GMR
The Original

BIOCHEMICAL AND MECHANISTIC INVESTIGATION OF DEBREGEASIA SAENEB MEDIATED TIO_2 NANOPARTICLES IN OXIDATIVE STRESS INDUCED LIVER INJURY

Zaib-un-Nisa[1], Syed Mubashar Sabir[1], Saima shafique[2], Zubaida Umar Zehri[3], Ali Jan[3], Izhar Ullah[4*], Qurrat ul Aain[5]

[1]Department of Chemistry, University of Poonch, Rawalakot, Azad Kashmir, Pakistan
[2]Department of Plant breeding and molecular genetics, University of Poonch, Rawalakot, Azad Kashmir, Pakistan
[3]Department of Pharmacology and Therapeutics, Basic Medical Science Institute JPMC Karachi, University of Karachi, Sindh Pakistan
[4]Department of Pharmacy, University of Poonch Rawalakot, Azad Kashmi, Pakistan
[5]Department of physics, University of Gujrat ,Punjab, Pakistan
*Corresponding Author: Dr. Izhar Ullah, Email: izharpharma@upr.edu.pk

Abstract
The worldwide increase in hepatic diseases and the side effects associated with many existing treatments has emphasized the importance for safer and more effective therapeutic alternatives. Although currently available treatments such as N-acetylcysteine, Silymarin, and Ursodeoxycholic acid are routinely used for liver disorders, poor patient tolerance and associated side effects have encouraged increasing interest in natural plant based therapies. However, the therapeutic use of traditional herbal extracts is often limited by poor stability, low bioavailability, and lack of dose standardization. Current study is the first to report green synthesis and hepatoprotective evaluation of TiO_2 nanoparticles mediated by Debregeasia saeneb. The present study investigated the hepatoprotective potential of green synthesized TiO_2 nanoparticles prepared from Debregeasia salicifolia (D. saeneb) leaf extract collected from Azad Jammu Kashmir, a medicinal plant known for its rich antioxidant phytochemicals and traditional therapeutic value against oxidative stress-related disorders.The novelty of this work lies in the development of a safer and eco-friendly nanoparticle system designed to improve the stability, delivery, and biological activity of plant-derived compounds for liver protection. Successful nanoparticle synthesis was confirmed through UV–Vis, FTIR, DLS, zeta potential, and SEM analyses, which demonstrated stable nano sized particles with granular morphology. In APAP intoxicated mice, TiO_2 nanoparticles significantly improved liver biomarkers, restored antioxidant defenses, reduced lipid peroxidation, and improved hepatic histology with minimal necrosis. These findings suggest that plant-mediated TiO_2 nanoconjugates may serve as promising therapeutic candidates for the management of oxidative stress associated liver disorders.

KEYWORDS: Debreagasia seneb mediated TiO_2, Nanoparticals, Oxidative stress, Hepatoprotective Potential, SEM

INTRODUCTION

Liver diseases continue to be a major public health challenge worldwide and are responsible for significant morbidity and mortality. Oxidative stress is considered one of the major mechanisms involved in the initiation and progression of hepatic injury, particularly in drug induced liver toxicity. Excessive generation of reactive oxygen species (ROS) disrupts cellular antioxidant balance, leading to lipid peroxidation, mitochondrial dysfunction, inflammation, and hepatocellular necrosis (Rotundo & Pyrsopoulos, 2020). Among hepatotoxic agents, paracetamol overdose is widely used as an experimental model of liver injury because its toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI), causes glutathione depletion and oxidative hepatic damage (Yoon et al., 2016). Although conventional therapies such as N-acetylcysteine, Silymarin, and Ursodeoxycholic acid are available for liver disorders, their long term use may be associated with adverse effects, limited tolerance, and inconsistent therapeutic outcomes. These limitations have increased scientific interest in safer plant based therapeutics with antioxidant and hepatoprotective potential.

For centuries, healers across different cultures have turned to medicinal plants for managing liver problems and inflammation. Debregeasia saeneb is, commonly referred to as D. saeneb, is a plant species found in the hilly regions of Pakistan and other areas within South Asia, and has been used in folk medicine for its anti inflammatory properties, which help alleviate various inflammatory conditions associated with oxidative stress and swelling. The leaves and other parts of the plant are rich in natural compounds including phenolics, flavonoids, and tannins, all known for their antioxidant properties. Previous studies have already demonstrated that plants rich in phenolics can protect the liver

by scavenging free radicals and restoring the natural antioxidant defense mechanisms of the body (Fakurazi et al., 2012).

Nanotechnology has created new possibilities in the past few years for better assimilation of herbal medicines. Raw plant extracts often tend to lose their efficacy due to their instability, low bioavailability and poor dose control in practice. Nanoparticles prepared by "green" synthesis provide a more reliable and cleaner alternative due to its ability to preserve the stability of phytochemicals, delivering them to the target area and enhancing their biological activity. In the green method, the plant's own compounds can be used to reduce and stabilise the particles, eliminating the need to use the harsh chemicals that are usually used for standard preparation of nanoparticles (Iravani, 2011). The use of plants to synthesize the nanoparticles of titanium dioxide has garnered significant attention due to their stability, biocompatibility, and potential application in the field of medicine. Previously, it had been seen that plant phytochemicals alone have weaker antioxidant and healing properties as compared to TiO_2 nanoparticles (Sundrarajan et al., 2015).

