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MOLECULAR AND MECHANISTIC INSIGHT TO THE ROLE OF MORINGA OLEIFERA (DRUMSTICK TREE) MEDIATED TiO_2 NANOPARTICLES IN HEPATIC CYTOPROTECTION

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ABSTRACT
Liver diseases are one of the main health issues affecting the whole world, and oxidative stress has a central role in the pathogenesis of liver diseases. Due to adverse reactions and poor clinical tolerance, treatment using traditional antioxidants such as N-acetylcysteine is gradually shifting toward plantderived alternatives. Despite hepatoprotective potential of plants, the improper dosage forms of crude extracts, patient non compliance and buffering remain major challenges in their therapeutic use. These reasons demand safer, proper dosage and more bioavailable plant derived alternatives. This research was aimed to address these challenges. The novelty of this study resides in evaluation of the liver protective tendency of green synthesized TiO_2 nanoparticles using Pakistan origin Moringa oleifera leaf extract, it is not clearly reported in previous studies. It was hypothesized that green synthesized TiO_2 nanoparticles will not only retain efficacy of phytochemicals but will exhibit greater antioxidant, hepatoprotective activity due to increased bioavailability and calculated dosage. Nanoparticles formation was successful, and stable, bioactive TiO_2 NPs were produced and were confirmed by UV-Vis (270-320 nm), FTIR (Ti-O-Ti at 500-600 cm-1) and DLS (200-500 nm; (PDI < 0.5), Zeta potential -24 mV)., SEM showed granular morphology. Hepatoprotective activity in mice intoxicated with APAP was tested in vivo (n = 5/group). TiO_2 NPs treatment improved biochemical measurements significantly reversing the toxic effects (ALT dropped to 48.07 ± I.22 U/L , AST to 56.55 ± 1.63 U/L , Restoration of CAT and GSHs, as well as a decreased MDA by more than 60% ($p < 0.001$) were also observed in NPs. Histopathological examination showed almost standard hepatic lobular architecture, and less necrosis. The findings bridge ethnomedicine and nanotechnology, highlighting Moringa oleifera TiO2 nanoconjugates as promising biocompatible and specific dose dependant therapy for oxidative liver damage.These results build the bases for the future translational applications of plant based, eco friendly nanomedicines for the hepatic diseases.
KEYWORDS: Moringa oleifera, TiO_2 nanoparticals, oxidative stress, Hepatoprotective Potential, SEM

INTRODUCTION
Liver is the key organ in metabolism, detoxification, and managing oxidative stress. It is highly susceptible to chemically induced injury. Drug induced liver damage remains a major concern because excessive exposure to hepatotoxic agents can disturb antioxidant defenses, impair cellular integrity, and trigger progressive tissue injury. Experimental models widely use acetaminophen to induce hepatotoxicitydue to well established oxidative liver damage mechanism.whenacetaminophen is used above safe doses,is converted to the active intermediate N-acetyl-p-benzoquinone imine (NAPQI), that causes depletion of glutathione, reacts to cellular proteins, and induces necrosis of hepatocytes (Hinson et al., 2010; Mitchell et al., 1973).
Increase in serum liver marker enzymes and decrease in intracellular antioxidant storage are hall marks of injured liver. That is the reason experimental model have been widely using acetaminophine to evaluate bioactive compounds and different dosage forms having potential liver protective effects. Now a days nanoparticles have gained increasing interest, due to certain distinct surface charachteristics, high chemical reactivity, and adjustable physicochemical properties that may increase biological efficacyin comparison with conventional bulk materials. Among these nanomaterials , titanium dioxide nanoparticles (TiO_2 NPs) have gained significant attention owing to their stability, surface reactivity, and wider applications in biomedical and pharmaceutical field.
By Conventional approaches, synthesis of TiO_2 nanoparticles can be achieved by physical and chemical techniques, but such strategies often are energy intensive, require harsh reaction conditions, or synthetic reagents compromise their environmental and biomedical safety. On the other hand green approach has been recognized as more simple and environment friendly alternative. Plant based synthesis is attractive due to the presence of

