

RESEARCH ARTICLE

***Moringa oleifera* leaf nanoemulsion attenuates polystyrene nanoplastic-induced hepatotoxicity and dysregulation of hepatic glucose metabolism in rats**

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ABSTRACT

Background and Aim: Polystyrene nanoplastics (PS-NPs) are emerging environmental contaminants that can induce hepatic oxidative stress, inflammation, apoptosis, and metabolic dysfunction. Although *Moringa oleifera* leaves contain diverse antioxidant and hepatoprotective phytochemicals, conventional extracts may have limited aqueous solubility and oral bioavailability. This study aimed to develop a *M. oleifera* leaf nanoemulsion and evaluate its protective effects against PS-NP-induced hepatotoxicity and dysregulation of hepatic glucose metabolism in rats.

Materials and Methods: Forty-eight male Wistar rats were randomly assigned to six groups (n = 8 per group): normal control, PS-NP control receiving 200 µg/kg body weight (BW)/day, positive control receiving PS-NPs with quercetin at 20 mg/kg BW/day, and three treatment groups receiving PS-NPs with *M. oleifera* leaf nanoemulsion at extract-equivalent doses of 10, 20, or 40 mg/kg BW/day for 28 days. The extract and nanoemulsion were characterized using phytochemical analyses, antioxidant assays, Fourier-transform infrared spectroscopy, dynamic light scattering, and transmission electron microscopy. Hepatoprotective effects were assessed using serum liver enzymes, hepatic antioxidant, inflammatory, and apoptotic markers, histopathology, quantitative gene expression analysis, and exploratory molecular docking.

Results: The nanoemulsion had a mean hydrodynamic diameter of 72.1 nm, a polydispersity index of 0.33, and a zeta potential of -36.09 mV. PS-NP exposure increased liver injury biomarkers and histopathological damage, suppressed superoxide dismutase and catalase, and increased tumor necrosis factor-α, interferon-γ, caspase-9, and caspase-3. PS-NPs also downregulated *Slc2a2*, *Pklr*, and *Gys2* and upregulated *Pck1*. Nanoemulsion treatment attenuated these biochemical, histological, inflammatory, apoptotic, and transcriptional alterations, with the most consistent protective responses observed at 10–20 mg/kg. Exploratory molecular docking indicated potential multitarget interactions of selected phytoconstituents, particularly β-sitosterol.

Conclusion: *Moringa oleifera* leaf nanoemulsion attenuated PS-NP-induced hepatotoxicity and partially normalized glucose metabolism-related gene expression. The findings provide experimental proof-of-concept for nanoemulsion-based delivery of *M. oleifera* phytoconstituents against nanoplastic-associated liver injury, although further pharmacokinetic and dose-response studies are warranted.

Keywords: antioxidant defense, glucose metabolism, hepatotoxicity, *Moringa oleifera*, nanoemulsion, oxidative stress, polystyrene nanoplastics, Wistar rats.

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INTRODUCTION

Polystyrene nanoplastics (PS-NPs) are among the most prevalent and extensively investigated nanoplastic pollutants because of their widespread environmental occurrence and increasing detection in biological systems, including human blood and liver tissue [1, 2]. Following ingestion, PS-NPs can cross the intestinal barrier, enter the portal circulation, and accumulate in the liver, a major organ responsible for xenobiotic metabolism and detoxification [2, 3]. Consequently, the liver is considered an important target organ for nanoplastic-induced toxicity. Accumulating evidence indicates that PS-NP exposure induces hepatic injury through oxidative stress, inflammatory activation, and apoptosis, resulting in hepatocellular damage and impaired liver function [4, 5].

Recent studies have further demonstrated that PS-NPs may disrupt hepatic metabolic homeostasis by altering the expression of genes involved in glucose uptake, glycolysis, glycogen synthesis, and gluconeogenesis [6, 7]. In particular, PS-NP exposure has been associated with suppression of glucose utilization pathways, characterized by reduced expression of *Slc2a2*, *Gck*, and *Pklr*, together with increased expression of gluconeogenic genes such as *Pck1* and *G6pc* [7]. These transcriptional alterations suggest a shift from glucose utilization toward glucose production, potentially contributing to hepatic dysfunction beyond conventional oxidative and inflammatory injury. Oxidative stress may play a pivotal role in this metabolic imbalance because excessive reactive oxygen species (ROS) generated following PS-NP exposure can impair mitochondrial function, activate inflammatory signaling pathways, and alter transcriptional regulators governing hepatic glucose metabolism [8, 9]. Thus, oxidative, inflammatory, apoptotic, and metabolic abnormalities may represent interconnected processes rather than isolated toxicological effects [3, 10].

Moringa oleifera Lam. (Moringaceae) is a well-documented medicinal plant with a long history of traditional use in South and Southeast Asia, including Indonesia. Its leaves have been used for the management of liver disorders, inflammatory conditions, and metabolic diseases [11]. These pharmacological properties have been attributed to the diverse phytochemical constituents of *M. oleifera* leaves, including phenolic acids, flavonoids, glucosinolate/isothiocyanate derivatives, terpenoids, tocopherols, and phytosterols [12, 13]. Previous experimental studies using conventional *M. oleifera* leaf extracts have demonstrated hepatoprotective effects and short-term tolerability at oral doses of 200–400 mg/kg under the evaluated experimental conditions [13]. However, these findings cannot be considered direct evidence of the safety of the nanoemulsion formulation evaluated in the present study. In addition to their hepatoprotective properties, constituents of *M. oleifera* have been reported to modulate hepatic metabolic pathways involved in glucose utilization and energy homeostasis, suggesting their potential utility in conditions associated with metabolic dysregulation [11].

Despite these potential biological activities, conventional *M. oleifera* extracts may be limited by poor aqueous solubility, low oral bioavailability, and degradation during gastrointestinal transit [14]. Nanoemulsion-based delivery systems offer a rational formulation strategy for improving the solubilization and physicochemical stability of bioactive phytochemicals and may facilitate their oral delivery [15, 16]. However, gastrointestinal stability, release kinetics, and systemic or hepatic exposure to phytochemicals were not evaluated in the present study. Therefore, enhanced bioavailability of the nanoemulsion formulation cannot be inferred from the current findings.

Despite growing evidence regarding PS-NP-induced hepatotoxicity and the hepatoprotective properties of *M. oleifera*, important knowledge gaps remain. Most investigations of nanoplastic-associated liver injury have focused primarily on conventional toxicological outcomes, including oxidative stress, inflammation, apoptosis, and histopathological damage, whereas the accompanying disruption of hepatic glucose metabolism at the transcriptional level has received comparatively limited attention [3, 6, 7, 10]. Moreover, although conventional *M. oleifera* extracts and nanoemulsion-based phytodelivery systems have demonstrated biological potential [13, 17], their capacity to simultaneously attenuate PS-NP-induced hepatic injury and modulate the associated transcriptional dysregulation of glucose metabolism remains largely unexplored. In particular, evidence integrating biochemical and histopathological indicators of hepatotoxicity with antioxidant, inflammatory, apoptotic, and glucose metabolism-related molecular responses is limited. Furthermore, the potential interactions between phytoconstituents of *M. oleifera* and molecular targets implicated in these toxicological processes have not been sufficiently explored in the context of PS-NP-induced hepatic injury. Addressing these gaps may provide a more comprehensive understanding of the protective potential of nanoemulsified *M. oleifera* against nanoplastic-associated hepatotoxicity.

Therefore, this study aimed to develop and characterize a *M. oleifera* leaf nanoemulsion and evaluate its

protective effects against PS-NP-induced hepatotoxicity in rats. Specifically, the study assessed changes in liver injury biomarkers and histopathological features; hepatic antioxidant defenses; inflammatory and apoptotic responses; and the expression of genes involved in glucose uptake, glycolysis, glycogen synthesis, and gluconeogenesis. In addition, an *in silico* molecular docking approach was used to explore potential interactions between selected *M. oleifera* phytoconstituents and molecular targets associated with oxidative stress, inflammation, and apoptosis. By integrating physicochemical characterization, biochemical assessment, histopathology, gene expression analysis, and *in silico* investigation, the study sought to provide experimental evidence regarding the potential of *M. oleifera* leaf nanoemulsion to attenuate PS-NP-associated hepatic injury and metabolic dysregulation.

MATERIALS AND METHODS

Ethical approval

All experimental procedures involving animals were conducted in accordance with the institutional ethical standards for the care and use of laboratory animals. The experimental protocol was reviewed and approved by the Ethical Clearance Commission, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia (Approval No. 0720/HRECC.FODM/VII/2025). The study used male Wistar rats (*Rattus norvegicus*), and animal handling, housing, treatment administration, anesthesia, sample collection, and euthanasia-associated experimental procedures were conducted under the approved protocol. Measures were taken throughout the experiment to ensure appropriate animal husbandry and to minimize unnecessary discomfort during experimental handling and sample collection.

Study period and location

The experimental work was conducted from July to October 2025 at Molecular Genetics Laboratory, Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia.

Study design

Male Wistar rats were obtained from the Faculty of Pharmacy, Universitas Airlangga. This experimental study included 48 male Wistar rats (*R. norvegicus*), aged 8–10 weeks and weighing 200–250 g. The animals were acclimatized and maintained under controlled environmental conditions at 22 ± 2 °C, 50–60% relative humidity, and a 12 h light/dark cycle, with *ad libitum* access to standard chow and water. Following acclimatization, the rats were randomly allocated to six groups (n = 8/group) (Figure 1): (i) normal control (C), receiving distilled water; (ii) PS-NP control (CN), receiving PS-NPs at 200 µg/kg BW/day; (iii) positive control (CP), receiving PS-NPs at 200 µg/kg BW/day plus quercetin at 20 mg/kg BW/day; and (iv–vi) treatment groups receiving PS-NPs at 200 µg/kg BW/day together with *M. oleifera* leaf nanoemulsion at target extract-equivalent doses of 10 mg/kg BW/day (P1), 20 mg/kg BW/day (P2), or 40 mg/kg BW/day (P3).

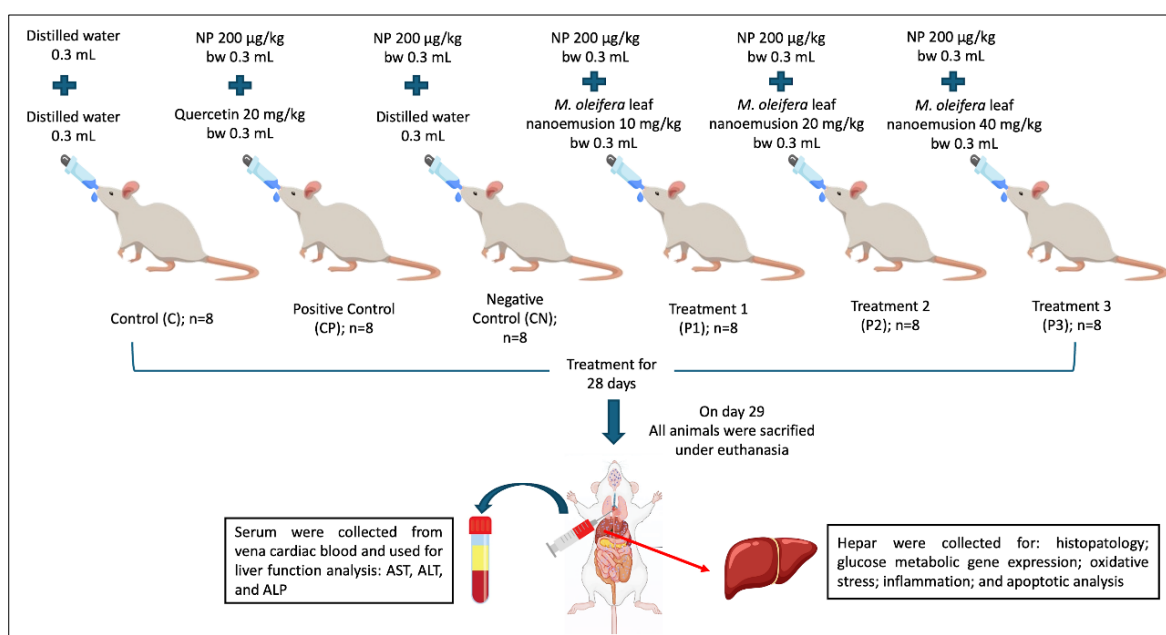


Figure 1: Experimental design

The 20 mg/kg BW/day dose was prospectively selected as the central exploratory nanoemulsion dose, corresponding to one-tenth of the previously reported effective dose of 200 mg/kg BW/day for conventional *M. oleifera* leaf extract [13], without assuming pharmacokinetic equivalence or greater bioavailability or potency. The 10 and 40 mg/kg BW/day doses represented one-half and twofold of the central dose, respectively. Quercetin served as the reference antioxidant comparator.

