Hepatoprotective and Antioxidant Activity of Standardized Herbal Extract of *Moringa Oleifera* in Paracetamol and Isoniazid Induced Hepatotoxicity in Rats

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ABSTRACT

Background: Drug-induced liver injury remains a major clinical challenge, with oxidative stress playing a central role in hepatocellular damage. *Moringa oleifera* is a medicinal plant rich in antioxidant phytoconstituents that may offer hepatoprotective benefits.

Objective: This study evaluated the hepatoprotective and antioxidant potential of the ethanolic extract of *Moringa oleifera* (MOEE) against isoniazid- and paracetamol-induced hepatotoxicity in rats.

Methods: Hepatotoxicity was induced using isoniazid (50 mg/kg) and paracetamol (60 mg/kg). Rats received MOEE orally at doses of 100, 200, and 400 mg/kg for 21 days, while silymarin (50 mg/kg) served as the reference drug. Hepatoprotective activity was assessed by estimating serum biochemical markers, endogenous antioxidant parameters, histopathological alterations, and in vitro free radical scavenging activity.

Results: MOEE significantly attenuated drug-induced hepatotoxicity by reducing serum liver biomarkers and improving antioxidant status. In the isoniazid model, the 400 mg/kg dose reduced ALT from 245.95 ± 2.15 to 201.63 ± 0.13 IU/L, ALP from 110.58 ± 3.21 to 55.21 ± 1.25 IU/L, and total bilirubin from 4.42 ± 0.18 to 3.02 ± 0.25 mg/dL. In the paracetamol model, ALT decreased from 93.15 ± 2.02 to 64.05 ± 0.83 IU/L, ALP from 260.71 ± 4.41 to 152.24 ± 2.02 IU/L, and total bilirubin from 4.61 ± 0.04 to 2.91 ± 0.30 mg/dL. MOEE also restored endogenous antioxidant defenses, reduced lipid peroxidation, and markedly improved liver histopathology. In vitro antioxidant assays demonstrated potent free radical scavenging activity, with IC_{50} values of 62.8 µg/mL for hydroxyl radicals, 65.0 µg/mL for lipid peroxidation, 116 µg/mL for DPPH radicals, and 362 µg/mL for nitric oxide radicals.

Conclusion: The ethanolic extract of *Moringa oleifera* exhibited significant hepatoprotective and antioxidant activities against isoniazid- and paracetamol-induced liver injury. The observed effects are likely mediated through attenuation of oxidative stress, restoration of endogenous antioxidant defenses, and preservation of hepatic architecture, supporting its potential as a natural therapeutic candidate for drug-induced hepatotoxicity.

Keywords: *Moringa oleifera*; Hepatoprotection; Drug-induced liver injury; Oxidative stress; Antioxidant activity; Isoniazid; Paracetamol.

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1. INTRODUCTION

The liver is a vital organ responsible for the metabolism, detoxification, and elimination of endogenous and exogenous substances. Owing to its central role in xenobiotic metabolism, the liver is highly susceptible to injury caused by drugs, toxins, alcohol, and environmental chemicals. Drug-induced liver injury (DILI) remains one of the leading causes of acute liver failure worldwide and continues to be a major concern in clinical practice and pharmaceutical development [1,2].

Isoniazid and paracetamol are among the most widely used drugs associated with hepatotoxicity. Isoniazid, a first-line antitubercular agent, undergoes hepatic metabolism to generate reactive intermediates that induce oxidative stress, mitochondrial dysfunction, and hepatocellular necrosis [3]. Similarly, excessive paracetamol administration results in the formation of the toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI), which depletes intracellular glutathione reserves and promotes oxidative damage, ultimately leading to severe liver injury [4]. Oxidative stress and lipid peroxidation are therefore recognized as major mechanisms underlying chemically induced hepatotoxicity [5].