phenolics, flavonoids, proteins, terpenoids, and other bioactive metabolites in plant extracts that might be involved in reducing, capping, and stability of nanoparticles in one step. This limits dependence on toxic materials and may inhance the biocompatibility of the final nanomaterial product (Ahmad et al., 2022).
Many research studies have confirmed successful synthesis of TiO_2 nanoparticles using medicinal bio active plants. For example, leaf extract of Azadirachta indica has been used to synthesize TiO_2 nanoparticles with stabilization support of bioactive compounds of plants. (Thakur et al., 2019), similarly leaf extract of Luffa acutangula has been evaluated to generate bio active TiO_2 nanoparticles by sustainable approach (Anbumani et al., 2022). Another study demonstrated that TiO_2 nanoparticles synthesized from Ocimum sanctum extract had specific UV-visible and FTIR characteristics indicating nanoparticle generation and surface interaction with plantderived phytochemicals (Ahmad et al., 2022). These reports suggest that bioactive plant chemicals do not only help in synthesis, but also determine particle size, surface interactions , and stability.
Such characteristics are highly relatable when nanoparticles are used in biomedicines. TiO_2 nanoparticles with plant capping may associate the functional characteristics of the core metal oxide with the protective and stabilizing effect of plant bioactive compounds present on the surface. This might enhance the possibility of the green TiO_2 nanoparticles to decrease oxidative stress and to conserve antioxidant defense of liver under toxic environment. On this basis, the present study was designed to synthesize TiO_2 nanoparticles through a Moringa oleifera mediated synthesis to evaluate their hepatoprotective capacity against acetaminophen-induced liver toxicity using biochemical parameters of liver damage and oxidative stress.
Although many studies describe different application of green synthesized Moringa oleifera nanoparticles but experimentation for M. oleifera TiO_2 nanoparticlesis still limited.
Moreover, there is no obvious research that used Pakistani origin Moringa oleifera, which makes this study regionally and scientifically significant.

MATERIALS AND METHODS

Collection of Plant

The leaves of Moringa oleifera were collected from Sialkot, Pakistan and its identification was done in Department of Botany, University of Poonch Rawalakot, Azad Jammua Kashmir (Pakistan).

Experimentation

Preparation of Leaf Extract

The fresh leaves of M. oleifera, were water cleaned, dried in shade, grounded to powder. Extraction was done through maceration. Powdered material of M. oleiferawas soaked in distilled water for three days with frequent shaking and stirring. After three days the material was filtered using muslin cloth first and then used WhatmanNo.1 filter paper. The residues were soaked again in water while the obtained filtrate was concentrated and dried through rotary evaporator. This process was repeated three times.The filtrates were converted to semi solid form under reduced pressure at 40 ºC in rotary evaporator (Abubakar & Haque, 2020)The percentage yield of plants extracts were calculated by the following formula. Percentage Yield = Extract weight/Powder Weight X100 Final concentration of stock solution calculated was 1mg/ml

Synthesis of TiO2 NPs

TTIP (10ml) was added in 70 ml of distilled water to prepare 80 ml solution of titanium tetra-isopropoxide (TTIP). This solution (80 ml) was mixed with 30 ml already prepared aquous extract of Moringa oleifera and magnetic stirrer was used to stir mixture for 3 hours at 50 °C until the color was changed from light brown to dark brown and showing the generation of nanoparticles.
The synthsized NPs were cleaned with excess water in the reaction flask and each time liquid was decanted. The mixture was filtered to obtain the prepared TiO_2 NPS and were dried at 100ºC for 24 hours and calcination was done in a muffle furnace for 3 hours at 400 ºC to obtain the final product of TiO_2 NPs (Hussain et al., 2024).

Fig 1(a) *M. oleifera* Leaf Powder | **Fig 1(b)** *M. oleifera* Leaf Extract | **Fig 1(c)** Extract+ TTIP stirring for 3 hrs at 50°C | **Fig1(d)** TiO2 nanoparticles of *M. oleifera*

Figure1. Green synthesis of Moringa oleifera mediated TiO2 nanoparticles using TTIP

Characterization
Synthesized nanoparticles were characterized by Zetasizer (ZS 90,Zetasizer Malvern, UK) SEM(JSm-6940-A, Tokyo, Japan) and FTIR (Shimadzu). Nanoparticle FTIR spectroscopy was used to identify the functional groups in TiO2. The biosynthesized TiO2 NPs were analyzed for particle size, polydispersity index (PDI), and hydrodynamic diameter using dynamic light scattering (DLS) with the help of a Nano ZS90 zeta sizer from Malvern Instruments, UK.(Reddy et al., 2003) A small quantity of TiO2 dispersion was combined with 5 mL of doubledistilled water for DLS analysis.