The nanoemulsion formulations were freshly vortexed before administration to ensure uniform dispersion. All treatments were administered by oral gavage once daily for 28 days. BW was recorded at baseline and every 2 weeks during treatment, and the clinical condition of each animal was monitored throughout the experiment. Dosing concentrations of PS-NPs, quercetin, and nanoemulsion formulations were adjusted according to the most recent individual BW, while the gavage volume was maintained at 0.3 mL/rat/day.

Twenty-four hours after the final administration, the rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally. Terminal blood collection was performed by cardiac puncture under anesthesia. Death was confirmed before organ collection, after which the livers were excised, rinsed with ice-cold saline, blotted, and divided for subsequent analyses. Aliquots were stored at -80°C for biochemical and gene expression analyses, whereas the remaining tissue was processed for histopathological examination. Liver weight was recorded at necropsy, and relative liver weight (%) was calculated as $(\text{liver weight}/\text{final BW}) \times 100$.

Chemicals and reagents

Polystyrene nanoplastics (PS-NPs; nominal size, ~ 100 nm; Cat. No. 43302), quercetin ($\geq 95\%$; Cat. No. Q4951), Folin–Ciocalteu reagent (Cat. No. 47641), gallic acid (Cat. No. G7384), aluminum chloride (AlCl_3 ; Cat. No. 206911), potassium acetate (Cat. No. 236497), and 2,2-diphenyl-1-picrylhydrazyl (DPPH; Cat. No. D9132) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical-grade virgin coconut oil (VCO), Span 80, Tween 80, phenoxyethanol, and ethanol (96%) were obtained from local commercial suppliers for nanoemulsion preparation, whereas pharmaceutical-grade 0.5% sodium carboxymethyl cellulose (Na-CMC) was used as the quercetin vehicle. Commercial kits for serum alanine aminotransferase (ALT; Cat. No. GD-GPT100), aspartate aminotransferase (AST; Cat. No. GD-GOT100), and alkaline phosphatase (ALP; Cat. No. GD-ALP100) determination were obtained from Linear Chemicals S.L.U. (Barcelona, Spain). Enzyme-linked immunosorbent assay (ELISA) kits for superoxide dismutase (SOD; Cat. No. E0168Ra), catalase (CAT; Cat. No. E0869Ra), tumor necrosis factor- α (TNF- α ; Cat. No. E0764Ra), interferon- γ (IFN- γ ; Cat. No. E0103Ra), caspase-3 (Cat. No. E1648Ra), and caspase-9 (Cat. No. AP01212) were purchased from Shanghai Korain Biotech Co., Ltd. (Shanghai, China). Total RNA was isolated using the Total RNA Mini Kit (Tissue; Cat. No. RT050; Geneaid Biotech Ltd., New Taipei City, Taiwan). Complementary DNA (cDNA) synthesis was performed using ReverTra Ace™ qPCR RT Master Mix (Code No. FSQ-201; Toyobo Co., Ltd., Osaka, Japan), whereas quantitative polymerase chain reaction (qPCR) was performed using THUNDERBIRD™ Next SYBR® qPCR Mix (Code No. QPX-201; Toyobo Co., Ltd., Osaka, Japan).

M. oleifera extraction and nanoemulsion preparation

Fresh *M. oleifera* leaves were obtained from and authenticated by Balai Materia Medica Batu, East Java, Indonesia (authentication No. 01/III.3.AU/D/2025), where a voucher specimen was deposited. The leaves were washed, shade-dried at room temperature ($\sim 25^{\circ}\text{C}$) to a constant weight, ground into powder, and stored in an airtight container until extraction. The powdered leaves were extracted by maceration in ethanol at a ratio of 1:10 (w/v) for 72 h at $\sim 25^{\circ}\text{C}$ with occasional stirring. The extract was filtered through Whatman No. 1 filter paper, concentrated under reduced pressure at 40°C , and stored at 4°C until further use. Qualitative phytochemical screening was performed using standard tests for flavonoids, terpenoids, sterols, alkaloids, and phenolics to characterize the extract profile [18].

Three nanoemulsion formulations with different extract loads (P1, P2, and P3) were prepared by varying the mass of the extract while maintaining a constant excipient composition. For each 100 mL batch, *M. oleifera* extract (0.6 g for P1, 1.3 g for P2, or 2.6 g for P3) was dissolved in VCO (19 mL), followed by the addition of Span 80 (1.4 mL), Tween 80 (14 mL), 96% ethanol (2.5 mL), and phenoxyethanol (0.1 mL). Distilled water was then added to a final volume of 100 mL. These quantities represented the extract loads used for formulation preparation; dosing concentrations were subsequently adjusted according to the most recent individual BW to maintain the target extract-equivalent doses described in the study design. The oil phase containing the extract and surfactants was homogenized, after which the aqueous phase was added dropwise under continuous stirring to produce a coarse emulsion. The resulting mixture was sonicated at 40–60% amplitude for 5–10 min.

Extract profiling and nanoemulsion characterization

Gas chromatography–mass spectrometry (GC–MS) analysis: The phytochemical composition of the *M. oleifera* leaf extract was analyzed using GC–MS to characterize the nonpolar and semivolatile phytochemical fractions, including terpenoids, sterols, and fatty acid derivatives. GC–MS was used for targeted phytochemical profiling and exploratory in silico prioritization rather than comprehensive metabolomic characterization. A 1 µL sample was injected into the GC–MS system, and chromatographic separation was monitored using the total ion chromatogram. The resulting chromatogram contained multiple peaks corresponding to different phytochemical constituents.

The detected compounds were tentatively identified by comparing their mass spectra with spectra available in the NIST mass spectral library database. Identification was based on the similarity index, retention time, and relative peak area obtained from the chromatographic profile. Compounds with greater relative abundance were considered major constituents and selected for further analysis. Fatty acid derivatives were not prioritized for molecular docking because of their widespread occurrence and potentially nonspecific hydrophobic interactions; therefore, structurally distinctive phytochemicals were selected for exploratory docking. Because GC–MS analysis was performed without authentic standards, retention-index confirmation, or complementary LC–MS/MS, compound identification was considered tentative and was not intended to represent a comprehensive phytochemical profile.

Total phenolic and flavonoid contents: Total phenolic content (TPC) was determined using the Folin–Ciocalteu method. Briefly, 0.5 mL of extract (1 mg/mL in ethanol) was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and incubated for 5 min. Subsequently, 2.0 mL of 7.5% Na₂CO₃ was added, and the mixture was incubated in the dark for 30 min. Absorbance was measured at 765 nm using a Multiskan GO microplate reader. TPC was quantified against a gallic acid standard and expressed as mg gallic acid equivalents (GAE)/g extract.

Total flavonoid content (TFC) was determined using the AlCl₃ assay. Briefly, 0.5 mL of extract (1 mg/mL) was mixed with 1.5 mL of ethanol, 0.1 mL of 10% AlCl₃, 0.1 mL of 1 M potassium acetate, and 2.8 mL of water. After incubation for 30 min, absorbance was measured at 415 nm. TFC was quantified against a quercetin standard and expressed as mg quercetin equivalents (QE)/g extract. All assays were performed in triplicate, and the results were expressed as mean ± Standard deviation (SD) [19].

DPPH radical scavenging activity: The DPPH assay was used as a preliminary cell-free assessment of radical scavenging activity to complement the TPC and TFC analyses. Antioxidant activity was evaluated using 0.1 mM DPPH in methanol. Extract solutions at concentrations of 20–100 µg/mL were mixed with DPPH and incubated for 30 min in the dark at ~25 °C. Absorbance was measured at 517 nm, and radical scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = (A_0 - A_s)/A_0 \times 100.$$

IC₅₀ values were derived from concentration–response curves. Measurements were performed in triplicate.

Fourier-transform infrared spectroscopy (FTIR) analysis: Functional groups in the dried extract were characterized using attenuated total reflectance-FTIR (ATR–FTIR) over a spectral range of 4000–400 cm^{–1}, at a resolution of 4 cm^{–1} and 32 scans. This analysis was performed as a qualitative complement to the TPC, TFC, and DPPH analyses.

Nanoemulsion characterization: Droplet size (Z-average), polydispersity index (PDI), and zeta potential were determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments, UK). Before analysis, samples were diluted 1:10–1:100 (v/v) with distilled/deionized water. Measurements were performed at ~25 °C with at least three independent readings per formulation, and the results were expressed as mean ± SD. Droplet morphology was examined by transmission electron microscopy (TEM). Diluted nanoemulsion samples were deposited onto carbon-coated copper grids, stained with phosphotungstic acid, air-dried, and imaged at an accelerating voltage of 80–120 kV [20].

Nanoemulsion stability assessment: A preliminary short-term refrigerated physicochemical stability assessment was performed by monitoring particle size and zeta potential during storage. Baseline measurements (Day 0) were obtained immediately after formulation preparation at ~25 °C. Subsequent measurements were performed after refrigerated storage at 4 °C on Days 14 and 28. Particle size and zeta potential were determined by DLS under the same analytical conditions described above.

The Day 0 measurement represented the immediate post-preparation baseline and did not constitute an

assessment of stability during ~25 °C storage. Long-term, room-temperature, accelerated, and freeze–thaw stability were not evaluated.

Preparation of PS-NPs and quercetin

Polystyrene nanoplastics (nominal size, ~100 nm; Sigma-Aldrich) were diluted from a 100 mg/mL stock solution with distilled/deionized water to provide an oral dose of 200 µg/kg BW/day, based on previous literature demonstrating reproducible hepatotoxic effects at this exposure level [6]. The suspension was sonicated for 10 min immediately before administration to minimize aggregation and improve dosing consistency.

Quercetin (≥95%; Sigma-Aldrich) was used as the reference antioxidant (positive control) and administered orally at 20 mg/kg BW/day. Because of its poor aqueous solubility, quercetin was suspended in 0.5% Na-CMC to provide a uniform gavage preparation. The doses and vehicles were selected based on previous reports of PS-NP toxicity and quercetin efficacy [6, 21].

Serum liver function analysis

ALT, AST, and ALP activities were quantified using commercial assay kits (Glory Kits, China) according to the manufacturer's instructions. Serum was obtained by allowing whole blood to clot, followed by centrifugation, and measurements were performed using freshly prepared reagents. ALT and AST activities were determined using kinetic spectrophotometric methods at 340 nm, whereas ALP activity was measured at 405 nm according to the kit protocols and previously reported procedures [14]. Each sample was analyzed in duplicate, and enzyme activities were calculated using the calibration or kinetic factors provided by the manufacturer.

ELISA analysis

Hepatic antioxidant enzymes (SOD and CAT), inflammatory cytokines (TNF-α and IFN-γ), and apoptotic markers (caspase-3 and caspase-9) were quantified in liver homogenates using commercial ELISA kits (BT-Lab, Shanghai, China) according to the manufacturer's protocols. Liver tissues were homogenized under cold conditions, and the resulting supernatants were collected for analysis. Protein-normalized aliquots were used to minimize intersample variability. Standards and samples were assayed in duplicate, and concentrations were calculated from standard curves generated for each plate. Absorbance was measured at 450 nm using a Thermo Scientific Multiskan GO microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Gene expression analysis

Total RNA was isolated from approximately 25 mg of liver tissue using the Mini Total RNA Kit (Geneaid, Taiwan) according to the manufacturer's instructions. RNA quantity and purity were assessed spectrophotometrically, and only samples with A260/A280 ratios of 1.8–2.0 were used for subsequent analyses. For cDNA synthesis, 1 µg of total RNA was reverse-transcribed using ReverTra Ace™ qPCR RT Master Mix (Toyobo, Japan) according to the recommended thermal program.