Among the plants explored for making nanoparticles, Moringa oleifera has caught the eye of many researchers because of its strong antioxidant make up and proven ability to safeguard the liver. The antioxidant enzyme activity was found to significantly recover and the level of liver injuries due to oxidative stress was significantly reduced in animals following the preparation of TiO_2 nanoparticles from Moringa (Fakurazi et al., 2012) Compared with Moringa, D. saeneb remains relatively underexplored despite its rich phytochemical composition and traditional medicinal use. This highlights the need to investigate whether D. saeneb mediated nanoparticles may exhibit comparable or superior hepatoprotective effects.

Therefore, the present study was designed to investigate the molecular and mechanistic role of green synthesized TiO_2 nanoparticles prepared from the state of Azad Jammu and Kashmir, Pakistani D.saeneb leaf extract in paracetamol induced hepatotoxicity. The novelty of this work lies in the development of an eco friendly nanoparticle system utilizing D. saeneb phytochemicals and the evaluation of its antioxidant, biochemical, and histopathological protective mechanisms against oxidative liver injury.

To the best of current knowledge, limited information is available regarding the hepatoprotective efficacy of D. saeneb mediated TiO_2 nanoparticles. The study therefore attempts to bridge traditional ethnomedicine with modern nanotechnology and may contribute toward the future development of safer plant based nanomedicines for oxidative stress associated liver diseases.

MATERIALS AND METHODS:

Collection of Plant

The leaves of Debregeasia saeneb were collected from Azad Jmmu Kashmir and its identification was done in Department of Botany, University of Poonch Rawalakot.

Experimentation

Sample Preparation

The fresh leaves of Debregeasia saeneb were washed with water, dried in shade, and then ground into powder. Maceration was used to carry out extraction. For three days, the powdered substance was steeped in distilled water while being frequently shaken and stirred. Three days later, the mixture was filtered through Whatman No. 1 filter paper after first passing through muslin cloth. A rotary evaporator was used to concentrate and dry the collected filtrate while the residual residue was once more soaked in water. This procedure was carried out three times. A rotary evaporator was then used to transform the filtrates into a semi-solid state at 40°C under low pressure (Abubakar & Haque, 2020). The following formula was used to determine the plant extract's % yield:

Percentage Yield = Extract weight/Powder Weight X100

Synthesis of TiO2 NPs

TTIP (10 ml) was added to 70 ml of distilled water to prepare 80 ml of titanium tetra-isopropoxide solution. This solution was then mixed with 30 ml of already prepared aqueous extract of Debregeasia saeneb. The mixture was stirred using a magnetic stirrer for 3 hours at 50°C until the color changed from light brown to dark brown, indicating the formation of nanoparticles.

The prepared nanoparticles were washed with excessive distilled water in reaction flask, and each time the liquid was decanted . The mixture was then filtered to get TiO2 nanoparticles, which were dried at 100°C for 24 hours. Finally, calcination was done in a muffle furnace at 400°C for 3 hours and the final TiO_2 nanoparticles were obtained. (Hussain et al., 2024).

Characterization

The prepared TiO_2 nanoparticles were analyzed using different characterization techniques, including a Zetasizer (ZS 90, Malvern Instruments, UK), SEM (JSM-6940-A, Tokyo, Japan), and FTIR (Shimadzu). FTIR analysis was

carried out to identify the functional groups present in the nanoparticles. Particle size, polydispersity index (PDI), and hydrodynamic diameter were determined by DLS using a Nano ZS90 Zetasizer following the method of Reddy et al.(2003). For analysis, the TiO_2 nanoparticle dispersion was diluted in 5 mL of double distilled water.

In vivo hepatoprotective activity:

In vivo hepatoprotective study was followed here with slight modifications (Zakaria et al., 2019).

Model animals grouping and dosage

All animal experiments performed in this study were conducted with great care to ensure the welfare and ethical treatment of the animals, following internationally recognized guidelines for laboratory animal research. The study protocol was reviewed and approved by the Ethical Committee of University of Poonch Rawalakot (No: UPR/AEC/16/2025) before the experimental work was initiated.

Male albino mice weighing 25–35 g and aged approximately 2.5 months were procured from the breeding facility of Centre of Excellence in Molecular Biology for the in vivo experiments. The animals were housed in separate cages under controlled environmental conditions with unrestricted access to food and water.

Adult mice were randomly divided into three groups (n = 5). Before the experiment, all animals were fasted overnight with free access to water. Group 1 (Normal Control) was given normal saline. Group 2 (Hepatotoxic Control) was administered paracetamol (60 mg/kg). Group3 (Test Group) received TiO_2 nanoparticles, which were prepared as a colloidal solution in distilled water and administered orally at a dose of 100 mg/kg (Zhou et al., 2024).

Biochemical Analysis of Blood and Liver Tissues:

On the 10th and last day of the trial, the animals were humanly sacrificed and blood was drawn directly by puncturing the heart for measurement of ALT and AST levels. The livers were then removed, were washed out with cold saline and weighed before further proceeding. Liver was cut into two parts, fixed in 10% formalin for histological analysis and the second part was ground up for biochemical testing. The clear liquid after centrifugation was saved for the assays, while the remaining portion was stored at −80°C foruse in future .

ALT, AST level Evaluation

Serum ALT and AST activities were measured using AMD diagnostic kits following the standardized protocol recommended by the International Federation of Clinical Chemistry (IFCC) (Clin Chem Lab Med, 2002; 40(7): 718–724) and the method described by Ozer et al. (2008). All analyses were performed according to the manufacturer's instructions under controlled laboratory conditions.

Liver Tissue TBARS Assessment

TBARS production was determined using a slightly modified protocol based on the method described by Sabir et al.(2017). This procedure was applied for the in vitro evaluation of lipid peroxidation during the study.