Natural products have gained considerable attention as potential hepatoprotective agents because of their antioxidant, anti-inflammatory, and membrane-stabilizing properties. Among these, *Moringa oleifera* Lam. (Moringaceae), commonly known as

the drumstick tree, is widely used in traditional medicine and is rich in bioactive phytochemicals including flavonoids, phenolic acids, tannins, glucosinolates, vitamins, and carotenoids [6,7]. These phytoconstituents exhibit potent antioxidant activity by scavenging reactive oxygen species, inhibiting lipid peroxidation, and enhancing endogenous antioxidant defense systems, thereby protecting tissues from oxidative damage [8].

Although several studies have reported the pharmacological potential of *M. oleifera*, comparative evaluation of its hepatoprotective efficacy against different chemically induced liver injury models remains limited. Therefore, the present study was designed to investigate the hepatoprotective and antioxidant effects of the ethanolic extract of *Moringa oleifera* leaves using both isoniazid- and paracetamol-induced hepatotoxicity models in rats. Hepatic function was evaluated through serum biochemical markers, endogenous antioxidant status, histopathological examination, and in vitro free radical scavenging assays to provide a comprehensive assessment of its hepatoprotective potential.

Figure 1. ***Moringa oleifera*** **plant**

2. Materials and Methods

2.1 Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. Isoniazid, paracetamol, and silymarin were procured from Yarrow Chem (Mumbai, India). Ethanol and other analytical reagents were purchased from standard commercial suppliers, including Merck (Mumbai, India), S.D. Fine Chemicals (Mumbai, India), SRL Chemicals (Mumbai, India), Nice Chemicals (Cochin, India), Sigma-Aldrich (USA), and Loba Chemie (Mumbai, India). Commercial diagnostic kits for the estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, and direct bilirubin were obtained from Erba Diagnostics Mannheim GmbH (Germany). All reagents were freshly prepared before use.

2.2 Plant Material and Authentication

2.2 Plant Material and Authentication

Fresh leaves of *Moringa oleifera* Lam. were collected from Dausa District, Rajasthan, India. The plant was taxonomically authenticated by the Botanical Survey of India (BSI), Jodhpur Regional Centre, Rajasthan, India. A voucher specimen (Ref. No. A.12012/Tech./2024-25(Pl.Id.)/492) was deposited in the institute herbarium for future reference [9]. The authenticated plant used in the present investigation is shown in Figure 1.

2.3 Preparation of Ethanolic Extract

The freshly collected leaves were washed thoroughly with running tap water followed by distilled water to remove adhering dust and extraneous matter. The leaves were shade-dried at room temperature (25–30°C) until a constant weight was achieved and subsequently pulverized into a coarse powder using a mechanical grinder. Approximately 500 g of the powdered plant material was extracted with 90% ethanol using a Soxhlet extraction apparatus. Extraction was continued until the siphon tube became colorless, indicating exhaustive extraction of phytoconstituents. The resulting extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary vacuum evaporator at a temperature not exceeding 45°C. The concentrated extract was further dried in a vacuum desiccator to obtain a semisolid mass. The dried ethanolic extract was transferred into an amber-colored airtight container and stored at 4°C until further pharmacological evaluation [10].

2.4 Preliminary Phytochemical Screening

The ethanolic extract of *Moringa oleifera* leaves was subjected to preliminary qualitative phytochemical screening using standard methods to identify major classes of secondary metabolites, including alkaloids, carbohydrates, glycosides, flavonoids, tannins, saponins, steroids, proteins, and triterpenoids. Preliminary phytochemical screening was performed according to standard methods described by Harborne and Trease & Evans [11].