Quantitative PCR was performed using THUNDERBIRD™ Next SYBR® qPCR Mix (Toyobo, Japan) in a final reaction volume of 20 µL containing 2 µL of cDNA template, gene-specific forward and reverse primers (Table 1) [6, 22], SYBR master mix, and nuclease-free water.

Expression of the glucose metabolism-related genes *Slc2a2*, *Gck*, *Pklr*, *Gys2*, *G6pc*, and *Pck1* was normalized to *Actb* (β-actin), which served as the reference gene. These genes represented pathways involved in glucose transport/phosphorylation, glycolysis, glycogen synthesis, and gluconeogenesis. The qPCR program consisted of an initial denaturation at 95 °C for 10 min, followed by 40 cycles at 95 °C for 10 s and 57.5 °C for 5 s. Each biological sample was analyzed in triplicate technical reactions, and relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method with MyGo Pro software (version 3.5.2; <https://mygopcr.com/>).

Table 1: Primer sequences used for quantitative polymerase chain reaction.

Gene/function	Forward primer (5'–3')	Reverse primer (5'–3')	Reference
Glucose uptake <i>Slc2a2</i>	TTGGTGCCATCAACATGATCTTC	AGATGGCCGTCATGCTCACATA	[22]
Glycolysis <i>Gck</i>	AGTATGACCGGATGGTGGATGAA	CCAGCTTAAGCAGCACAAAGTCGTA	[22]
<i>Pklr</i>	ATCTGGGCAGATGATGTGGA	ATAGGGTGTAAGTGGTCAAGATGG	[22]
Glycogen metabolism <i>Gys2</i>	CATGAATGGCAGGCTGGAAC	GCTCCATGCAGTAGCGGTGA	[6]
Gluconeogenesis <i>G6pc</i>	TTAGAGGCAAAGGAGCCCAAG	GGGTGGAAACACAGGCATCA	[22]

Gene/function	Forward primer (5'–3')	Reverse primer (5'–3')	Reference
<i>Pck1</i> Housekeeping gene	CAGCCAATGTCCCATTATTGACC	TGCCAGCTGAGAGCTTCGTAGA	[6]
<i>Actb</i> (β-actin)	AGGCCCTCTGAACCTAAG	ATGTCACGCACGATTTCCCT	[6]

Histopathological analysis

Liver tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 4–5 µm, and stained with hematoxylin and eosin (H&E). Sections were examined using light microscopy (Olympus CX22, Japan) for hepatocellular swelling/ballooning, sinusoidal dilatation/congestion, nuclear pyknosis, necrosis, and inflammatory-cell infiltration.

For quantitative assessment, three lobules per liver were randomly selected, and three nonoverlapping fields per lobule were analyzed at a constant magnification, resulting in nine fields per animal. In each field, 100 hepatocytes were classified as normal, swollen/ballooned, or necrotic. Percentages were averaged across the nine fields to obtain a single value for each animal, and group data were expressed as mean ± SD. Inflammatory-cell infiltration was manually estimated as the percentage of the analyzed tissue-field area occupied by inflammatory-cell aggregates, identified as clusters of small hyperchromatic nuclei with scant cytoplasm. Inflammation was graded as normal (0–5%), mild (6–15%), moderate (16–30%), or moderate-to-severe (31–45%) based on an adapted liver inflammation scoring system [23]. All histopathological assessments were performed with the evaluator blinded to group allocation.

In silico screening and molecular docking

An *in silico* workflow was used to prioritize *M. oleifera* phytochemicals and explore their putative interactions with molecular targets associated with oxidative stress, inflammation, and apoptosis. Target proteins included SOD (UniProt P07895), CAT (P04762), IFN-γ (P01581), TNF-α (P16599), caspase-3 (P55213), and caspase-9 (Q9JHK1). Corresponding three-dimensional protein structures were obtained from public structural databases, including the Protein Data Bank, and prepared for molecular docking by removing nonessential heteroatoms and water molecules, adding polar hydrogens, assigning charges, and converting the structures to the required file format.

Candidate compounds were compiled from Dr. Duke's Phytochemical and Ethnobotanical Databases, and three-dimensional ligand structures were obtained from PubChem. Quercetin (PubChem CID: 5280343) was included as a reference compound. Candidate compounds were initially screened using PASS Online (<https://way2drug.com/PassOnline/predict.php>) based on Pa versus Pi values, and the three highest-ranked candidates were subsequently evaluated for drug-likeness according to Lipinski's Rule-of-Five using SCFBio (<https://scfbio-iitd.res.in/software/drugdesign/lipinski.jsp>) [24].

Molecular docking was performed using PyRx 0.8 (<https://sourceforge.net/projects/pyrx/>) with AutoDock Vina (<https://vina.scripps.edu/>), and protein–ligand interactions were visualized using PyMOL version 1.74 (<https://pymol.org/>) and BIOVIA Discovery Studio (<https://www.3ds.com/products/biovia/discovery-studio>). The best-scoring complexes were further explored using CABS-flex 3.0 (<https://lcbio.pl/cabsflex3/>) to assess residue-level flexibility based on root-mean-square fluctuation profiles. This analysis was exploratory and was not interpreted as confirmation of complex stability or molecular target engagement.

Statistical analysis

Data are presented as mean ± SD. Each endpoint was analyzed separately using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparisons post hoc test. Statistical significance was set at $p < 0.05$. No additional global multiplicity correction was applied across the distinct endpoints. Statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software (<https://www.graphpad.com/>), USA).

RESULTS

GC–MS analysis of *Moringa oleifera* leaf extract

GC–MS analysis of the *M. oleifera* leaf extract generated a complex chromatographic profile containing numerous peaks corresponding to volatile and semivolatile constituents (Figure 2a). Several hundred chromatographic peaks were detected, indicating a chemically diverse GC–MS-detectable fraction. To focus on the major constituents, compounds with a relative peak area >0.5% were selected for further evaluation. Based on this criterion, 13 compounds were tentatively identified as the predominant GC–MS-detected constituents of

the extract (Table 2).

The identified compounds represented several chemical classes, including terpenoids, fatty acid derivatives, sterols, and tocopherols. Constituents with comparatively high relative peak areas included 3,7,11,15-tetramethyl-2-hexadecen-1-ol, ethyl linoleate, tocopherol, hexadecanoic acid ethyl ester, neophytadiene, and β -sitosterol. These identifications represent tentative annotations based on mass spectral matching and should not be interpreted as definitive structural confirmation.

Table 2: Major phytochemical constituents of *Moringa oleifera* leaf extract identified by Gas chromatography–mass spectrometry.

No.	Compound	CAS	RT	Area	Area (%)
1	Loliolide	5989-02-6	27.820	82,718	0.74
2	Neophytadiene	504-96-1	28.391	364,872	3.29
3	Hexadecanoic acid, ethyl ester	628-97-7	30.220	461,663	4.16
4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	7541-49-3	31.758	1,288,838	11.60
5	Ethyl linoleate	544-35-4	32.515	1,078,309	9.71
6	Octadecanoic acid, ethyl ester	111-61-5	32.866	137,724	1.24
7	Pentacosane	629-99-2	40.295	68,633	0.62
8	Squalene	111-02-4	41.716	94,388	0.85
9	Triacontane	638-68-6	43.030	66,880	0.60
10	γ -Tocopherol	7616-22-0	45.435	153,051	1.38
11	Tocopherol	59-02-9	46.815	923,031	8.31
12	β -Sitosterol	83-46-5	51.004	328,641	2.96
13	Ergosta-5,24(28)-dien-3-ol	474-63-5	51.381	135,017	1.22

RT = Retention time; CAS = Chemical Abstracts Service registry number.

Phytochemical profile of *M. oleifera* leaf extract

Qualitative phytochemical screening demonstrated the presence of multiple classes of secondary metabolites in the *M. oleifera* leaf extract (Table 3). Positive reactions were observed for flavonoids, phenolic compounds, terpenoids, sterols, and alkaloids under the applied test conditions, confirming that these phytochemical classes were detectable in the crude extract before nanoemulsion formulation and *in vivo* administration.

Table 3: Phytochemical screening of *Moringa oleifera* leaf extract.

Phytochemical class	Result
Flavonoids	+
Phenolics	+
Terpenoids	+
Sterols	+
Alkaloids	+

(+) = Present.

Antioxidant capacity and FTIR profile of *M. oleifera* leaf extract

The *M. oleifera* leaf extract exhibited concentration-dependent DPPH radical scavenging activity (Figure 2b). The IC_{50} was 55.7 ± 2.3 μ g/mL (Table 4), demonstrating measurable radical scavenging activity under the applied cell-free assay conditions. Quantitative analysis showed a TPC of 47.0 ± 0.8 mg GAE/g extract and a TFC of 54.0 ± 0.6 mg QE/g extract.

FTIR analysis demonstrated several characteristic absorption bands in the extract (Figure 2c). A broad band at $3200\text{--}3500$ cm^{-1} was attributable to O–H stretching. Signals at $1600\text{--}1650$ cm^{-1} and $1450\text{--}1510$ cm^{-1} were consistent with aromatic C=C and carbonyl-related vibrations, whereas bands within $1200\text{--}1000$ cm^{-1} were attributable to C–O stretching.

Table 4: Phytochemical quantification and antioxidant properties of *Moringa oleifera* leaf extract.

Parameter	Value (mean $\pm$ SD)	Unit
Total phenolic content	47.0 ± 0.8	mg GAE/g extract
Total flavonoid content	54.0 ± 0.6	mg QE/g extract
DPPH radical scavenging activity (IC_{50})	55.7 ± 2.3	μ g/mL

GAE = Gallic acid equivalents; QE = Quercetin equivalents; DPPH = 2,2-diphenyl-1-picrylhydrazyl; IC_{50} = Half-maximal inhibitory concentration; SD = Standard deviation.

M. oleifera nanoemulsion characterization

Macroscopic examination showed a clear visual difference between the crude extract and the nanoemulsion. The crude extract appeared dark and turbid, whereas the nanoemulsion showed a lighter appearance and greater optical homogeneity (Figure 2d). TEM analysis demonstrated predominantly spherical, well-dispersed nanodroplets without apparent aggregation (Figure 2e). DLS analysis showed a Z-average hydrodynamic diameter of 72.1 nm and a PDI of 0.33 (Figure 2f), indicating a moderately narrow droplet size distribution. Zeta potential analysis showed a single peak centered at -36.09 mV (Figure 2g), consistent with appreciable electrostatic stabilization of the dispersion.

