previous studies indicating that silver nanoparticles can induce structural alterations and disrupt hepatic microcirculation, even when synthesized via plant-based methods. However, the comparatively mild degree of tissue damage observed in this study suggests that the phytochemical constituents of the Rosa extract may have exerted a protective effect by neutralizing reactive oxygen species.

Although the observed histological alterations in the liver—such as cellular degeneration, vascular congestion, and mild to moderate hemorrhage—suggest a degree of hepatotoxicity, they appear to fall within a tolerable range. The relatively limited severity of these changes implies that the liver may retain the ability to recover or adapt once exposure is discontinued, as the tissue damage was not indicative of irreversible hepatic failure

Accordingly, when balanced against their potential therapeutic advantages, the hepatic effects noted in this study could be considered acceptable, particularly if such biogenically synthesized silver nanoparticles are intended for use in managing severe or treatment-resistant conditions. Nevertheless, further investigations are essential to ensure safe biomedical application, with emphasis on optimizing dosage, exploring the role of antioxidant co-therapy, and assessing the capacity for tissue recovery without compromising liver function

Even though the liver tissue showed histological changes such as degeneration, congestion, and moderate bleeding, these changes were still within a manageable and reversible range. There was no indication of severe necrosis or irreparable damage, and the liver structure mostly remained intact. This implies that after being exposed to the biogenic silver nanoparticles, the organism can adapt and recover physiologically(Salah, *et al.*, 2025).

The differential silver staining results shown in Figure 4 indicate a clear accumulation of silver crystals within the liver tissue. These deposits appear as dark spots ranging in color from brown to dark brown, concentrated primarily within hepatic sinusoids and hepatocytes. This deposition pattern reflects the liver's ability to accumulate and concentrate silver following increased exposure. Furthermore, this accumulation may be associated with cellular pathological changes. This is attributed to the liver's pivotal role in detoxification, making it particularly susceptible to heavy metal accumulation, which may contribute to structural and functional disturbances in the liver with increasing dose levels (Grizzle, 1996).

Interestingly, the experimental animals showed a significant level of tolerance and survival even at the 400 concentrations, which is regarded as hazardous or high-dose. The results show that the hepatic tissue could live with the nanoparticles without suffering a catastrophic loss of function, even though the hepatocellular stress was created at this level.

Thus, the reported level of hepatotoxicity may be considered clinically manageable in the context of therapeutic uses for resistant or severe illnesses, particularly if the dose is closely monitored and backed by antioxidant or protective medicines. These findings lend support to the possible therapeutic application of green-synthesised nanoparticles, so long as their concentration and exposure time are carefully calibrated to strike a compromise between safety and effectiveness.

In line with the normal toxicological profile of silver nanoparticles (AgNPs) that has been documented in previous studies Lakkim, *et al.*, (2023) reported similar hepatic changes, showing that AgNPs can cause oxidative stress, which results in lipid peroxidation, mitochondrial dysfunction, and hepatocellular membrane disruption.

The buildup of nanoparticles within hepatocytes and Kupffer cells, which causes reactive oxygen species (ROS) formation and compromises antioxidant defense mechanisms, is probably the cause of the cellular deterioration observed in our work. The loss of normal hepatocyte architecture and cytoplasmic vacuolization are caused by this oxidative imbalance figure 4 (Khan, *et al.*, 2025).

Several studies indicate that the phytochemical constituents of *Rosa damascene* notably phenolic acids (e.g., gallic acid), flavonoids (e.g., quercetin), and related antioxidant compounds—exert significant effects on hepatic cells and liver health, primarily through modulation of oxidative stress and inflammation. These compounds are known for their free-radical scavenging and antioxidant activities, which help protect hepatocytes from oxidative injury by reducing reactive oxygen species and lipid peroxidation, thereby maintaining cellular integrity and function (Chroho, *et al.*, 2022).

In experimental models of induced liver injury (e.g., cadmium chloride exposure), administration of *Rosa damascena* extracts was shown to increase endogenous antioxidant enzyme levels such as glutathione, superoxide dismutase (SOD), and catalase, while decreasing markers of oxidative damage and inflammation including malondialdehyde (MDA), ALT, AST, TNF- α , and IL-6. This suggests a hepatoprotective effect, where plant constituents mitigate oxidative stress-mediated cellular damage and inflammatory responses in liver tissue (Nourozi, *et al.*, (2024).

Furthermore, in models of nonalcoholic fatty liver disease (NAFLD), *Rosa damascena* supplementation was associated with improvements in liver function biomarkers and reductions in hepatic fat accumulation and enzyme elevations, likely linked to its antioxidant and anti-inflammatory phytochemicals. The phenolic components identified (such as gallic acid, quercetin, and syringic acid) are specifically implicated in enhancing total antioxidant capacity, reducing lipid peroxidation, and improving histological liver architecture, which supports their protective role at the cellular level in the liver (Rostami, *et al.*, 2023).

Overall, the literature suggests that the phenolic and flavonoid constituents of *Rosa damascena* contribute to reduced oxidative stress, enhanced antioxidant defenses, and attenuation of inflammatory damage in hepatocytes, which may underlie observed hepatoprotective effects in both clinical and animal studies. Damask rose (*Rosa damascena*) is a valuable source of bioactive phytochemicals that facilitate the green synthesis of nanoparticles. Its phenolic compounds function as effective reducing agents, converting metal ions into stable nanoparticles, while flavonoids provide antioxidant activity and contribute to particle stabilization by preventing aggregation (Farhadi-Azar, *et al.*, 2023).

Additionally, terpenes, volatile oils, organic acids, and sugars act synergistically as natural capping and stabilizing agents, enhancing the physicochemical properties and uniformity of the synthesized nanoparticles. This multifaceted composition allows Damask rose extract to mediate both reduction and stabilization in a single, eco-friendly step, producing nanoparticles with controlled size, improved dispersity, and enhanced biological activity. Such properties underscore the potential of Damask rose as a sustainable and efficient plant-based platform for biomedical and nanotechnological applications.

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