Hepatoprotective Activity (In-vivo model):
Zakaria et al.,2019 PERFORMED in-vivo hepatoprotective evaluation; it will be followed with slight changes

Study Animals
All animal procedures were carried according to the NIH Guide for the Use of Laboratory Animals and were certified by the University of poonch Rawalakot Ethical committee . Male albino mice (25–35 g) and age (2.5 months) from breeding center in CEMB University of Panjab were used for in vivo research. The animals were kept in the separate cages with food and water access under controlled conditions.

Dosage
In this study, adult mice were divided into 3 groups, (n = 5), and were fasted overnight before the initiation of the experiment.
Group I (Normal Control) normal saline was given.
Group II (Hepatotoxic Control) was administered Paracetamol(70 mg/kg).
Group III(Test Groups) Distilled water was used to create a colloidal solution of TiO2 NPs, which was then orally given to the animals were administered oral doses (100 mg/kg) (Zhou et al., 2024).

Blood and Tissue Biochemistry
On 10th day after 48 hrs of PCM exposure, all animals were anesthetized and blood was drawn from heart to determine level of ALT and AST.Livers were cut, washed in the ice-cold saline, weighed, and sectioned. Mid-lobe liver tissues were placed in 10% formalin for histopathological studies, a part of tissues was used to make homogenate.clear supernatent from centrifuged homogenate was used for the biochemical analysis.While the remaining tissues were frozen using dry ice and was stored at −80°C for the further studies.

ALT,AST Determination
This method has been designed according to the standard method described by IFCC.Clin Chem lab Med 2002;40(7):718-724(1)(Ozer et al., 2008).AMD diagnostic kits were used.

AST Determination
Reagent R1 includes TRIS buffer, aspartate, Malate dehydrogenase, Lactate dehydrogenase, while Reagent: R2 includes NADH, Oxoglutarate biocides. Working reagent was made by mixing the 4 volumes of the R1 with 1 volume of R2. The working reagent, samples and controls were pre incubated at 37°C. Photometer was set to zero using distilled water. Blank was made by adding 1000 µl of the working reagent and 100 µl of the distilled water. Test Sample was given incubation for 1 minute and starting absorbance reading was noted. The absorbance reading was recorded three times exactly and difference between the absorbance values was determined.

ALT Determination
Working reagents from MDA diagnostic kits were formed (4 volume of R1 +volume of R2) were protected from light. Reagents were mixed gently by inversion and were incubated for the 1 minute and recorded the initial absorbance reading. Absorbance reading was repeated three times exactly one minute after the previous reading and determined the difference between the absorbance values. Average result was obtained to determine the average change of absorbance per minute.

TBARS Levels From Liver Tissue
TBARS production was evaluated by repeating slightly different method of sabir et al.(2017) that was used for in-vitro TBARS production in this study.

Reduced Glutathione Assay
200µl of liver tissuehomogenate was taken in test tube 300 µl of 3% sulfocylic acid was added and centrifugation was done on 1500rpm for 10 minutes. 200µl of supernatant was then mixed into 50 µl of DNTB and 980 µl of phosphate buffer. Absorbance was recorded at 412 nm. This method was taken from Salbitani, Bottone, and Carfagna (Salbitani et al., 2017)

Catalase Assay
65 µl of H_2O_2 was added into 10 ml of phosphate buffer 980 µl of the solution was added into 20 µl of tissue homogenate and finally absorbance at 240nm was recorded **.**(Aebi, 1984)

Histopathological Examination
Mice were humanely euthanized and the abdominal cavity was opened through a midline incision. The liver was excised and placed in the 10% neutral buffered formalin for 24–48 hrs. Water content of preserved tissues was removed through series of ethanol concentrations, tissues were treated with xylene, and embedded in molten paraffin. 4-5 µm thick sections of parafifin blocks were cut with microtome and were stained with the hematoxylin and eosin. Then water content was removed again, cleared, and were placed with DPX. Histological examination was performed under a light microscope to evaluate liver architecture and pathological changes (Mamun et al., 2015)

RESULTS
The Nanoparticles synthesis reaction mixture changed from dark brown to light brown (Fig:1), this change in color indicated the synthesis of nanoparticles, after calcination colour of nanoparticles changed to off white .ess.