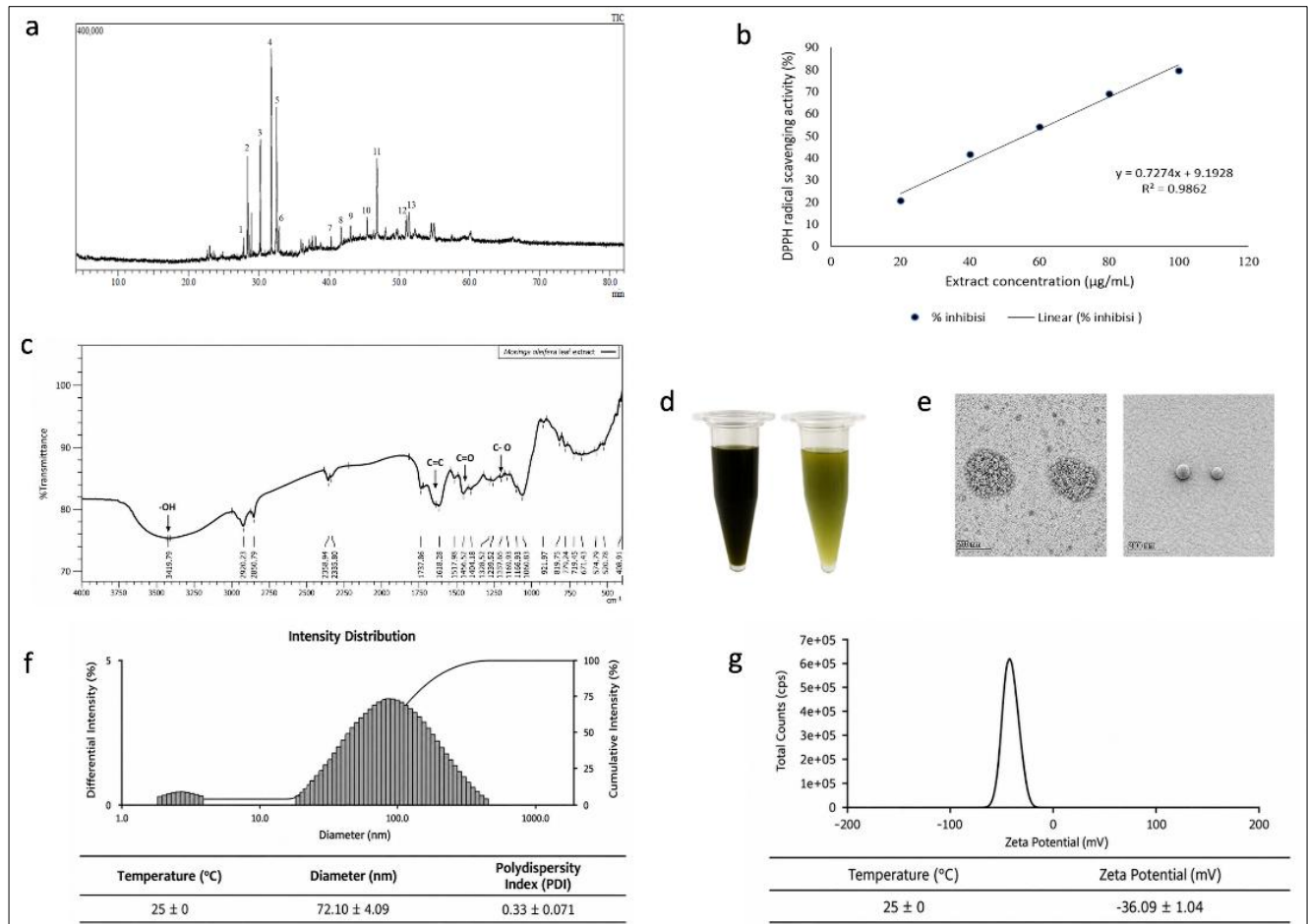


Figure 2: Physicochemical and antioxidant characterization of *M. oleifera* leaf extract and nanoemulsion. (a) GC–MS chromatogram showing numerous peaks corresponding to GC–MS-detectable phytochemical constituents. (b) Concentration–response profile of DPPH radical scavenging by *M. oleifera* leaf extract at 20–100 µg/mL, with IC_{50} derived from regression of percentage inhibition against concentration. (c) FTIR spectrum of the ethanolic *M. oleifera* leaf extract showing bands attributable to O–H stretching, aromatic C=C vibrations, carbonyl-related absorptions, and C–O stretching. (d) Representative photographs of the crude extract (left) and nanoemulsion (right), showing differences in optical appearance and homogeneity after formulation. (e) TEM micrograph of the nanoemulsion showing predominantly spherical and well-dispersed droplets without visible aggregation (scale bar = 200 nm). (f) DLS particle size distribution of the nanoemulsion showing a unimodal droplet population within the nanoscale range. (g) Zeta potential distribution of the nanoemulsion showing a uniform negative surface charge. GC–MS = Gas chromatography–mass spectrometry; DPPH = 2,2-diphenyl-1-picrylhydrazyl; IC_{50} = Half-maximal inhibitory concentration; FTIR = Fourier-transform infrared spectroscopy; TEM = Transmission electron microscopy; DLS = Dynamic light scattering.

Short-term physicochemical stability measurements showed only limited variation in particle size, PDI, and zeta potential during the evaluated storage period (Table 5). Particle size increased from 72.1 ± 4.09 nm at Day 0 to 76.3 ± 5.18 nm at Day 28, whereas the zeta potential changed from -36.09 ± 1.04 mV to -34.72 ± 1.17 mV.

Although the baseline measurement was obtained immediately after preparation at approximately 25 °C, subsequent measurements after refrigerated storage at 4 °C for 14 and 28 days showed maintenance of nanoscale particle dimensions and negative zeta potential. These findings indicate short-term physicochemical stability under the tested refrigerated conditions. The Day 0 measurement represented an immediate post-preparation

baseline only; room-temperature, accelerated, long-term, and freeze–thaw stability were not evaluated. **Table 5:** Physical stability profile of *Moringa oleifera* leaf nanoemulsion during storage.

Temperature (°C)	Storage time (day)	Particle size (nm)	PDI	Zeta potential (mV)
25	0	72.1 ± 4.09	0.33 ± 0.071	−36.09 ± 1.04
4	14	74.5 ± 2.37	0.34 ± 0.036	−35.21 ± 0.92
4	28	76.3 ± 5.18	0.35 ± 0.064	−34.72 ± 1.17

PDI = Polydispersity index. Day 0 measurements were obtained immediately after formulation preparation at room temperature (25 °C), whereas Day 14 and Day 28 measurements were obtained after refrigerated storage at 4 °C. Values are presented as mean ± SD.

Effects of PS-NP exposure and *M. oleifera* nanoemulsion on body weight (BW) and liver index

PS-NP exposure was associated with significant changes in final BW and liver indices. Final BW was significantly lower in the CN group than in the normal control group ($p < 0.0001$). Co-administration of the *M. oleifera* nanoemulsion significantly increased final BW in P1 and P2 compared with CN ($p = 0.0007$ and $p < 0.0001$, respectively), whereas P3 did not differ significantly from CN (Figure 3a).

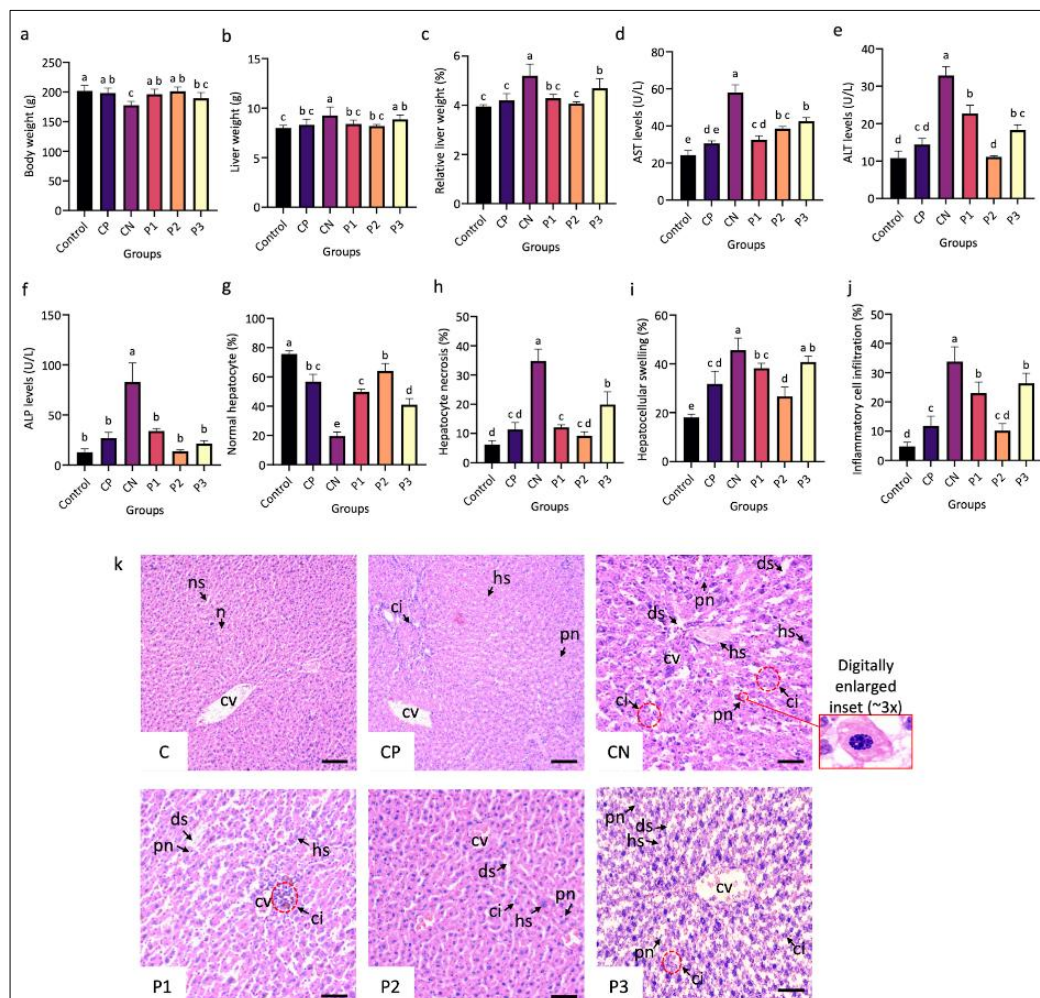


Figure 3: Effects of *M. oleifera* nanoemulsion on PS-NP-induced liver injury. (a) Final BW, (b) absolute liver weight, and (c) relative liver weight (liver index, %). Serum liver injury biomarkers: (d) AST, (e) ALT, and (f) ALP. Quantitative histomorphometry: (g) normal hepatocytes, (h) hepatocyte necrosis, (i) hepatocellular swelling/ballooning, and (j) inflammatory-cell infiltration (% area). Data are presented as mean ± SD; different superscript letters indicate significant differences according to one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). (k) Representative H&E-stained liver sections from C, CP, CN, and P1–P3. Arrows indicate swelling/ballooning (hs), pyknotic nuclei (pn), inflammatory-cell infiltration (ci), and sinusoidal dilatation/congestion (ds); cv = central vein; n = normal hepatocytes; ns = normal sinusoids. Dashed circles indicate representative inflammatory-cell aggregates. The CN inset shows a digitally enlarged region containing a pyknotic nucleus. Scale bar = 50 µm. C = Normal control; CP = Quercetin-treated positive control; CN = PS-NP control; P1–P3 = PS-NPs plus *M. oleifera* nanoemulsion at 10, 20, and 40 mg/kg BW/day, respectively; BW = Body weight; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALP = Alkaline phosphatase; H&E = Hematoxylin and eosin.

Absolute liver weight was significantly higher in CN than in the normal control group ($p = 0.0002$). This increase was attenuated in P1 and P2 ($p = 0.0221$ and $p = 0.0018$ versus CN, respectively), whereas P3 did not

differ significantly from CN (Figure 3b).

Relative liver weight was significantly higher in CN than in the normal control group ($p < 0.0001$). Nanoemulsion co-treatment significantly reduced relative liver weight in all treatment groups compared with CN (all $p \leq 0.0102$). Relative liver weights in P1 and P2 did not differ significantly from the normal control group, whereas that in P3 remained significantly elevated (Figure 3c).

Effects of *M. oleifera* nanoemulsion on PS-NP-induced liver enzyme dysregulation

PS-NP exposure significantly increased serum ALT, AST, and ALP activities compared with the normal control group (Figure 3d–f). Co-administration of the *M. oleifera* leaf nanoemulsion significantly reduced all three enzyme activities compared with CN. In P2, ALT and ALP activities did not differ significantly from those of the normal control group (both $p > 0.9999$), whereas AST activity remained significantly higher than that of the normal control group ($p = 0.0001$).

Effects of *M. oleifera* nanoemulsion on PS-NP-induced hepatic histopathological damage

Histopathological evaluation demonstrated marked hepatic alterations following PS-NP exposure. Quantitative histomorphometry showed that CN had a significantly lower proportion of normal hepatocytes and significantly greater hepatocellular swelling and necrosis than the normal control group (Figure 3g–i). Histological examination of CN showed ballooning degeneration/hepatocellular swelling, sinusoidal dilatation/congestion, inflammatory-cell infiltration, pyknotic nuclei, and focal necrosis (Figure 3k).