2.5 Experimental Animals

Healthy adult Wistar albino rats of either sex weighing 150–200 g were used for the experimental study. The animals were procured from Bilwal Medchem and Research Laboratory Pvt. Ltd., India,

and housed in clean polypropylene cages containing sterile rice-husk bedding under standard laboratory conditions (temperature 23 ± 2°C, relative humidity 50–60%, and a 12 h light/12 h dark photoperiod). Animals had free access to standard pellet diet and filtered drinking water *ad libitum*. Prior to the initiation of the experiment, the animals were acclimatized to laboratory conditions for one week. All experimental procedures were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Bilwal Medchem and Research Laboratory Pvt. Ltd. (Registration No. 2304/PO/Rc/S/2024/CCSEA).

2.6 Acute Oral Toxicity Study

The acute oral toxicity of the ethanolic extract of *Moringa oleifera* (MOEE) was evaluated according to the Organisation for Economic Co-operation and Development (OECD) Guideline 425 (Up-and-Down Procedure). Healthy adult Wistar albino rats of either sex were fasted overnight with free access to water before dosing. The extract was administered orally at a limit dose of 2000 mg/kg body weight using an oral gavage. Following administration, animals were continuously observed during the first 4 h for any behavioral or neurological abnormalities and periodically monitored for 14 days for signs of toxicity or mortality. Based on the safety profile observed during the acute toxicity study, three doses of MOEE (100, 200, and 400 mg/kg body weight) were selected for subsequent hepatoprotective evaluation.

2.7 Experimental Design

The hepatoprotective activity of the ethanolic extract of *Moringa oleifera* (MOEE) was evaluated using isoniazid- and paracetamol-induced hepatotoxicity models. For each model, animals were assigned to six groups comprising a normal control, hepatotoxic control, silymarin-treated (50 mg/kg), and MOEE-treated groups receiving 100, 200, and 400 mg/kg [12].

2.7.1 Isoniazid-Induced Hepatotoxicity Model

Animals received their respective treatments orally once daily for 21 consecutive days. Hepatotoxicity was induced from Day 17 by oral administration of isoniazid (50 mg/kg) to all groups except the normal control. The standard group received silymarin (50 mg/kg, p.o.), whereas the treatment groups received MOEE at doses of 100, 200, or 400 mg/kg together with isoniazid. Animals were sacrificed on Day 21, and blood and liver samples were collected for biochemical, antioxidant, and histopathological analyses [13].

2.7.2 Paracetamol-Induced Hepatotoxicity Model

Animals received their respective treatments orally once daily for 21 consecutive days. Hepatotoxicity was induced from Day 17 by oral administration of paracetamol (60 mg/kg) to all groups except the normal control. The standard group received silymarin (50 mg/kg, p.o.), whereas the treatment groups received MOEE at doses of 100, 200, or 400 mg/kg together with paracetamol. Animals were sacrificed on Day 21, and blood and liver samples were collected for biochemical, antioxidant, and histopathological analyses [14].

2.8 Sample Collection

At the end of the experimental period (Day 21), animals were anesthetized with light ether, and blood samples were collected through the retro-orbital plexus. Serum was separated by centrifugation at 3000 rpm for 15 min and stored at −20°C until biochemical analysis. The animals were then euthanized, and the liver was excised, washed with ice-cold normal saline, blotted dry, and weighed. Liver tissues were divided for histopathological examination and antioxidant analysis [15].

2.9 Serum Biochemical Analysis

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, and direct bilirubin were determined using commercially available diagnostic kits (Erba Diagnostics Mannheim GmbH, Germany) according to the manufacturer's instructions [16].

2.10 Preparation of Liver Homogenate

Immediately after sacrifice, liver tissues were excised, washed thoroughly with ice-cold normal saline to remove residual blood, blotted dry, and weighed. Approximately 10% (w/v) liver homogenate was prepared in ice-cold phosphate buffer (0.1 M, pH 7.4) using a Teflon-glass homogenizer under chilled conditions. The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C, and the clear supernatant was collected for the estimation of endogenous antioxidant enzymes and oxidative stress markers. All analyses were performed immediately or the samples were stored at −20°C until biochemical analysis [17].