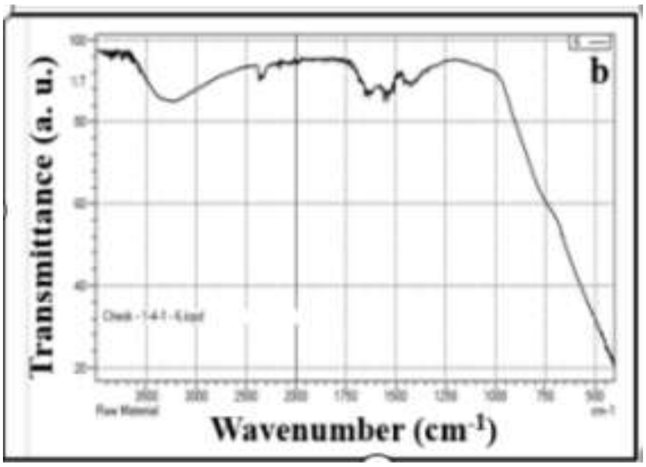

Fig. 2.1 FTIR spectra of Moringa oleifera TiO_2 nanoparticles

The presence of characteristic phytochemical and Ti-O-Ti bands in FTIR spectra (Figure 2.1) ascertained the presence of TiO_2 nanoparticles which were successfully bioreduced and stabilized.
The TiO_2 of Moringa oleifera (Figure 4.13b) exhibited a good hydroxyl peak of 3350 cm^{-1} and aliphatic C-H 2920 cm^{-1} and amide-related peaks of 1640 cm^{-1} showing the appearance of proteins and polyphenols in the reduction. Peaks of C-O-C were shown between 1050-1250 cm^{-1} of glycosides.

Scanning Electron Microscopy (SEM)

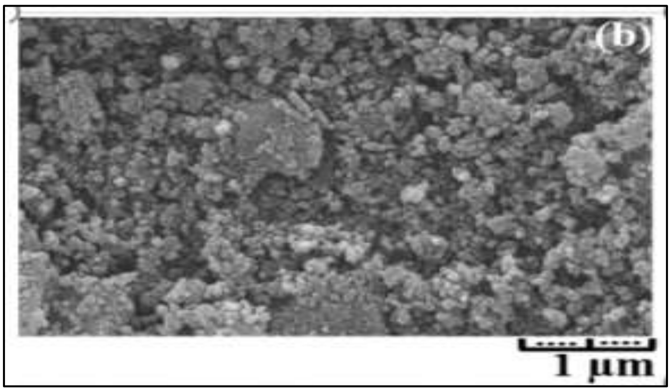

Figure 2.2: SEM micrographs of greensynthesized Moringa oleifera TiO_2 nanoparticles at 1 µm scale

The TiO_2 particles of Moringa oleifera seemed to be bigger (550-650 nm) and irregular in shape which can be attributed to the effects of complex phytoconstituents on the nucleation process. The morphology is consistent with other literature on the green production of TiO_2, in which the biomolecular residues enhance aggregation without affecting the nano crystallinity (Sahraoui et al., 2025). Although necessary in phytochemical pathways, particle clustering enhances the surface area and reactive oxygen species regulation which are essential to biomedical success.

Table 1: In vivo hepatoprotective activities using plant synthesized TiO_2 nanoparticles

Values are expressed as means of six replicates, data is different significantly P<0.05 when compared to paracetamol control group showing the effect of NPS on liver function markers and oxidative stress parameters

Treatments	ALT (IU/L)	AST (IU/L)	TBARS (nmol/g.tissue)	Catalase (IU/g.tissue)	NPSH µmol/g.tissue)
Normal	39.5±3	64.7±3.0	150.48±0.34	36.86±1.79	57.32±5.63
Paracetamol (60 mg/kg)	78±2[a]	114±1.2[a]	774.44± 2.3[a]	4.1±0.9[a]	21.247+-0.45[a]
NPS (100mg/kg)	48.07±1.22[b]	56.55±1.63[b]	170.44±5[b]	17.8± 0.85[b]	52.1+-1.32[b]

in paracetamol induced hepatotoxicity.