The *M. oleifera* leaf nanoemulsion-treated groups showed higher proportions of normal hepatocytes and lower proportions of swollen and necrotic hepatocytes than CN, accompanied by better-preserved hepatic architecture in the representative sections (Figure 3k). P1 and P2 showed the greatest improvement among the nanoemulsion-treated groups. P3 showed partial improvement; however, residual hepatocellular swelling, inflammatory-cell infiltration, and necrotic changes remained significantly greater than those in the normal control group ($p < 0.0001$).

Effects of *M. oleifera* nanoemulsion on endogenous hepatic antioxidant defenses

PS-NP exposure significantly reduced hepatic SOD and CAT levels in CN compared with the normal control group (both $p < 0.0001$) (Figure 4a and b). Co-administration of the *M. oleifera* leaf nanoemulsion increased both antioxidant markers relative to CN.

SOD levels were significantly higher in P1 and P2 than in CN ($p = 0.0005$ and $p < 0.0001$, respectively), whereas P3 did not differ significantly from CN ($p = 0.3388$). CAT levels were significantly higher in all nanoemulsion-treated groups than in CN, and the P2 value did not differ significantly from that of the normal control group ($p > 0.9999$).

Effects of *M. oleifera* nanoemulsion on PS-NP-induced pro-inflammatory cytokine production

PS-NP exposure significantly increased hepatic TNF- α and IFN- γ levels in CN compared with the normal control group (both $p < 0.0001$) (Figure 4c and d). Co-treatment with the *M. oleifera* leaf nanoemulsion reduced both cytokines compared with CN. Among the nanoemulsion-treated groups, P2 showed the greatest reductions in TNF- α and IFN- γ relative to CN (both $p < 0.0001$), with values approaching those of the normal control group.

Effects of *M. oleifera* nanoemulsion on intrinsic apoptosis-related markers in the liver

PS-NP exposure significantly increased hepatic caspase-9 and caspase-3 levels compared with the normal control group (both $p < 0.0001$) (Figure 4e and f). Co-administration of the *M. oleifera* leaf nanoemulsion significantly reduced both markers relative to CN. P2 showed the lowest caspase-3 and caspase-9 levels among the nanoemulsion-treated groups, whereas significant reductions relative to CN were also observed in P1 and P3 ($p \leq 0.0005$).

Effects of *M. oleifera* nanoemulsion on PS-NP-induced dysregulation of hepatic glucose metabolism-related genes

PS-NP exposure significantly altered the hepatic expression of several genes involved in glucose metabolism. Compared with the normal control group, *Slc2a2* and *Pklr* expression was significantly lower in CN ($p = 0.0039$ and $p = 0.0004$, respectively), whereas *Gck* showed a nonsignificant reduction ($p = 0.3945$) (Figure 5a–c). *Pck1* was significantly upregulated in CN ($p < 0.0001$), whereas *G6pc* showed a nonsignificant increase ($p = 0.1822$). Expression of the glycogen synthesis-related gene *Gys2* was significantly reduced ($p = 0.0101$) (Figure 5d–f).

Co-administration of the *M. oleifera* leaf nanoemulsion modulated these transcriptional alterations. P1 and P2 showed the most consistent expression patterns relative to CN, characterized by higher expression of *Slc2a2*,

Gck, *Pklr*, and *Gys2* and lower expression of *G6pc* and *Pck1*.

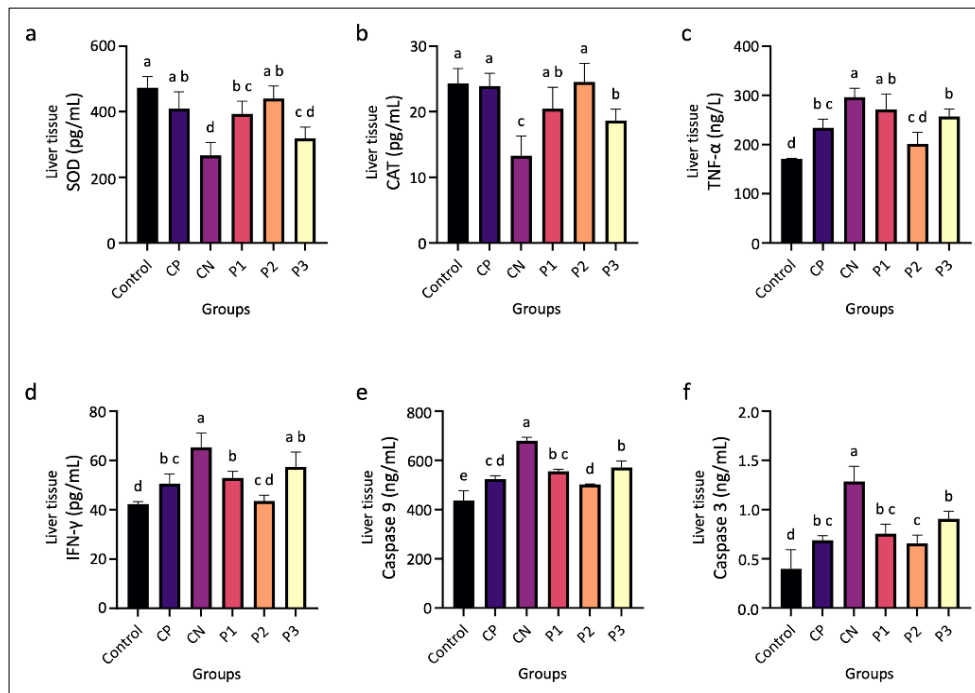


Figure 4: Hepatic antioxidant, inflammatory, and apoptotic markers following PS-NP exposure and nanoemulsion treatment. Levels of (a) SOD, (b) CAT, (c) TNF- α , (d) IFN- γ , (e) caspase-9, and (f) caspase-3 in liver tissue from C, CP, CN, and P1–P3. Data are presented as mean $\pm$ SD (n = 8/group). Bars with different superscript letters differ significantly (p < 0.05). C = Normal control; CP = Quercetin-treated positive control; CN = PS-NP control; P1–P3 = PS-NPs plus *M. oleifera* nanoemulsion at 10, 20, and 40 mg/kg BW/day, respectively; SOD = Superoxide dismutase; CAT = Catalase; TNF- α = Tumor necrosis factor- α ; IFN- γ = Interferon- γ .

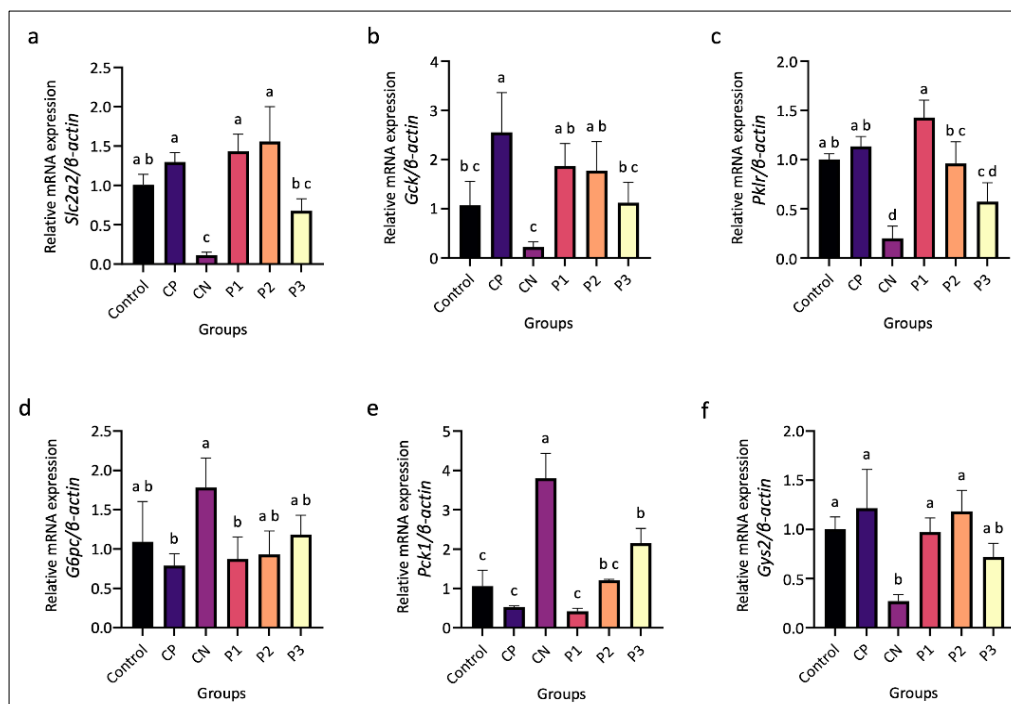


Figure 5: Hepatic glucose metabolism-related gene expression following PS-NP exposure and nanoemulsion treatment. Relative mRNA expression, normalized to *Actb* (β -actin), of (a) *Slc2a2*, (b) *Gck*, (c) *Pklr*, (d) *G6pc*, (e) *Pck1*, and (f) *Gys2* in liver tissues from C, CP, CN, and P1–P3. Data are presented as mean $\pm$ SD (n = 8/group). Bars with different superscript letters differ significantly (p < 0.05). C = Normal control; CP = Quercetin-treated positive control; CN = PS-NP control; P1–P3 = PS-NPs plus *M. oleifera* nanoemulsion at 10, 20, and 40 mg/kg BW/day, respectively.

In silico prediction and molecular docking

PASS screening predicted antioxidant-related activity for the evaluated *M. oleifera* phytochemicals, with Pa

values exceeding P_i for all three compounds (Table 6). Tocopherol showed the highest predicted probability of activity ($P_a = 0.927$), followed by β -sitosterol ($P_a = 0.815$) and neophytadiene ($P_a = 0.615$). These compounds were subsequently evaluated for drug-likeness using Lipinski's Rule-of-Five.

Table 6: PASS-predicted antioxidant activity of selected *M. oleifera* phytochemicals.

Compound	SMILES code	P_a	P_i
Neophytadiene	<chem>CC(C)CCCC(C)CCCC(C)CCCC(=C)C=C</chem>	0.615	0.021
β -Sitosterol	<chem>CCC@HC(C)C</chem>	0.815	0.004
Tocopherol	<chem>CC1=C(C=C2CCC(OC2=C1C)(C)CCCC(C)CCCC(C)CCCC(C)C)O</chem>	0.927	0.003

P_a = Probability of activity; P_i = Probability of inactivity; PASS = Prediction of Activity Spectra for Substances.

Neophytadiene, β -sitosterol, and tocopherol were further evaluated for drug-likeness according to Lipinski's Rule-of-Five (Table 7). All three compounds met the molecular weight criterion (<500 g/mol) and remained within the specified hydrogen-bond donor and acceptor limits. However, each compound exceeded the cLogP threshold, resulting in one Rule-of-Five violation for neophytadiene and two violations each for β -sitosterol and tocopherol.

Table 7: Drug-likeness assessment of selected compounds according to Lipinski's Rule-of-Five.

Compound	Compound ID	Molecular mass (g/mol)	HBA	HBD	LogP	Molar refractivity	Ro5 violations (n)
Neophytadiene	10446	278.52	0	0	7.17	97.31	1
β -Sitosterol	222284	414.71	1	1	8.02	133.23	2
Tocopherol	14986	416.68	2	1	8.53	134.31	2

HBA = Hydrogen-bond acceptor; HBD = Hydrogen-bond donor; Ro5 = Rule-of-Five. Thresholds: molecular weight ≤ 500 g/mol, HBA ≤ 10 , HBD ≤ 5 , cLogP ≤ 5 , and molar refractivity 40–130.

Exploratory molecular docking showed that β -sitosterol had the most favorable predicted binding energies across SOD, CAT, IFN- γ , TNF- α , caspase-3, and caspase-9, ranging from -6.4 to -8.1 kcal/mol. Neophytadiene showed comparatively weaker predicted binding energies, ranging from -3.8 to -6.1 kcal/mol (Figures 6a–f; Table 8). Tocopherol and quercetin showed intermediate or target-dependent binding energies. Several complexes showed high RMSD values, limiting interpretation of the docking poses and predicted interactions.