Reduced glutathione assay:

For the assay, 200 µL of liver tissue homogenate was mixed with 300 µL of 3% sulfosalicylic acid and centrifuged at 1500 rpm for 10 minutes. Following centrifugation, 200 µL of the collected supernatant was combined with 50 µL of DTNB and 980 µL of phosphate buffer. The absorbance of the reaction mixture was then measured at 412 nm. The procedure was carried out according to the method described by Salbitani et al. (2017).

Catalase activity assay:

For catalase activity analysis, 65 µL of H_2O_2 was added to 10 mL of phosphate buffer to prepare the reaction mixture. Subsequently, 980 µL of this solution was mixed with 20 µL of tissue homogenate, and the absorbance was measured at 240 nm(Aebi, 1984).

Histopathological Evaluation

Following completion of the experiment, the mice were humanely sacrificed and the abdominal cavity was carefully opened through a midline incision. Liver tissues were excised and fixed in 10% neutral buffered formalin for 24–48 hours. The preserved liver tissues were then dehydrated through graded ethanol , cleared with xylene, and were fixed in molten paraffin wax. Paraffin blocks were cut into 4–5 µm thick slices with a microtome and were stained with hematoxylin and eosin (H&E). The sections were dehydrated after staining, , cleared, mounted with DPX, and examined under a light microscope to detect hepatic architecture and pathological changes according to the method reported by Mamun et al.(2015).

RESULTS

Extraction Yield

A total of 6.7 g of dried extract was obtained from 120 g of powdered Debregeasia saeneb leaves, giving a percentage yield of 6.7%. The amount of extract recovered may vary depending on the polarity of the solvent and the extraction procedure, as both factors influence the extraction efficiency of phytochemical compounds. This yield is similar to those reported for comparable aqueous extractions of medicinal plants from polyphenol-rich species (e.g., 5-8%; Sabir et al., 2017)

Nanoparticles synthesis and chrachterization

During the synthesis process, the reaction mixture showed a visible color change, which indicated the formation of nanoparticles. After calcination, the nanoparticles appeared off-white in color.The nanoparticles were formed through the bioreduction of Ti^{4+} ions mediated by the polyphenolic and flavonoid compounds present in the plant extract (Narayanan et al., 2021).

FTIR Spectrum

FTIR reading of Debregeasia saeneb indicated the presence of several common functional groups associated with the active plant compounds. The broad band between 3200 and 3400 cm^{-1} could be attributed to the O–H stretching, suggesting phenolics and flavonoids. The highest peak around 2920 cm^{-1} is probably caused by aliphatic C–H stretching. Signals in the 1600–1650 cm^{-1} region are typically associated with C=C bonds of aromatic structures or carbonyl (C=O) groups, which are frequently found in polyphenolic molecules. Additionally, the 1000–1200 cm^{-1} bands indicate C–O stretching, typical of alcohols, ethers, or sugar based structures. The combination of these groups indicates that the extract contains a diverse array of phytochemicals that could have antioxidant and healing properties.

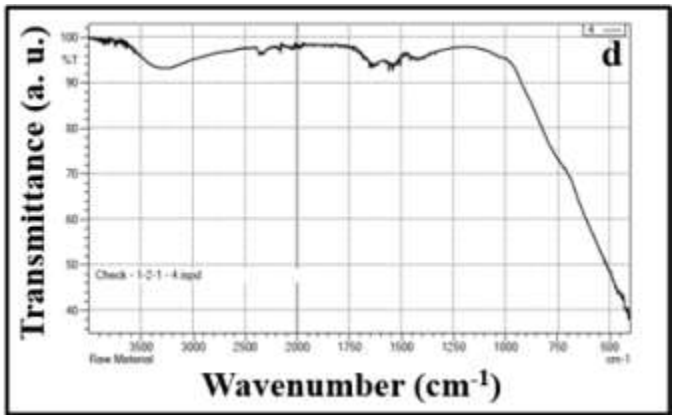

Fig. 1FTIR spectra of TiO_2 nanoparticles

Scanning Electron Microscopy (SEM)

The SEM micrograph revealed the formation of densely distributed TiO_2 nanoparticles with irregularly spherical morphology and noticeable agglomeration. The particles appeared nanosized and were closely packed, forming clustered structures across the surface. Such aggregation is commonly observed in green synthesized nanoparticles due to intermolecular interactions between phytochemical capping agents present in plant extracts and the nanoparticle surface. Despite partial clustering, the particles retained fine granular morphology, indicating successful nanoscale synthesis.

The rough and compact surface texture observed in the image suggests high surface area, which may enhance the biological and antioxidant activity of the nanoparticles. The particle distribution further indicates effective stabilization of the nanostructures by bioactive compounds present in the plant extract.