Dynamic Light Scattering (DLS) Analysis

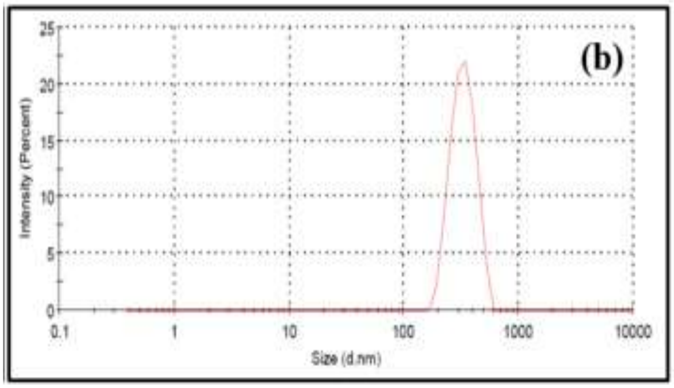

Figure 2.3: Dynamic light scattering (DLS) particle-size distribution of green synthesized TiO_2 nanoparticles

The DLS size distribution of plantsynthesized TiO_2 nanoparticles showed a single narrow peak around 250–350 nm, (Fig 2.3) indicating a relatively uniform particle population. The lack of multiple peaks suggests low polydispersity and limited aggregation. The measured size represents hydrodynamic diameter and may be enlarged by agglomeration and phytochemical capping on the nanoparticle surface.

The size and dispersity were measured by a Zetasizer, showed a narrow and single peak at 600 nm, indicating good colloidal stability.

The biochemical findings demonstrated a clear protective effect of NPS (100 mg/kg) against paracetamol induced liver damage. In the paracetamol treated group, serum liver enzymes were markedly elevated, with ALT increasing from 39.5±3 IU/L in the normal group to 78±2 IU/L and AST rising from 64.7±3.0 IU/L to 114±1.2 IU/L. This sharp increase indicates significant hepatocellular injury and leakage of these enzymes from damaged liver cells into the bloodstream.

Paracetamol administration also caused high oxidative stress, as confirmed by the substantial rise in TBARS levels from 150.48 ±0.34 nmol/g tissue in the normal group to 774.44 ±2.3 nmol/g tissue, reflecting enhanced lipid peroxidation and membrane damage. At the same time, the antioxidant defense system was severly affected, with catalase activity decreasing from 35.86 ±1.79 to 4.1 ±0.9 IU/g tissue and NPSH levels falling from 57.32 ±5.63 to 21.247 ±0.455 µmol/g tissue. These reductions suggest depletion of endogenous antioxidants and impaired ability of the liver to neutralize reactive oxygen species.

Treatment with NPS (100 mg/kg) considerably improved these altered biochemical parameters. ALT and AST levels decreased to 48.07 ± 1.22 and 56.55±1.63 IU/L, respectively, approaching normal values, which indicates restoration of liver integrity and reduced hepatocellular damage. Likewise, TBARS levels were reduced to 170.44±5 nmol/g tissue, showing a marked decline in lipid peroxidation in comparison with the paracetamol group. In addition, catalase activity increased to 17.8±0.85 IU/g tissue, while NPSH levels improved to 52.1±1.32 µmol/g tissue, suggesting partial recovery of the antioxidant defense system.

Overall, these results indicate that NPS exerts a significant hepatoprotective effect, possibly through its antioxidant potential, by reducing lipid peroxidation, preserving endogenous antioxidant status, and preventing paracetamol-induced liver cell damage. However, although NPS markedly restored the disturbed parameters, some valuesdid not return completely to the normal control level, suggesting partial but substantial protection.

Histopathological Analysis

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

Group	Treatment	Lesion score	Interpretation
A	Normal saline (control)	0.00 ±0.00[c]	Normal hepatic tissue morphology
B	Acetaminophen Toxicity	2.67 ±0.21[a]	Acetaminophen-induced hepatotoxicity
C	M.oleifera Nps treatment	1.00 ±0.26[b]	Liver protective and ameliorative action

Values are stated as mean ± SEM. Different superscript letters show significant differences among groups at $p < 0.05$. n=6.