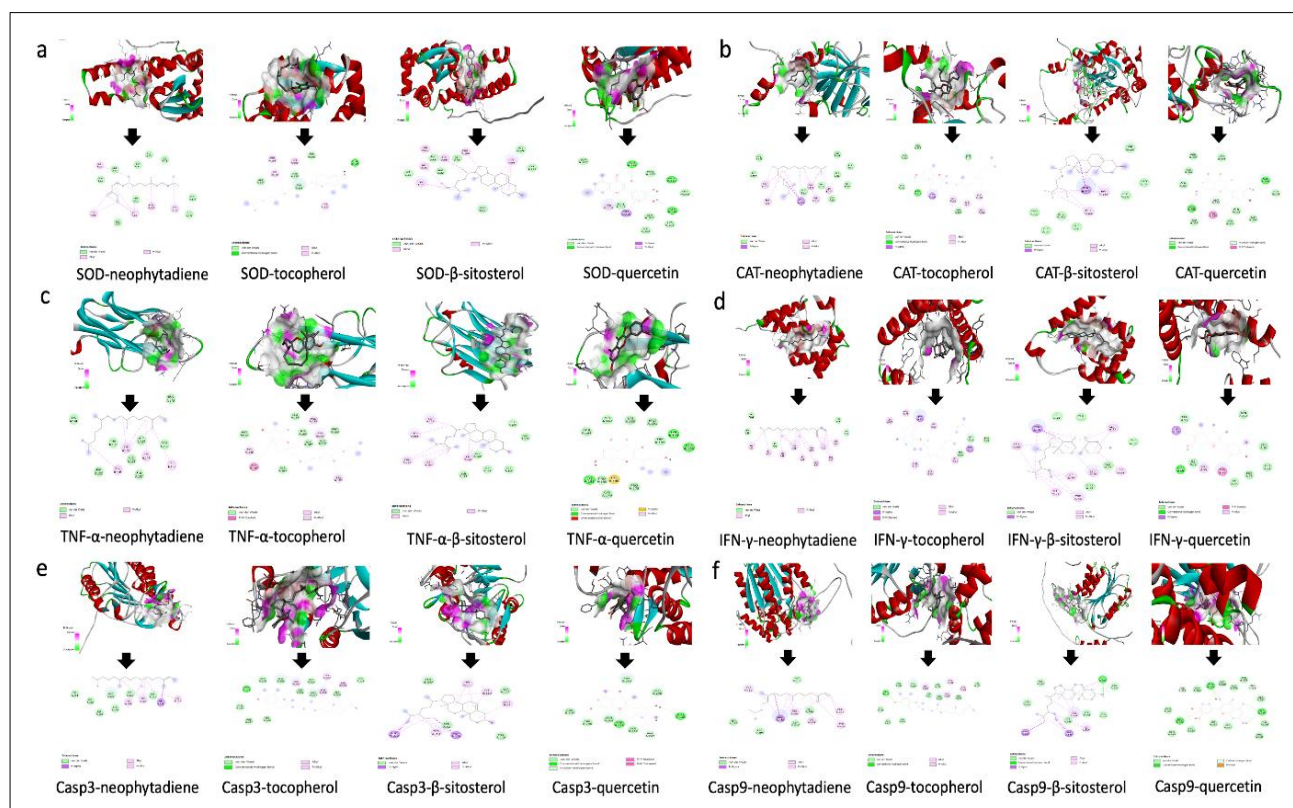


Figure 6: Molecular docking of selected *M. oleifera* phytoconstituents and quercetin with redox, inflammatory, and apoptotic targets. Predicted binding modes (top) and corresponding two-dimensional interaction maps (bottom) are shown for neophytadiene, tocopherol, β -sitosterol, and quercetin docked to (a) SOD, (b) CAT, (c) TNF- α , (d) IFN- γ , (e) caspase-3, and (f) caspase-9. Ligands are displayed within the predicted binding pockets, and interaction maps summarize predicted contacts, including hydrogen bonds, hydrophobic interactions, π - π interactions, and van der Waals contacts.

Table 8: Molecular docking of *M. oleifera* compounds and quercetin with SOD, CAT, IFN- γ , TNF- α , caspase-3, and caspase-9.

Compound	Receptor	CBA (kcal/mol)	RMSD	Type of Interaction	Amino Acids Involved in Interaction
Neophytadiene	SOD	-4.2	19.180	vdw	Arg23(A), Leu62(A), Glu66(A), Gln85(A), Gly92(A), Gly93(A), Ile96(A), Gly172(A), Thr173(A)
				HI	His26(A), Tyr69(A), Leu88(A), Lys89(A), Pro169(A)
	CAT	-5.5	10.674	vdw	Gln11(A), Ala79(A), Gly80(A), Ala81(A), Asp259(A), Arg263(A), Ile269(A), Asn321(A), Val329(A)
				HI	Ala8(A), Leu262(A), Leu265(A), Pro322(A), Tyr325(A)
	IFN- γ	-6.1	2.479	Pi-sigma	Phe266(A)
				vdw	Lys34(A), Ser39(A), Glu58(A), Ile71(A), Phe108(A)
				HI	Tyr75(A), Leu33(A), Phe37(A), Trp55(A), Ile72(A), Tyr75(A), Leu78(A), Phe79(A), Ile95(A), Leu99(A)
	TNF- α	-3.8	9.306	vdw	Gly147(A), Pro178(A), Lys181(A), Asp182(A), Thr183(A), Ala187(A), Glu188(A), Pro191(A)
				HI	Cys148(A), Pro184(A), Lys190(A), Trp192(A)
	Caspase-3	-5.1	7.582	vdw	Ser205(A), Arg207(A), Asn208(A), Ser209(A), Trp214(A), Phe250(A), Ser250(A)
				HI	Cys163(A), Tyr204(A), Trp206(A)
	Caspase-9	-5.7	24.737	Pi-sigma	Phe256(A)
				vdw	Asp347(A), Ser350(A), Asp394(A), Ser397(A), Gly433(A)
				HI	Trp392(A), Trp400(A), Lys432(A), Val434(A), Tyr435(A)
Tocopherol	SOD	-5.5	45.056	Pi-Sigma	Phe346(A)
				vdw	Thr136(A), Ala137(A), Glu133(A), Trp205(A)
				HI	Pro198(A), Lys202(A), Val140(A), Lys132(A)
	CAT	-7.1	4.186	PHI	Glu129(A)
				vdw	Gln11(A), Ala323(A), Asn324(A), Asn321(A)
				HI	Ala8(A), Phe266(A), Arg263(A), Leu262(A)
	IFN- γ	-6.7	5.459	PHI	Pro322(A)
				Pi-sigma	Tyr325(A)
				vdw	Ser39(A), Leu78(A), Leu82(A)
	TNF- α	-4.4	1.606	HI	Leu99(A), Phe79(A), Ile95(A), Leu30(A), Lys34(A), Leu33(A)
				Pi-sigma	Tyr75(A), Phe37(A)
				vdw	Pro178(A), Ala187(A), Leu189(A), Thr183(A), Gly147(A), Glu188(A), Pro180(A)
	Caspase-3	-6.4	1.450	HI	Lys190(A), Pro184(A), Cys148(A), Lys181(A)
				Pi-stacked	Trp192(A)
				vdw	Glu248(A), Phe247(A), Ser249(A), Asn208(A), Phe250(A), Ser251(A), Asp253(A), Arg207(A), Ser205(A), His121(A)
				HI	Trp206(A), Phe256(A), Tyr204(A), Cys163(A)
				PHI	Trp214(A)

Compound	Receptor	CBA (kcal/mol)	RMSD	Type of Interaction	Amino Acids Involved in Interaction
β -sitosterol	Caspase-9	-6.4	1.599	vdw	Ser280(A), His281(A), Ala279(A), Gln358(A), Ser297(A), Ser295(A), Arg13(A), Gly293(A)
				HI	Arg10(A), Tyr357(A), Val14(A), Cys294(A)
				UAA	Glu359(A)
	SOD	-6.9	13.001	vdw	Arg23(A), Glu66(A), Gln85(A), Gly92(A), His95(A), Gly172(A), Thr173(A)
				HI	His26(A), Leu62(A), Leu88(A), Lys89(A), Ile96(A), Pro169(A)
	CAT	-8.0	13.008	vdw	Ala8(A), Arg68(A), Gly80(A), Ser120(A), Arg263(A), Pro322(A), Phe326(A)
				HI	Leu262(A), Phe266(A)
	IFN- γ	-8.1	5.006	Pi-sigma	Tyr325(A)
				vdw	Phe37(A), Ser39(A), Glu68(A), Phe79(A), Ile95(A)
	TNF- α	-6.4	27.503	HI	Leu30(A), Leu33(A), Lys34(A), Trp55(A), Ile71(A), Ile72(A), Leu99(A)
				Pi-sigma	Tyr75(A)
				vdw	Gly119(A), Asp121(A), Pro130(A), Gln166(A), Lys206(A), Gly207(A)
				HI	Lys123(A), Val128(A), Phe161(A), Ile163(A), Leu209(A)
Quercetin	Caspase-3	-7.2	35.607	vdw	Ser62(A), Gly122(A), Phe128(A), Arg207(A)
				HI	Met61(A), His121(A), Cys163(A), Tyr204(A)
	Caspase-9	-7.6	32.054	Pi-sigma	Trp206(A), Phe256(A)
				vdw	Asp331(A), Gly333(A), Glu335(A), Phe338(A), Ser430(A), Lys436(A)
				HI	Phe334(A), Ile438(A)
				PHI	Val336(A)
	SOD	-6.6	2.171	Pi-sigma	Phe386(A), Phe389(A)
				vdw	Ile208(A), Lys108(A), Gly109(A), Glu211(A), Gly111(A), Pro113(A), Gly115(A)
				HI	Lys114(A)
				PHI	Gly110(A), Ser214(A), Glu112(A)
	CAT	-7.9	1.392	Pi-sigma	Trp210(A)
				vdw	Pro368(A), Asn369(A), Gln387(A), Leu371(A), Ala384(A), Asp396(A), Gln398(A), Asn397(A), Asp389(A)
	IFN- γ	-6.1	4.344	PHI	Arg388(A), Val383(A), Gln372(A)
				Pi-pi T-shaped	His395(A)
				vdw	Asn102(A), Trp55(A), Leu30(A), Leu33(A), Phe79(A), Ile95(A), Ile71(A)
				HI	Leu99(A)
	TNF- α	-6.1	44.138	PHI	Glu68(A)
				Pi-pi stacked	Tyr75(A)
				Pi-sigma	Ile72(A)
				vdw	Trp192(A), Pro191(A), Lys190(A), Gly147(A), Thr183(A), Pro180(A), Cys179(A), Pro178(A)
				PHI	Lys181(A), Glu194(A), Tyr193(A)
				Pi-sulfur	Cys148(A)

Compound	Receptor	CBA (kcal/mol)	RMSD	Type of Interaction	Amino Acids Involved in Interaction
	Caspase-3	-6.8	1.587	vdw	Asn208(A), Ser249(A), Trp206(A), Arg207(A), Phe250(A), Glu248(A)
	Caspase-9	-7.4	10.219	PHI	Glu246(A), Trp214(A), Phe247(A)
				vdw	Arg362(A), Gly360(A), Ser280(A), Ala279(A), Tyr357(A), Ala287(A), Val296(A), Arg300(A), Ser297(A), His281(A), Leu282(A), Gln283(A)
				PHI	Ser295(A), Glu359(A), Glu299(A), Gln358(A), Pro361(A)

Vdw = Van der Waals interaction; HI = Hydrophobic interaction; PHI = Polar hydrogen interaction; UAA = Unfavorable acceptor-acceptor; RMSD = Root Mean Square Deviation; CBA = Complex binding affinity; SOD = Superoxide dismutase; CAT = Catalase; IFN- γ = Interferon- γ ; TNF- α = Tumor necrosis factor- α .