2.11 Evaluation of Hepatic Antioxidant Status

2.11.1 Estimation of Reduced Glutathione (GSH)

The reduced glutathione content in liver homogenate was determined using Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid), DTNB). Briefly, an aliquot of liver homogenate was mixed with trichloroacetic acid to precipitate proteins and centrifuged to obtain a clear supernatant. The supernatant was reacted with DTNB in phosphate buffer, and the absorbance of the resulting yellow-colored complex was measured spectrophotometrically at 412 nm. The glutathione concentration was calculated from a standard calibration curve and expressed relative to tissue protein content.

2.11.2 Estimation of Superoxide Dismutase (SOD)

Superoxide dismutase activity was estimated by measuring the inhibition of epinephrine auto-oxidation under alkaline conditions. The reaction mixture consisted of carbonate buffer, EDTA, liver homogenate, and epinephrine solution. The increase in absorbance due to adrenochrome formation was monitored at 480 nm using a UV-visible spectrophotometer. One unit of SOD activity was defined as the amount of enzyme required to inhibit epinephrine auto-oxidation by 50% under the assay conditions [18].

2.11.3 Estimation of Catalase (CAT)

Catalase activity was determined by monitoring the decomposition of hydrogen peroxide in phosphate buffer. The reaction was initiated by adding liver homogenate to freshly prepared hydrogen peroxide solution, and the decrease in absorbance was measured spectrophotometrically at 240 nm at predetermined time intervals. Catalase activity was expressed as units per milligram of protein.

2.11.4 Estimation of Lipid Peroxidation (TBARS)

Lipid peroxidation in liver tissue was estimated by measuring thiobarbituric acid reactive substances (TBARS). Liver homogenate was mixed with trichloroacetic acid and thiobarbituric acid reagent and heated in a boiling water bath to facilitate color development. After cooling and centrifugation, the absorbance of the pink chromogen was recorded at 532 nm. The extent of lipid peroxidation was expressed as malondialdehyde (MDA) equivalents [19].

2.11.5 Estimation of Total Protein

The total protein concentration of liver homogenate was determined by the Biuret method using commercially available reagents. Protein concentration was measured spectrophotometrically at 540 nm with bovine serum albumin as the reference standard. The measured protein concentration was used for normalization of antioxidant enzyme activities [20].

2.11.6 Estimation of Total Thiol Content

Total thiol content was estimated using Ellman's reagent (DTNB). Liver homogenate was mixed with phosphate buffer and DTNB solution, and the absorbance of the yellow-colored product was measured spectrophotometrically at 412 nm. Total thiol concentration was calculated using the molar extinction coefficient of DTNB and expressed relative to tissue protein content [21].

2.12 Histopathological Examination

Following blood collection, liver tissues were immediately excised, rinsed with ice-cold normal saline to remove residual blood, and fixed in 10% neutral buffered formalin for at least 24 h. The fixed tissues were processed by dehydration through ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Paraffin blocks were sectioned into 4–5 µm thick slices using a rotary microtome [22]. The tissue sections were mounted on clean glass slides, deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E). The stained sections were examined under a light microscope by a histopathologist blinded to the treatment groups. Histopathological evaluation focused on hepatic architecture, hepatocellular degeneration, necrosis, inflammatory cell infiltration, sinusoidal congestion, fatty changes, and other treatment-related morphological alterations. Representative photomicrographs were captured using a digital microscope for qualitative assessment.

2.13 Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism software (Version 8.0 or later, GraphPad Software Inc., San Diego, CA, USA). Comparisons among experimental groups were carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison post hoc test to compare each treatment group with the hepatotoxic control group. A value of $p < 0.05$ was considered statistically significant [22].