Our results are validating the findings of Rajibul et al., (2019). The results of histopathology revealed that control group showed hepatic lobules with typical architecture, central veins, intact hepatocyte cords, and minimal cytoplasmic vacuolation. The APAP treated group showed severe foci of hepatocellular necrosis, ballooning degeneration, sinusoidal dilation and inflammatory cell infiltrationclassical signs of acute hepatotoxicity. However, moderately protective sections of TiO_2 of Moringa oleifera treated groups exhibited less necrosis, less severe cytoplasmic vacuolation, and partial lobular reconstruction (Table 2).

DISSCUSSION

The reduction and capping agents of the natural reducing and capping agents were successfully used to synthesize titanium dioxide (TiO_2) nanoparticles (NPs) using aqueous leaf extracts of Moringa oleifera. The precursor was titanium isopropoxide (TTIP) and all the conditions of pH, temperature, and stirring were optimized to provide uniform hydrolysis and condensation. The color change was the confirmation of the presence of nanoparticles, which were formed by the process of bioreduction of Ti^{4+} ions with the help of polyphenolic and flavonoid substances of the extracts (Hussain et al., 2024).

This green synthesis avoids the use of toxic solvents or surfactants thereby making it more biocompatible and biomedical applications a possibility. The same bio fabrication strategy has been used in Azadirachta indica and Luffa acutangula, and it has been validated that phytochemical can be used to reduce and stabilize TiO_2 (Thakur et al., 2019; Anbumani et al., 2022).

The UV-visible spectrum demonstrated TiO_2 nanoparticle formation, with absorption in the UV region characteristic of nanosized TiO_2. The shift toward lower wavelength relative to bulk anatase TiO_2 and the presence of additional phytochemical-associated bands suggest that plant metabolites contributed to nanoparticle reduction, stabilization, and modulation of optical properties (Ahmad et al., 2022; Thakur et al., 2019).

The FTIR spectrum supports the successful formation of TiO_2 nanoparticles. A broad band in the 3200–3400 cm^{-1} region suggests O–H stretching frome hydroxyl groups on surface or adsorbed water, while the signal near 1600–1630 cm^{-1} is consistent with bending vibrations of bound water molecules. Small bands around 1370–1450 cm^{-1} may indicate residual plant-derived organic compounds involved in reduction and capping during green synthesis. The intense absorption in the low-wavenumber region below 1000 cm^{-1}, particularly within 400–800 cm^{-1}, is characteristic of Ti–O–Ti vibrations and confirms the TiO_2 framework (Ahmad et al., 2022; Aravind et al., 2021; Dobrucka, 2017).

The morphology showed in SEM is consistent with other literature on the green production of TiO_2, in which the biomolecular residues enhance aggregation without affecting the nano crystallinity (Sahraoui et al., 2025). Although necessary in phytochemical pathways, particle clustering enhances the surface area and reactive oxygen species regulation which are essential to biomedical success.

All formulations had polydispersity index (PDI) < 0.5, confirming uniformity and stability suitable for biomedical applications (Batool et al., 2025). The negatively charged surface (Zeta potential ≈ −24.3 mV) implies electrostatic repulsion preventing aggregation. The nanometric size range and colloidal stability are favorable for intestinal and lymphatic uptake, aligning with prior findings by Fawad et al. (2025). M. oleifera extracts produced fine and stable nanostructures, consistent with their higher phenolic and flavonoid contents. Their smaller particle size (200–400 nm) implies better bioavailability and surface reactivity, corroborating literature where plant polyphenols enhance TiO_2 NP antioxidant and antimicrobial activities (Hussain et al., 2020; Khan et al., 2022)

Paracetamol toxicity is mainly caused by the formation of a harmful metabolite that disturbs the redox balance and ultimately damages liver cells (Yoon et al., 2016; Ramachandran & Jaeschke, 2017). In the current study, animals treated with paracetamol showed clear increases in ALT and AST levels, which indicates injury to hepatocytes and leakage of these enzymes into the bloodstream. Such elevations are widely considered important biochemical signs of hepatic damage (Hamza & Al-Harbi, 2015; Yoon et al., 2016; Ilavenil et al., 2016; Ramachandran & Jaeschke, 2017).