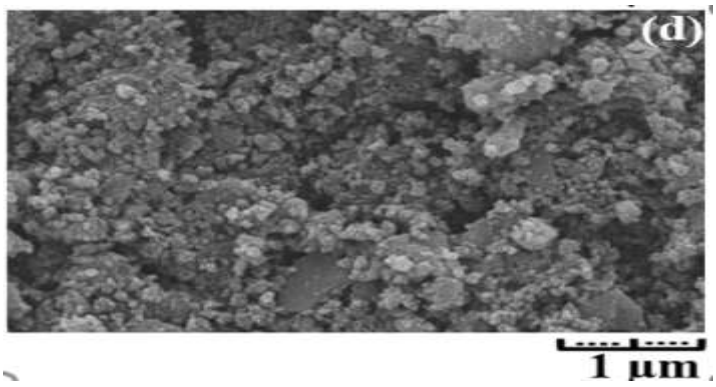

Figure 2: SEM micrographs of greensynthesized TiO_2 nanoparticles at 1 µm scale

Dynamic Light Scattering (DLS) Analysis

The Dynamic Light Scattering (DLS) Analysis was done using Zetasizer instrument . Debregeasia saeneb TiO_2 nanoparticles revealed a predominant particle population within the nanoscale range, indicating successful nanoparticle synthesis. The intensity distribution graph displayed a major peak between approximately 100–1000 nm, suggesting relatively uniform particle distribution and effective stabilization by phytochemical constituents present in the plant extract. The narrow distribution pattern reflects acceptable colloidal behavior and moderate particle homogeneity.

A minor secondary peak observed at a larger size range may indicate slight nanoparticle aggregation, which is commonly reported in green synthesized nanomaterials due to intermolecular interactions between phytochemical capping agents and nanoparticle surfaces. Despite this minor aggregation, the nanoparticles retained suitable nanoscale properties important for biological applications. The zeta potential findings further suggest that the particles possessed adequate surface charge to maintain colloidal stability through electrostatic repulsion, thereby reducing excessive aggregation and supporting their potential use in antioxidant and hepatoprotective applications.

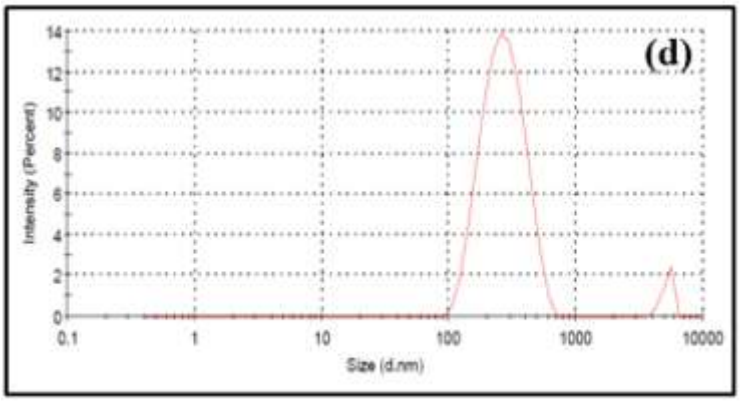

Figure 3: Dynamic light scattering (DLS) particle-size distribution of Debregeasia saeneb green synthesized TiO_2 nanoparticles

Table No. 1. In vivo hepatoprotective activities Using Plant Synthesized TiO_2 Nanoparticles

Treatment	ALT(IU/L)	AST (IU/L)	TBARS (nmol/g/.tissue)	Catalase (IU/g.tissue)	NPSH (µmol/g.tissue)
Normal	40.54 ±2	63.37 ±2.5	149.50 ±0.24	37.06 ±1.59	59.48 ±4.53
Paracetemol (60 mg/Kg)	79.20 ±2[a]	113.6 ±1.2[a]	775.00 ±0.78[a]	4.5 ±1.42[a]	20.54 ±1.40[a]
NPS (100 mg/Kg)	63.90 ±0.31[b]	81.79 ±0.72[b]	277.62 ±1.49[b]	8.17 ±0.60[b]	40.42 ±1.67[b]

Values are presented as mean ± SD of five replicates. Superscript "a" indicates significant difference with normal group and superscript "b" indicates significant difference when compared with the paracetamol group at $P < 0.05$.

Table: 1 result demonstrated a marked hepatotoxic effect of paracetamol administration compared with the normal control group. Mice treated with paracetamol (60 mg/kg) showed significant elevations in serum liver marker enzymes ALT and AST, increasing from 40.54 ± 2 to 79.20 ± 2 IU/L and from 63.37 ± 2.5 to 113.6 ± 1.2 IU/L, respectively. These elevations indicate severe hepatocellular injury and leakage of intracellular enzymes into circulation. Lipid peroxidation, assessed through TBARS levels, was also markedly increased in the paracetamol treated group (775.00 ± 0.78 nmol/g tissue) compared with the normal group (149.50 ± 0.24 nmol/g tissue), confirming enhanced oxidative stress and membrane damage.

In contrast, antioxidant defense parameters were significantly reduced following paracetamol intoxication. Catalase activity decreased sharply from 37.06 ± 1.59 to 4.5 ± 1.42 IU/g tissue, while NPSH content declined from 59.48 ± 4.53 to 20.54 ± 1.40 µmol/g tissue, indicating depletion of endogenous antioxidant reserves and impairment of cellular redox balance.

Treatment with NPS (100 mg/kg) considerably ameliorated paracetamol sinduced hepatic damage. ALT and AST levels were reduced to 63.90 ± 0.31 IU/L and 81.79 ± 0.72 IU/L, respectively, indicating stabilization of hepatocyte

membranes and reduced hepatic injury. Similarly, TBARS levels decreased substantially to 277.62 ± 1.49 nmol/g tissue, demonstrating inhibition of lipid peroxidation and oxidative damage. Furthermore, antioxidant status was significantly restored by NPS treatment, as evidenced by increased catalase activity (8.17 ± 0.60 IU/g tissue) and elevated NPSH levels (40.42 ± 1.67 µmol/g tissue) compared with the paracetamol group.
Overall, these findings suggest that NPS exerts significant hepatoprotective and antioxidant effects against paracetamol induced liver toxicity, likely through attenuation of oxidative stress, suppression of lipid peroxidation, and restoration of endogenous antioxidant defense mechanisms.