CABS-flex analysis of the β -sitosterol-protein complexes showed mean RMSF values of approximately 1.191–2.932 Å across the evaluated targets (Figures 7a–f). These values provide an exploratory estimate of residue-level flexibility under the modeled conditions and were not considered evidence of confirmed ligand-protein stability or target engagement.

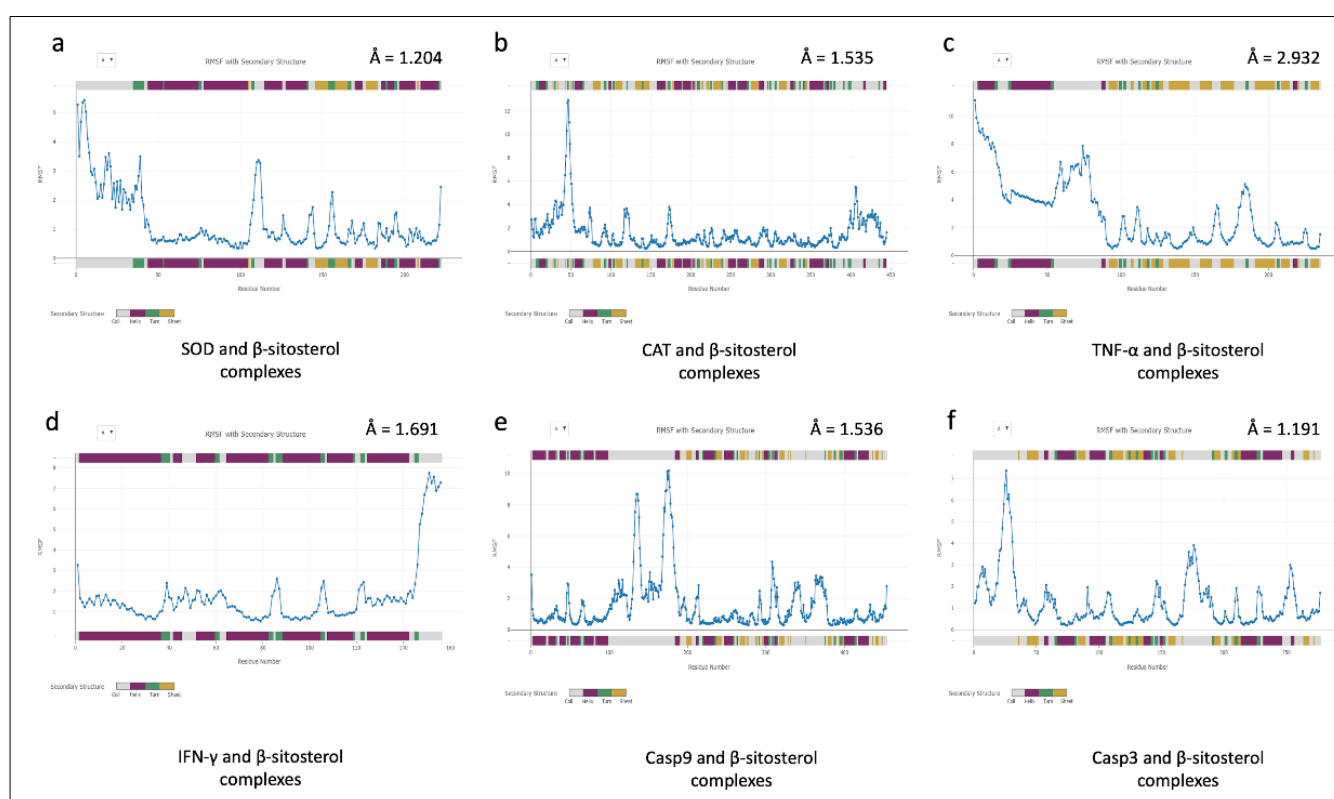


Figure 7: Residue-level flexibility of β -sitosterol-protein complexes predicted using CABS-flex. RMSF profiles are shown for β -sitosterol complexed with (a) SOD, (b) CAT, (c) TNF- α , (d) IFN- γ , (e) caspase-9, and (f) caspase-3. Secondary-structure tracks indicate residue segments classified as coil, helix, turn, or β -sheet. Mean RMSF values are reported in the corresponding panels. These profiles represent modeled residue-level flexibility and do not confirm ligand-protein binding stability or *in vivo* target engagement.

DISCUSSION

Phytochemical characteristics and nanoemulsion formulation

The present study provides experimental evidence that nanoemulsion-based delivery of *M. oleifera* leaf extract attenuated PS-NP-induced hepatotoxicity, accompanied by changes in oxidative, inflammatory, apoptotic, and glucose metabolism-related transcriptional endpoints. Previous studies on *M. oleifera* have predominantly evaluated conventional extracts in other models of hepatotoxicity [13, 17], whereas earlier PS-NP studies have primarily focused on toxicity mechanisms without evaluating a *M. oleifera* nanoemulsion [6, 10]. Thus, the present study extends existing evidence by integrating phytochemical characterization with biochemical, histopathological, transcriptional, and exploratory *in silico* assessments in a PS-NP-induced hepatotoxicity model.

To characterize the phytochemical properties potentially associated with the observed hepatoprotective

effects, the *M. oleifera* leaf extract was evaluated before formulation. Qualitative screening demonstrated the presence of flavonoids, phenolics, terpenoids, sterols, and alkaloids, indicating retention of several major phytochemical classes before *in vivo* administration. This profile is consistent with previous reports showing that 96% ethanolic extracts of *M. oleifera* leaves contain polyphenolic and terpenoid constituents with antioxidant and anti-inflammatory properties [25]. The extract also demonstrated measurable DPPH radical scavenging activity and substantial total phenolic and flavonoid contents. In addition, FTIR analysis revealed bands associated with hydroxyl-rich and aromatic functional groups characteristic of phenolic and flavonoid structures [26]. Nevertheless, the DPPH assay represents a cell-free assessment of radical scavenging capacity and should not be interpreted as direct evidence of inhibition of lipid peroxidation *in vivo*. Collectively, these findings establish the presence of antioxidant-associated phytochemical constituents that could plausibly contribute to the biological responses observed following treatment.

Flavonoids and phenolic compounds may protect hepatocytes through ROS scavenging, limitation of lipid peroxidation, and preservation of membrane integrity, thereby reducing enzyme leakage during toxic injury [27]. Terpenoids and sterols have also been associated with modulation of inflammatory pathways, membrane properties, and mitochondrial function, which may influence hepatocyte survival during xenobiotic stress [28, 29]. Alkaloids may contribute through regulation of intracellular pathways associated with redox homeostasis and inflammation rather than acting predominantly as direct radical scavengers [30]. However, these mechanisms were not individually evaluated in the present study and should therefore be considered potential explanations rather than demonstrated mechanisms.

Nanoemulsion formulation may facilitate phytochemical delivery by improving the solubilization and physicochemical stability of lipophilic and amphiphilic constituents [16, 31]. The nanoscale droplet size, negative zeta potential, and short-term refrigerated physicochemical stability observed in this study support the formation of a relatively uniform dispersion with limited aggregation under the evaluated conditions [32]. However, gastrointestinal stability, release kinetics, mucosal absorption, pharmacokinetics, and systemic or hepatic phytochemical exposure were not evaluated. Consequently, improved oral bioavailability, intracellular availability, or hepatic delivery cannot be concluded from the present findings.

Effects on BW and liver indices

PS-NP exposure reduced final BW and increased absolute and relative liver weights, indicating systemic effects accompanied by hepatic alterations [10]. Increased liver weight and liver index have been associated with hepatocellular swelling, edema, congestion, and inflammatory remodeling during toxicant-induced hepatic injury [3]. In related PS-NP exposure models, increased liver weight or hepatosomatic index has been reported together with inflammatory infiltration and metabolic disturbances, supporting the utility of organ-weight alterations as indicators of hepatic responses to sustained nanoplastic exposure [33].

In the present study, nanoemulsion co-treatment attenuated these changes, with P1 and P2 generally showing the most consistent responses. These effects occurred alongside improvements in serum liver enzyme activities and histopathological findings, supporting concordance among organ-level and tissue-level outcomes. Self-nanoemulsifying drug delivery systems have similarly been investigated for phytochemical delivery in experimental hepatotoxicity models [34, 35]. Nevertheless, because pharmacokinetic parameters were not determined in the present study, the observed protective effects cannot be attributed specifically to enhanced bioavailability.

Attenuation of PS-NP-induced hepatic injury

PS-NP exposure produced a hepatotoxic phenotype characterized by increased serum ALT, AST, and ALP activities, inflammatory-cell infiltration, histopathological abnormalities, and increased apoptosis-related markers. ALT is predominantly a cytosolic hepatocellular enzyme and is commonly used as an indicator of hepatocellular injury, whereas AST occurs in both cytosolic and mitochondrial compartments and may increase during more extensive cellular and mitochondrial injury [8]. Increased ALP activity may indicate hepatobiliary involvement and has also been reported following nano- and microplastic exposure [36].

PS-NPs can accumulate in the liver and have been associated with disruption of mitochondrial membrane potential and increased lipid peroxidation, processes that may compromise cellular membrane integrity and promote leakage of intracellular enzymes into the circulation [6, 37]. In the present study, co-administration of the *M. oleifera* leaf nanoemulsion significantly reduced ALT, AST, and ALP activities relative to the PS-NP group, with P1 and P2 generally showing the most consistent effects. These biochemical changes occurred together with

improvements in antioxidant-associated, inflammatory, apoptosis-related, and histopathological endpoints, supporting an overall hepatoprotective response without establishing a specific causal mechanism [38].

Modulation of antioxidant defenses and inflammatory responses

Oxidative stress appears to be an important component of PS-NP-induced hepatotoxicity. PS-NP exposure significantly reduced hepatic SOD and CAT levels, indicating impairment of antioxidant-associated defenses and a potentially diminished capacity to counteract ROS accumulation. Similar alterations in antioxidant enzyme systems have been reported in experimental nanoplastic exposure models, in which persistent ROS generation has been associated with mitochondrial impairment, cytokine activation, and apoptotic signaling [7, 39]. Reduced SOD and CAT activity may facilitate oxidative damage to cellular membranes and mitochondrial components and activate redox-sensitive inflammatory pathways [40].

Co-administration of the *M. oleifera* nanoemulsion significantly increased hepatic SOD and CAT levels relative to the PS-NP group, indicating partial restoration of antioxidant-associated defenses [41]. These changes were accompanied by reductions in inflammatory markers, suggesting an association between improved antioxidant status and attenuation of inflammatory responses. Redox-sensitive pathways such as NF- κ B may participate in this interaction [8, 29]; however, NF- κ B activation and downstream signaling were not directly evaluated and therefore cannot be established as mechanisms in the present study.

Consistent with the oxidative alterations, PS-NP exposure significantly increased hepatic TNF- α and IFN- γ levels. Similar cytokine responses have been associated with NF- κ B-related inflammatory signaling in experimental nanoplastic toxicity models [42–44]. Sustained cytokine production may contribute to hepatic injury through recruitment and activation of inflammatory cells, maintenance of ROS-generating immune responses, and increased susceptibility of hepatocytes to cell death [45]. Consequently, inflammatory activation may amplify tissue injury through reciprocal interactions with oxidative stress [46].

Co-administration of the *M. oleifera* leaf nanoemulsion reduced TNF- α and IFN- γ levels relative to the PS-NP group, with P2 showing the most consistent reduction. Previous studies have reported anti-inflammatory effects of *M. oleifera* bioactive constituents, including flavonoids, through modulation of inflammatory signaling and cytokine production [13, 46, 47]. The concurrent improvement in antioxidant-associated markers and reduction in inflammatory cytokines observed in the present study are therefore consistent with attenuation of the oxidative stress–inflammation axis. However, the present data demonstrate associations among these endpoints rather than direct pathway-specific effects.

Attenuation of apoptosis-related responses

Oxidative and inflammatory stress may contribute to activation of intrinsic apoptosis during PS-NP-induced hepatic injury [8]. In the present study, PS-NP exposure significantly increased hepatic caspase-9 and caspase-3 levels. These findings are consistent with increased intrinsic apoptosis-related signaling reported following nanoplastic-associated mitochondrial dysfunction and cytochrome *c* release [38]. Caspase-9 functions as an initiator caspase in the intrinsic apoptotic pathway, whereas caspase-3 mediates downstream execution events associated with cellular dismantling and apoptotic cell death [48].