A marked increase in TBARS was also observed in the toxic group, showing that paracetamol exposure enhanced lipid peroxidation and placed the liver under severe oxidative stress (Du et al., 2016; Jaeschke & Ramachandran, 2024). At the same time, catalase activity declined, suggesting that the natural antioxidant defense system of the liver had been weakened (Du et al., 2016; Hassan et al., 2024). The reduction in NPSH further supports this finding, as it reflects depletion of intracellular sulfhydryl compounds, especially glutathione, which are consumed during detoxification of the active metabolite (Mitchell et al., 1973; Liu et al., 2024). Altogether, these changes confirm that paracetamol caused serious oxidative damage and disrupted normal liver function.

Treatment with NPS significantly improved these altered parameters. The decline in ALT and AST after nanoparticles administration suggests that the treatment helped preserve the structural integrity of hepatocytes and reduced enzyme leakage from damaged cells (Hamza & Al-Harbi, 2015; Chen et al., 2024). In a similar way, the lowering of TBARS in the treated group indicates that NPS was capable of reducing lipid peroxidation and limit oxidative injury in hepatic tissue (Du et al., 2016; Hassan et al., 2024).

The antioxidant results also support the protective role of NPS. The improvement in catalase activity in nanoparticletreated animals suggests recovery of the endogenous antioxidant system and indicates that the treatment was effective in reducing oxidative burden. Similar restoration of antioxidant enzymes has been described in previous studies involving herbal compounds and nanoparticlebased therapies in liver injury models (Ajith et al., 2007; Fuloria et al., 2022). This recovery may be linked with better breakdown of hydrogen peroxide, activation of antioxidant-response pathways, and protection of cell membranes from oxidative damage. Likewise, the rise in NPSH level after treatment suggests restoration of glutathione-related defense mechanisms, which are essential for protecting liver cells from reactive toxic intermediates (Mitchell et al., 1973; Liu et al., 2024).

Overall, the findings of the study suggest that NPS has a beneficial effect against paracetamolinduced liver injury. Its hepatoprotective action may be related to reduction of oxidative stress, suppression of lipid peroxidation, recovery of antioxidant enzyme activity, and restoration of intracellular sulfhydryl balance (Du et al., 2016; Ramachandran & Jaeschke, 2017; Hassan et al., 2024).

Histopathological finding of nanoparticle treated group corroborate the biochemical and molecular data. APAP induced hepatic necrosis and renal tubular injury are well documented outcomes of N-acetyl-p-benzoquinone imine (NAPQI) formation, leading to glutathione depletion and lipid peroxidation-mediated oxidative stress (Jaeschke et al., 2020; Hinson et al., 2010).

Treatment with the selected phytochemical naoparticles restored hepatic integrity, supporting their strong antioxidant and anti inflammatory properties. The obtained results are consistent with the fact that previous studies showed that phenolic enriched plant extracts prevent hepatotoxicity by increasing the antioxidant enzymes activity and preventing the involvement of proinflammatory mediators (Younis et al., 2015; El-Baz et al., 2023).

CONCLUSION

The results suggest that nanoparticles prepared using Moringa oleifera have promising antioxidant and liver protective properties. Their protective effect against paracetamol induced liver damage indicates that these nanoparticles may have potential as a natural supportive option for liver health. This also highlights their possible value for future use in food, nutraceutical, and pharmaceutical products. Still, further studies are needed to better understand how they work and to confirm their safety before practical application.

Conflict of Interest: The authors declare no conflict of interest financial or otherwise.

REFERENCES

1. 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://pmc.ncbi.nlm.nih.gov/articles/PMC7398001/
2. Aebi, H. (1984). Catalase in vitro. Methods in Enzymology, 105, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3
3. Ahmad, M. Z., Alqahtani, M. S., Akhtar, S., Alshahrani, S. M., Alhakamy, N. A., Kumar, A., Alshehri, S., & Prasher, P. (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
4. 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., & Alzahrani, S. A. (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
5. 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
6. 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(3), 101896. https://doi.org/10.1016/j.jksus.2022.101896