Histopathological analysis of liver tissue.
Histopathological analysis further supported the hepatoprotective potential of Debregeasia saeneb NPS against paracetamol induced liver injury. The control group (A) showed normal hepatic architecture with intact hepatocytes (HC), normal central vein (CV), and well-organized hepatic cords. In contrast, the APAP toxicity group (B) exhibited marked hepatic damage including hepatocellular degeneration, sinusoidal disorganization, inflammatory infiltration, and congestion around the central vein, indicating severe oxidative stress-mediated injury.
Treatment with Debregeasia saeneb NPS (F) markedly improved liver histology by restoring hepatic architecture, reducing cellular degeneration, and improving sinusoidal recovery (SR). Hepatocyte nuclei (N) appeared comparatively normal with reduced necrotic changes. These histological findings correlate with the biochemical results showing decreased ALT, AST, and TBARS levels along with restoration of catalase and NPSH contents. Overall, the results suggest that D. saeneb NPS exerts significant hepatoprotective activity through antioxidant and cytoprotective mechanisms against paracetamol induced hepatic toxicity.

Table 2: Score based histopathological analysis of liver lesions in different experimental groups

Hepatocellular Degeneration	Centrilobular Necrosis	Sinusoidal Distortion/Congestion	Inflammatory Infiltration	Overall Histopathological Score
Control	Absent (0)	Absent (0)	Normal (0)	0
APAP Toxicity	Severe (+++)	Severe (+++)	Severe (+++)	9
D.seneb NPS (100 mg/kg)	Mild (+)	Mild (+)	Mild (+)	4

Scoring criteria:0 = absent/normal; + = mild lesion; ++ = moderate lesion; +++ = severe lesion.

The liver of the control animals is healthy and is not damaged by inspecting it under the microscope. The APAP-treated group, on the other hand, had significant hepatic damage characterized by liver cell destruction, central vein tissue necrosis, sinusoidal occlusion, and accumulation of inflammatory cells, with the highest damage score. The results were different when the animals were given Debregeasia saeneb nanoparticles: only moderate amounts of liver cell damage and inflammation remained and the liver structure was relatively restored, demonstrating the nanoparticles' real protection against APAP induced damage.

DISSCUSSION
Debregeasia saeneb leaf extract was successfully used for the ecofriendly synthesis of titanium dioxide (TiO_2) nanoparticles, where naturally present photochemical acted to reduce and stabilize. Titanium isopropoxide (TTIP) acted as the precursor material, and reaction parameters including pH, temperature, and continuous stirring were managed to obtain controlled nanoparticle formation. Visible color change during the reaction confirmed nanoparticle synthesis, which may be attributed to the reduction of Ti^{4+} ions by bioactive compounds such as flavonoids and polyphenols in the plant extract (Hussain et al., 2024). Unlike conventional chemical methods, this green synthesis route avoids harmful solvents and surfactants, thereby improving the safety and biocompatibility of the synthesized nanoparticles. Comparable plant mediated synthesis strategies have also been reported using Azadirachta indica and Luffa acutangula, showing the important role of phytochemicals in nanoparticle reduction and stability (Thakur et al., 2019; Anbumani et al., 2022).
The UV–visible spectral analysis validated the successful synthesis of TiO_2 nanoparticles by showing characteristic absorption within the ultraviolet region. A slight shift toward shorter wavelengths compared with bulk anatase TiO_2 indicated the plant derived metabolites influenced nanoparticle stability and optical behavior (Ahmad et al., 2022; Thakur et al., 2019).

The FTIR spectrum of Debregeasia saeneb extract indicated the presence of many important bioactive functional groups that may support to its antioxidant and hepatoprotective chrachteristics. A broad absorption band observed around 3200–3400 cm^{-1} corresponds to O–H stretching vibrations of phenols and alcohols, indicating the presence of polyphenolic compounds and flavonoids. The peaks near 2900 cm^{-1} are attributed to C–H stretching of alkanes, while the absorption around 1600–1650 cm^{-1} suggests C=C or N–H stretching associated with aromatic compounds and amide groups. In addition, bands detected in the fingerprint region (1000–700 cm^{-1}) may be related to C–O, C–N, and aromatic ring vibrations, confirm the complex phytochemical constituents of the plant extract. These functional groups are known to act as anti oxidative and stabilizing agents during green synthesis of nanoparticles and are strongly linked with free radical scavenging and hepato protective activities (Saddick et al., 2023).

SEM observations indicated nanoparticle morphology comparable to previous reports on green synthesized TiO_2 nanoparticles, where biomolecular species may contribute to slight particle aggregation without disturbing nanocrystalline characteristics (Sahraoui et al., 2025). Such aggregation can inhance surface activity and may support regulation of reactive oxygen species , which is significant in biomedical applications.

The prepared nanoparticles also showed a polydispersity index (PDI) below 0.5, indicating satisfactory uniformity and colloidal stability (Batool et al., 2025). Moreover, the negative zeta potential value (approximately −24.3 mV) indicates sufficient electrostatic force of repulsion between particles, thereby decreasing aggregation capacities . The observed nanoscale distribution in current study is aligned with previous reports on plantmediated TiO_2 nanoparticles synthesized using medicinal plant extracts, where particle sizes range from 100–200 nm were linked with enhanced biological and antioxidant potential .Their nanoscale dimensions and colloidal stability may further support improved intestinal absorption and lymphatic transport, are in alignment with previous findings (Fawad et al., 2025).