Co-administration of the *M. oleifera* leaf nanoemulsion significantly reduced caspase-9 and caspase-3 levels relative to the PS-NP group, indicating attenuation of apoptosis-related responses. This effect occurred concurrently with improved antioxidant-associated markers and reduced inflammatory cytokines. Improved redox status and reduced inflammatory signaling could potentially decrease mitochondrial and inflammatory stimuli associated with apoptosis [49]. Nevertheless, mitochondrial membrane potential, cytochrome *c* release, and other upstream apoptotic regulators were not measured; therefore, a direct mechanism linking nanoemulsion treatment to inhibition of the intrinsic apoptotic pathway cannot be established.

Modulation of hepatic glucose metabolism-related gene expression

PS-NP exposure altered the expression of genes involved in hepatic glucose handling and energy metabolism. Expression of *Slc2a2* and *Pklr* was significantly reduced, whereas *Gck* showed a nonsignificant reduction. *Slc2a2* encodes GLUT2, which mediates high-capacity glucose transport across hepatocyte membranes, whereas *Gck* encodes glucokinase, which phosphorylates glucose to glucose-6-phosphate and facilitates its subsequent utilization [22]. The observed transcriptional pattern is consistent with reduced expression of components involved in hepatic glucose uptake and utilization, although the nonsignificant change in *Gck* should not be interpreted as definitive downregulation.

PS-NP exposure was also associated with significantly reduced *Gys2* expression and significantly increased

Pck1 expression, whereas *G6pc* showed a nonsignificant increase. Together, these findings indicate reduced expression of genes associated with glucose utilization and glycogen synthesis alongside increased expression of a key gluconeogenic gene. Similar alterations have been reported in experimental nanoplastic studies in which oxidative and mitochondrial stress were associated with disruption of hepatic glucose metabolism [6, 7, 9].

Although FoxO1 and PGC-1 α were not evaluated, the increased *Pck1* expression and directional increase in *G6pc* are compatible with transcriptional patterns that may occur during activation of gluconeogenic regulatory pathways [50]. However, involvement of FoxO1, PGC-1 α , insulin signaling, or related upstream pathways cannot be inferred directly from the present data. Similarly, although reduced *Pklr* expression could potentially influence glycolytic capacity and cellular energy metabolism [51], ATP production and metabolic flux were not measured.

Nanoemulsified *M. oleifera* partially modulated these transcriptional alterations, particularly in P1 and P2, which showed higher expression of *Slc2a2*, *Gck*, *Pklr*, and *Gys2* and lower expression of *Pck1* and *G6pc* relative to the PS-NP group. These changes occurred alongside improvements in antioxidant-associated and inflammatory markers, suggesting an association between attenuation of oxidative-inflammatory stress and modulation of glucose metabolism-related gene expression. However, functional hepatic glucose metabolism was not directly assessed, and the transcriptional findings should not be interpreted as evidence of restored glucose flux, insulin sensitivity, or glycogen metabolism.

Histopathological evidence of hepatoprotection

Histopathological findings supported the biochemical and molecular evidence of PS-NP-induced hepatic injury. PS-NP exposure resulted in hepatocellular ballooning/swelling, sinusoidal congestion and dilatation, inflammatory-cell infiltration, pyknotic nuclei, and focal necrosis. Similar hepatic alterations have been reported in rodent models of nanoplastic-induced liver injury [52]. Sinusoidal abnormalities may accompany microvascular and lobular disturbances, whereas inflammatory-cell infiltration has been reported together with increased cytokine production during toxicant-induced hepatic inflammation [53]. Furthermore, sustained oxidative and mitochondrial injury may contribute to progression from potentially reversible cellular degeneration to irreversible hepatocellular death [54].

The nanoemulsion-treated groups showed better preservation of hepatic architecture, fewer degenerative changes, reduced inflammatory-cell infiltration, and diminished necrotic alterations relative to the PS-NP group. Similar histological improvements have been reported following antioxidant- and polyphenol-based interventions in experimental models of plastic particle-associated hepatic injury [4, 40]. The concordance between histological improvement and reductions in serum liver enzymes, inflammatory cytokines, and apoptosis-related markers, together with improvement in antioxidant-associated endpoints, strengthens the evidence for an overall hepatoprotective effect rather than an isolated alteration in circulating biomarkers [55].

Non-monotonic dose-response pattern

The protective response was not strictly dose-proportional. P1 and P2 generally showed more consistent improvements than P3 across biochemical, inflammatory, apoptosis-related, transcriptional, and histopathological endpoints. Biphasic or non-monotonic responses have been described for phytochemical-rich preparations, in which biological effects may vary according to concentration and experimental context [17]. Higher formulation loads may also alter physicochemical behavior, cellular interactions, or biological handling of a formulation [56, 57].

However, the present study did not evaluate biodistribution, dose-dependent absorption, cellular uptake, or potential pro-oxidant effects at higher formulation loads. Consequently, the less consistent response observed with P3 should not be interpreted as evidence of toxicity or absorption saturation. Rather, the findings demonstrate a non-monotonic response pattern within the evaluated dose range and indicate the need for dedicated dose-response and formulation studies.

Exploratory *in silico* findings

Molecular docking provided exploratory information regarding potential interactions between selected *M. oleifera* phytoconstituents and proteins associated with antioxidant defense, inflammation, and apoptosis. The candidate ligands showed variable predicted binding energies toward SOD, CAT, TNF- α , IFN- γ , caspase-3, and caspase-9. β -Sitosterol generally showed the most favorable predicted binding energies across the evaluated targets, whereas neophytadiene showed weaker predicted affinities.

These docking results provide hypotheses regarding possible molecular interactions that could be investigated experimentally. However, predicted binding affinities varied among targets, and several complexes

exhibited high RMSD values, substantially limiting mechanistic interpretation. Furthermore, docking does not demonstrate biochemical inhibition, activation, binding stability, or cellular target engagement. Therefore, these findings should be regarded as exploratory and hypothesis-generating rather than mechanistic confirmation of the *in vivo* effects.

Study limitations and future perspectives

Several limitations should be considered when interpreting the present findings. Although the nanoemulsion demonstrated nanoscale physicochemical characteristics and short-term refrigerated stability over 28 days, encapsulation efficiency and release kinetics were not evaluated. Gastrointestinal stability, mucosal absorption, pharmacokinetics, biodistribution, and systemic or hepatic phytochemical exposure were also not determined. Consequently, enhanced bioavailability or hepatic delivery cannot be concluded. In addition, room-temperature, accelerated, long-term, and freeze–thaw stability were not evaluated.

Assessment of hepatic glucose metabolism was limited to transcriptional endpoints and did not include fasting blood glucose, insulin concentrations or signaling, hepatic glycogen content, protein expression, enzyme activity, or metabolic flux. Therefore, the gene expression findings cannot establish functional restoration of hepatic glucose metabolism. In addition, GC–MS compound identification was tentative because authentic standards and complementary confirmatory analytical approaches were not employed. Molecular docking and CABS-flex analyses were predictive and exploratory and require biochemical and experimental validation.

Future studies should incorporate comprehensive formulation characterization, including encapsulation efficiency and release profiles, together with pharmacokinetic and biodistribution analyses. Functional metabolic endpoints, protein-level validation, enzyme activity, insulin signaling, hepatic glycogen assessment, and metabolic flux measurements would help determine whether transcriptional modulation translates into functional metabolic improvement. Additional dose–response studies are also required to clarify the non-monotonic treatment pattern and determine an appropriate effective dose range.

Environmental-health relevance

From an environmental-health perspective, these findings are relevant to growing concerns regarding oral exposure to nanoplastics through contaminated food and water and their potential hepatic effects. The partial protection observed with a food-plant-derived nanoemulsion provides experimental proof-of-concept for investigating phytochemical-based approaches to mitigate nanoplastic-associated hepatic injury. However, the experimental exposure conditions and rat model do not establish effectiveness under environmentally relevant human exposure scenarios. Further studies incorporating environmentally relevant exposure levels, longer exposure periods, pharmacokinetic characterization, and appropriate translational models are required before broader implications for human health can be established.

Overall, the *in vivo* findings, complemented by exploratory *in silico* analyses, demonstrate that *M. oleifera* leaf nanoemulsion attenuated PS-NP-induced hepatotoxicity. The protective response was accompanied by improvement in antioxidant-associated markers, attenuation of inflammatory and apoptosis-related responses, preservation of hepatic architecture, and partial modulation of glucose metabolism-related gene expression, with P1 and P2 generally producing the most consistent responses.

CONCLUSION

The *M. oleifera* leaf nanoemulsion attenuated PS-NP-induced hepatotoxicity in rats, as demonstrated by improvements in serum liver enzyme activities, hepatic histopathology, antioxidant-associated markers, inflammatory cytokines, and apoptosis-related markers. PS-NP exposure significantly downregulated *Slc2a2*, *Pklr*, and *Gys2* and upregulated *Pck1*, whereas changes in *Gck* and *G6pc* were not statistically significant. Nanoemulsion co-treatment partially modulated these transcriptional alterations toward the normal control pattern, with P1 and P2 generally showing the most consistent protective responses. The treatment effect was not strictly dose-proportional.

The concurrent improvement in biochemical, histopathological, inflammatory, apoptosis-related, and transcriptional endpoints provides experimental proof-of-concept for the potential use of *M. oleifera* leaf nanoemulsion as a phytochemical-based approach for mitigating PS-NP-associated hepatic injury. The nanoscale droplet size, negative zeta potential, and short-term refrigerated physicochemical stability further support the suitability of the formulation for continued experimental development. However, these findings should not be extrapolated to human use or environmentally relevant exposure scenarios without further validation.

A major strength of this study was the integrated evaluation of PS-NP-induced hepatic injury using complementary biochemical, histopathological, antioxidant-associated, inflammatory, apoptosis-related, and glucose metabolism-related transcriptional endpoints. The study also combined phytochemical profiling and nanoemulsion characterization with *in vivo* assessment and exploratory *in silico* analysis. This integrated approach enabled comparison of tissue-level injury with molecular and biochemical responses while providing preliminary hypotheses regarding interactions of selected phytoconstituents with relevant molecular targets.

Overall, the *M. oleifera* leaf nanoemulsion attenuated PS-NP-induced hepatic injury and was associated with improvement in antioxidant defenses, attenuation of inflammatory and apoptosis-related responses, preservation of hepatic architecture, and partial modulation of glucose metabolism-related gene expression. P1 and P2 produced the most consistent responses within the tested dose range. These findings establish a foundation for further investigation of *M. oleifera* nanoemulsion as a potential strategy against nanoplastic-associated hepatotoxicity while emphasizing the need for functional metabolic, pharmacokinetic, formulation, and translational validation before broader conclusions can be drawn.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

GENERATIVE AI DECLARATION

The authors declare that generative artificial intelligence (AI) tools were used solely to improve language, grammar, and readability during manuscript preparation. All scientific content, data analysis, interpretation of results, and conclusions were developed and verified by the authors. The authors take full responsibility for the accuracy, integrity, and originality of the work presented, and no AI tool was listed as an author.

AUTHORS' CONTRIBUTIONS

PAIS: Conceptualization, investigation, formal analysis, data curation, and writing – original draft. AH: Conceptualization, supervision, data curation, and writing – review and editing. FS: Methodology, validation, writing – original draft, and writing – review and editing. MP: Investigation and data curation. MAH: Investigation and software. SM: Investigation, data curation, and formal analysis. MH: Investigation and data curation. LAA: Investigation, data curation, and methodology. AM: Investigation and formal analysis. AR: Investigation. SCD: Investigation and data curation. RJKS: Methodology and investigation. AA: Conceptualization and supervision. VL: Supervision, validation, and writing – review and editing. PAIS and FS: Contributed equally to this work and share first authorship. All authors have read and approved the final manuscript.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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