7. Aravind, M., Amalanathan, M., Michael Mary, M. S., & Baskaran, I. (2021). Synthesis of TiO_2 nanoparticles by chemical and green synthesis methods and their multifaceted properties. SN Applied Sciences, 3, 409. https://doi.org/10.1007/s42452-021-04281-5
8. 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 pathways. Mediators of Inflammation, 2024, Article 3938136. https://doi.org/10.1155/2024/3938136
9. 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
10. Hamza, R. Z., & Al-Harbi, M. S. (2015). Amelioration of paracetamol hepatotoxicity and oxidative stress in 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. 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 modulation of iron-mediated oxidative stress and SIRT-1 expression in mice. Frontiers in Pharmacology, 15, 1384834. https://doi.org/10.3389/fphar.2024.1384834
12. Hinson, J. A., Roberts, D. W., & James, L. P. (2010). Mechanisms of acetaminophen-induced liver necrosis. In J. Uetrecht (Ed.), Adverse drug reactions (pp. 369–405). Springer. https://doi.org/10.1007/978-3-642-00663-0_12
13. Hussain, S., Ashafaq, M., Alshahrani, S., Siddiqui, R., Ahmed, R. A., Khuwaja, G., & Islam, F. (2020). Cinnamon oil protects against acetaminophen-induced liver toxicity by attenuating inflammation, oxidative stress, and apoptosis. Toxicology Reports, 7, 1296–1304. https://doi.org/10.1016/j.toxrep.2020.09.008
14. 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
15. 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
16. 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
17. Khan, F., Shariq, M., Asif, M., Siddiqui, M. A., Malan, P., & Ahmad, F. (2022). Green nanotechnology: Plant-mediated nanoparticle synthesis and applications. Nanomaterials, 12(4), 673. https://doi.org/10.3390/nano12040673
18. 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
19. Mamun, M. A. A., Rahman, A., Belal, S. H., Islam, M. A., Sarker, M. E. H., & Arman, M. S. I. (2015). Histological study of the effect of malathion on liver and kidney tissues in mice. International Journal of Pharmaceutical Sciences and Research, 6(3), 1043–1048.
20. 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.
21. Mohsin, F., 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
22. Ozer, J., Ratner, M., Shaw, M., Bailey, W., & Schomaker, S. (2008). The current state of serum biomarkers of hepatotoxicity. Toxicology, 245(3), 194–205. https://doi.org/10.1016/j.tox.2007.11.021
23. Ramachandran, A., & Jaeschke, H. (2017). Mechanisms of acetaminophen hepatotoxicity and their translation to human pathophysiology. Journal of Clinical and Translational Research, 3(Suppl. 1), 157–169. https://doi.org/10.18053/jctres.03.2017S1.002
24. 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
25. Sabir, S. M., Rocha, J. B. T., Athayde, M. L., & Boligon, A. A. (2017). Antioxidant activities and phenolic profile of Baccharis trimera. South African Journal of Botany, 113, 318–323.

26. Sadeer, N. B., Montesano, D., Albrizio, S., Zengin, G., & Mahomoodally, M. F. (2020). Versatility of antioxidant assays in food science. Antioxidants, 9(8), 709. https://doi.org/10.3390/antiox9080709
27. Sahraoui, A., Hamlaoui, M., Chikhi, S., Harrat, S., Baghriche, O., Zertal, A., & Vernuccio, S. (2025). Green synthesis of titanium dioxide nanoparticles using Eucalyptus globulus. Materials Today Sustainability, 31, 101193. https://doi.org/10.1016/j.mtsust.2025.101193
28. Salbitani, G., Bottone, C., & Carfagna, S. (2017). Determination of glutathione content. Bio-protocol, 7(13), e2372.
29. Thakur, B. K., Kumar, A., & Kumar, D. (2019). Green synthesis of titanium dioxide nanoparticles using Azadirachta indica. South African Journal of Botany, 124, 223–227. https://doi.org/10.1016/j.sajb.2019.05.024
30. 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
31. Zakaria, Z. A., Kamisan, F. H., Nasir, N. M. M., Teh, L. K., & Salleh, M. Z. (2019). Protective effect of Dicranopteris linearis against liver damage. Nutrients, 11(12), 2945. https://doi.org/10.3390/nu11122945
32. Zhou, Y., Tang, J., Tan, X., & You, Y. (2024). Hepatoprotective activity of Ginkgo biloba-mediated TiO_2 nanoparticles. ChemistrySelect. https://doi.org/10.1002/slct.202403066