Paracetamol induced toxicity is basically associated with the formation of the reactive species N-acetyl-p-benzoquinone imine (NAPQI), which interferes cellular redox homeostasis and subsequently causes liver damage (Yoon et al., 2016). In this study, paracetamol administered animals showed significant elevations in serum ALT and AST activity, reflecting hepatocyte damage and leakage of these enzymes into the blood circulation. Such increases are well known biochemical markers of hepatic injury (Hamza & Al-Harbi, 2015; Yoon et al., 2016; Ilavenil et al., 2016; Ramachandran & Jaeschke, 2017). Moreover, a significant rise in TBARS levels was seens in the toxic control group, indicating increased lipid peroxidation and severe oxidative stress in liver tissues (Jaeschke & Ramachandran, 2024). Concurrently, catalase activity was significantly decreased, indicating disturbance in the endogenous antioxidant defense system (Hassan et al., 2024). The lowering level of NPSH further confirmed loss oxidative balance, as intracellular sulfhydryl compounds, particularly glutathione, are rapidly used during detoxification of NAPQI (Mitchell et al., 1973; Liu et al., 2024). Collectively, these alterations show that paracetamol exposure was a reason of pronounced oxidative stress and severe hepatic dysfunction.

Administration of Debregeasia saeneb nanoparticles significantly recovered these biochemical interruptions. The decrease in ALT and AST levels after nanoparticle treatment indicates stability of hepatocyte membranes and decrease in leakage of intracellular enzymes into the bloodstream (Hamza & Al-Harbi, 2015; Chen et al., 2024). Similarly, the significant decrease in TBARS levels shows that Debregeasia saeneb nanoparticles effectively lowered lipid peroxidation and decreased oxidative injury in hepatic tissues (Hassan et al., 2024). Recovery of catalase activity in treated groups further supports the antioxidant activity of the nanoparticles and showed recovery of the endogenous antioxidant recovery system. Comparable enhancement of antioxidant enzymes has been reported previously in studies involving plant based nanoparticles and herbal therapies against experimentally induced liver toxicity (Ajith et al., 2007; Fuloria et al., 2022). This protective effect may be aligned with increased scavenging of reactive oxygen species, higher level degradation of hydrogen peroxide, and increased activity of cellular antioxidant response mechanisms. In addition, the elevation of NPSH levels after treatment shows recovery of glutathione mediated detoxification agents that are essential for cellular protection against reactive species (Mitchell et al., 1973; Liu et al., 2024).

Overall, the results of the present study show that D. saeneb nanoparticles have significant hepatoprotective properties against paracetamol induced hepatotoxicity. The observed protective effects may be due to decrease in oxidative stress, lowering of lipid peroxidation, increase in antioxidant enzyme activity, and regaining of sulfhydryl balance in cells (Ramachandran & Jaeschke, 2017; Hassan et al., 2024). Histopathological analysis of the nanoparticle treated group finds the biochemical and molecular insight. APAP induced liver necrosis and kidney tubular degeneration are validated results of increased NAPQI formation, glutathione decrease, and membrane damage duento oxidative stress. (Jaeschke et al., 2020; Hinson et al., 2010). Treatment with D. saeneb phytochemical nanoparticles significantly improved hepatic structure, supporting their stron antioxidant and anti inflammatory chrachteristics. These findings are aligned with earlier studies reporting that phenol rich plant extracts protect against liver toxicity by increasing antioxidant enzyme systems and decreasing inflammatory agents involved in tissue injury (Chen et al., 2025)

When compared with Moringa oleifera mediated TiO_2 nanoparticles, Debregeasia saeneb nanoparticles exhibited comparatively moderate but significant hepatoprotective ability against paracetamol induced hepatotoxicity. Treatments with both showed improved hepatic biomarkers and antioxidant level by reducing oxidative stress and recovering liver function. However, the stronger antioxidant potential of M. oleifera may be attributed to its comparatively richer phenolic and flavonoid content. (Zaib-un-Nisa et al., 2026)

CONCLUSION

This study finds that Debregeasia saeneb mediated TiO_2 nanoparticles show valuable antioxidant and hepatoprotective chrachteristics against liver injury induced by Paracetamole. Although their hepatoprotection was comparatively less effective than those reported for Moringa oleifera nanoparticles, they still have promising biological potential. These results suggest their potential applicability in fields of nutraceutical and pharmaceutical however, further studies are required to confirm their mechanisms of action and safety profile.

REFERENCES:

1. Rotundo, L., & Pyrsopoulos, N. (2020). Liver injury induced by paracetamol and challenges associated with intentional and unintentional use. World Journal of Hepatology, 12(4), 125–136. https://doi.org/10.4254/wjh.v12.i4.125
2. Yoon, E., Babar, A., Choudhary, M., Kutner, M., & Pyrsopoulos, N. (2016). Acetaminophen-induced hepatotoxicity: A comprehensive update. Journal of Clinical and Translational Hepatology, 4(2), 131–142. https://doi.org/10.14218/JCTH.2015.00052
3. Fakurazi, S., Sharifudin, S. A., & Arulselvan, P. (2012). Moringa oleifera hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. Molecules, 17(7), 8334–8350. https://doi.org/10.3390/molecules17078334
4. Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. Green Chemistry, 13(10), 2638–2650. https://doi.org/10.1039/C1GC15386B
5. Sundrarajan, M., & Gowri, S. (2011). Green synthesis of titanium dioxide nanoparticles by Nyctanthes arbor-tristis leaf extract. Chalcogenide Letters, 8(8), 447–451.
6. Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy & Bioallied Sciences, 12(1), 1–10. https://doi.org/10.4103/jpbs.JPBS_175_19
7. Saddick, S., Ahmed, D., & Gul, H. (2023). Isolation of biomedically important bioactive compounds from Debregeasia salicifolia with extraordinary antioxidant potential hepato-protectivity. Journal of King Saud University – Science, 35(1), 102437. https://doi.org/10.1016/j.jksus.2022.102437
8. Narayanan, M., Devi, P. G., Natarajan, D., Kandasamy, S., Devarayan, K., Alsehli, M., Elfasakhany, A., & Pugazhendhi, A. (2021). Biosynthesized nanoparticles and environmental applications. Environmental Research, 200, 111333. https://doi.org/10.1016/j.envres.2021.111333
9. Ramachandran, A., & Jaeschke, H. (2016). Oxidative stress during acetaminophen hepatotoxicity: Sources, pathophysiological role and therapeutic potential. Redox Biology, 10, 148–156. https://doi.org/10.1016/j.redox.2016.10.001
10. Hamza, R. Z., & Al-Harbi, M. S. (2015). Amelioration of paracetamol hepatotoxicity and oxidative stress on mice liver with silymarin and Nigella sativa extract supplements. Asian Pacific Journal of Tropical Biomedicine, 5(7), 521–531. https://doi.org/10.1016/j.apjtb.2015.03.011
11. Ilavenil, S., Al-Dhabi, N. A., Srigopalram, S., Kim, Y. O., Agastian, P., Baru, R., Choi, K. C., & Arasu, M. V. (2016). Acetaminophen-induced hepatotoxicity in Wistar rats: A proteomic approach. Molecules, 21(2), 161. https://doi.org/10.3390/molecules21020161
12. Jaeschke, H., & Ramachandran, A. (2024). Central mechanisms of acetaminophen hepatotoxicity: Mitochondrial dysfunction by protein adducts and oxidant stress. Drug Metabolism and Disposition, 52(8), 712–721. https://doi.org/10.1124/dmd.123.001279
13. Hassan, H. M., Abdel-Halim, N. H. M., El-Shenbaby, I., Helmy, M. A., Hammad, M. O., Habotta, O. A., El Nashar, E. M., Alghamdi, M. A., Aldahhan, R. A., Al-Khater, K. M., Almohaywi, B., & Farrag, E. A. E. (2024). Phytic acid attenuates acetaminophen-induced hepatotoxicity via modulating iron-mediated oxidative stress and SIRT-1 expression in mice. Frontiers in Pharmacology, 15, 1384834. https://doi.org/10.3389/fphar.2024.1384834
14. Fuloria, S., Mehta, J., Chandel, A., Sekar, M., Rani, N. N. I. M., Begum, M. Y., Subramaniyan, V., Chidambaram, K., Thangavelu, L., Nordin, R., Wu, Y. S., Sathasivam, K. V., Lum, P. T., Meenakshi, D. U., Kumarasamy, V., Azad, A. K., & Fuloria, N. K. (2022). A comprehensive review on the therapeutic potential of Curcuma longa Linn. in

relation to its major active constituent curcumin. Frontiers in Pharmacology, 13, 820806. https://doi.org/10.3389/fphar.2022.820806
15. Ajith, T. A., Hema, U., & Aswathy, M. S. (2007). Zingiber officinale Roscoe prevents acetaminophen-induced acute hepatotoxicity by enhancing hepatic antioxidant status. Food and Chemical Toxicology, 45(11), 2267–2272. https://doi.org/10.1016/j.fct.2007.06.001
16. Liu, X., Liu, R., Wang, Y., et al. (2024). Indole-3-carboxaldehyde alleviates acetaminophen-induced liver injury via inhibition of oxidative stress and apoptosis. Biochemical and Biophysical Research Communications, 710, 149880. https://doi.org/10.1016/j.bbrc.2024.149880
17. Chen, H., Wang, S., Chen, Q., Yu, W., Nie, H., Liu, L., et al. (2024). Aloperine ameliorates acetaminophen-induced acute liver injury through HMGB1/TLR4/NF-κB and NLRP3/inflammasome pathway. Mediators of Inflammation, 2024, 3938136. https://doi.org/10.1155/2024/3938136
18. Mitchell, J. R., Jollow, D. J., Potter, W. Z., Gillette, J. R., & Brodie, B. B. (1973). Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. Journal of Pharmacology and Experimental Therapeutics, 187(1), 211–217.
19. Ramachandran, A., & Jaeschke, H. (2017). Mechanisms of acetaminophen hepatotoxicity and their translation to the human pathophysiology. Journal of Clinical and Translational Research, 3(Suppl. 1), 157–169. https://doi.org/10.18053/jctres.03.2017S1.002
20. Yoon, E., Babar, A., Choudhary, M., Kutner, M., & Pyrsopoulos, N. (2016). Acetaminophen-induced hepatotoxicity: A comprehensive update. Journal of Clinical and Translational Hepatology, 4(2), 131–142. https://doi.org/10.14218/JCTH.2015.00052
21. Anbumani, D., Dhandapani, K. V., Manoharan, J., Babujanarthanam, R., Bashir, A. K. H., Muthusamy, K., Alfarhan, A., & Kanimozhi, K. (2022). Green synthesis and antimicrobial efficacy of titanium dioxide nanoparticles using Luffa acutangula leaf extract. Journal of King Saud University – Science, 34, 101896. https://doi.org/10.1016/j.jksus.2022.101896
22. Thakur, B. K., Kumar, A., & Kumar, D. (2019). Green synthesis of titanium dioxide nanoparticles using Azadirachta indica leaf extract and evaluation of their antibacterial activity. South African Journal of Botany, 124, 223–227. https://doi.org/10.1016/j.sajb.2019.05.024
23. Sahraoui, A., Hamlaoui, M., Chikhi, S., Harrat, S., Baghriche, O., Zertal, A., & Vernuccio, S. (2025). Green synthesis and characterization of titanium dioxide nanoparticles using Eucalyptus globulus leaf extract: Impacts of the mild thermal treatment. Materials Today Sustainability, 31, 101193. https://doi.org/10.1016/j.mtsust.2025.101193
24. Hussain, S., Nazar, W., Tajammal, A., Nasreen, Z., Ahmad, T., Asghar, A., Mustafa, Z., Shahid, M., Batoo, K. M., & Hussain, S. (2024). Green synthesis of TiO_2 nanoparticles using Morus nigra leaves: Characterization and biological potential. Polish Journal of Environmental Studies, 33(3), 2707–2714. https://doi.org/10.15244/pjoes/175060
25. Fawad, M., Ali, Z., Zahid, F., Alamri, A. H., Al Fatease, A., Lahiq, A. A., Asiri, A., & Din, F. U. (2025). Fabrication of oral sustained release capecitabine loaded nanostructured lipid carriers with improved bioavailability and prolonged anticancer effects. Journal of Molecular Liquids, 421, 126852. https://doi.org/10.1016/j.molliq.2025.126852
26. Batool, S., Ali, Z., Alamri, A. H., Al Fatease, A., Lahiq, A. A., Asiri, A., Rehman, A. U., & Din, F. U. (2025). Development and statistical optimization of camptothecin-loaded hyaluronic acid and zein polymeric nanoparticles towards the treatment of melanoma. International Journal of Biological Macromolecules, 321, 146330. https://doi.org/10.1016/j.ijbiomac.2025.146330
27. Hinson, J. A., Roberts, D. W., & James, L. P. (2010). Mechanisms of acetaminophen-induced liver necrosis. In Handbook of Experimental Pharmacology (Vol. 196, pp. 369–405). Springer. https://doi.org/10.1007/978-3-642-00663-0_12
28. Ahmad, M. Z., Alasiri, A. S., Ahmad, J., Alqahtani, A. A., Abdullah, M. M., Abdel-Wahab, B. A., Pathak, K., Saikia, R., Das, A., Sarma, H., et al. (2022). Green synthesis of titanium dioxide nanoparticles using Ocimum sanctum leaf extract: In vitro characterization and its healing efficacy in diabetic wounds. Molecules, 27(22), 7712. https://doi.org/10.3390/molecules27227712
29. Sabir, S. M., Rocha, J. B. T., Athayde, M. L., & Boligon, A. A. (2017). Antioxidant activities and phenolic profile of Baccharis trimera, a commonly used medicinal plant from Brazil. South African Journal of Botany, 113, 318–323. https://doi.org/10.1016/j.sajb.2017.09.007
30. Zakaria, Z. A., Kamisan, F. H., Nasir, N. M. M., Teh, L. K., & Salleh, M. Z. (2019). Aqueous partition of methanolic extract of Dicranopteris linearis leaves protects against paracetamol-induced liver damage. Nutrients, 11(12), 2945. https://doi.org/10.3390/nu11122945

31. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. Toxicology. 2008;245(3):194-205. doi: 10.1016/j.tox.2007.11.02.
32. Salbitani G, Bottone C, Carfagna S. Determination of reduced and total glutathione content in extremophilic microalga Galdieria phlegrea. Bio Protoc. 2017;7(13):e2372. doi: 10.21769/BioProtoc.2372.
33. Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-126. doi: 10.1016/S0076-6879(84)05016-3.
34. Reddy, K. M., Manorama, S. V., & Reddy, A. R. (2003). Bandgap studies on anatase titanium dioxide nanoparticles. Materials Chemistry and Physics, 78(1), 239–245. https://doi.org/10.1016/S0254-0584(02)00343-7
35. Mamun MAA, Rahman A, Belal SH, Islam MA, Sarker MEH, Arman MSI, et al. Histological study of the effect of malathion on liver and kidney tissues of mice model. Int J Pharm Sci Res. 2015;6(3):1043-1048.
Online link: Open article
36. Abubakar AR, Haque M. Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. J Pharm Bioallied Sci. 2020;12(1):1-10. https://pmc.ncbi.nlm.nih.gov/articles/PMC7398001/
37. Zhou, Y., Tang, J., Tan, X., & You, Y. (2024). Hepatoprotective activity of Gingko biloba-mediated TiO2 NPs against acetaminophen-induced albino rats- In vitro studies. ChemistrySelect. Advance online publication. https://doi.org/10.1002/slct.202403066
38. Zaib-un-Nisa, Sabir, S. M., Ullah, I., Shafique, S., Iqbal, N., Mehmood, M., Khalid, S., & Abbas, S. R. (2026). Molecular and mechanistic insight to the role of Moringa oleifera (drumstick tree) mediated TiO2 nanoparticles in hepatic cytoprotection. Genetics and Molecular Research, 25(1s). https://doi.org/10.4238/vdqjs705
39. Chen, P., Zou, F., & Liu, W. (2025). Recent advancement in prevention against hepatotoxicity, molecular mechanisms, and bioavailability of gallic acid, a natural phenolic compound: Challenges and perspectives. Frontiers in Pharmacology, 16, 1549526. https://doi.org/10.3389/fphar.2025.1549526