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Preliminary phytochemical screening of the ethanolic extract of *Moringa oleifera* leaves revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, tannins, proteins, steroids, saponins, and triterpenoids (Table 1). Among these, flavonoids, tannins, and triterpenoids were detected in relatively higher abundance. These classes of phytochemicals are well documented for their antioxidant and hepatoprotective activities and may have contributed to the protective effects of the extract observed against isoniazid- and paracetamol-induced hepatic injury in the present study.

Table 1. Qualitative phytochemical screening of the ethanolic extract of *Moringa oleifera* leaves.

Phytochemical	Result
Alkaloids	+
Carbohydrates	+
Flavonoids	++
Glycosides	+
Tannins	++
Proteins	+
Steroids	+
Saponins	+
Triterpenoids	++

Note: (+) Present; (++) Abundantly present.

3.2 Effect of *Moringa oleifera* Ethanolic Extract on Isoniazid-Induced Hepatotoxicity

Isoniazid administration markedly increased liver weight and serum biochemical markers compared with the normal control group, indicating severe hepatic injury (Table 2). Liver weight increased from 5.80 ± 0.32 g in the normal group to 6.91 ± 0.11 g following isoniazid treatment. Similarly, ALT, a sensitive marker of hepatocellular damage, increased from 165.40 ± 0.41 IU/L to 245.95 ± 2.15 IU/L, while ALP almost doubled from 54.83 ± 2.12 IU/L to 110.58 ± 3.21 IU/L. Treatment with MOEE significantly improved these biochemical alterations in a dose-dependent manner. The 400 mg/kg dose produced the greatest hepatoprotective effect, reducing liver weight to 5.28 ± 0.19 g, AST to 157.56 ± 2.02 IU/L, ALT to 201.63 ± 0.13 IU/L, and ALP to 55.21 ± 1.25 IU/L, with values approaching those observed in the silymarin-treated group. A similar trend was observed for total and direct bilirubin levels, indicating restoration of normal hepatic function. The hepatoprotective activity of MOEE may be attributed to the flavonoids, tannins, and triterpenoids identified during phytochemical screening. These bioactive constituents are known to possess antioxidant properties that reduce oxidative stress and help preserve hepatocellular membrane integrity, thereby protecting against isoniazid-induced liver damage.

Table 2. Effect of MOEE on liver weight and serum biochemical parameters in isoniazid-induced hepatotoxicity

Treatment	Liver weight (g)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)
Normal Control	5.80 ± 0.32	156.70 ± 1.82	165.40 ± 0.41	54.83 ± 2.12	2.99 ± 0.17	1.75 ± 0.08
Isoniazid Control	6.91 ± 0.11	178.86 ± 1.09	245.95 ± 2.15	110.58 ± 3.21	4.42 ± 0.18	3.20 ± 0.32
Silymarin + INH	5.64 ± 0.14	161.53 ± 4.96	205.35 ± 1.10	57.15 ± 0.06	4.17 ± 0.08	1.78 ± 0.11
MOEE 100 mg/kg + INH	5.54 ± 0.13	164.52 ± 1.70	212.87 ± 0.14	60.25 ± 2.14	3.48 ± 0.12	2.08 ± 0.06
MOEE 200 mg/kg + INH	5.40 ± 0.18	162.15 ± 2.05	210.11 ± 2.11	57.10 ± 0.96	3.06 ± 0.40	2.03 ± 0.01
MOEE 400 mg/kg + INH	5.28 ± 0.19	157.56 ± 2.02	201.63 ± 0.13	55.21 ± 1.25	3.02 ± 0.25	2.01 ± 0.14

3.3 Effect of *Moringa oleifera* Ethanolic Extract on Paracetamol-Induced Hepatotoxicity

Paracetamol administration produced marked hepatic injury, as evidenced by increased liver weight and serum biochemical markers compared with the normal control group (Table 3). Liver weight increased from 5.65 ± 0.14 g to 6.85 ± 0.16 g, while AST and ALT increased from 58.78 ± 2.64 and 51.70 ± 0.55 IU/L to 109.43 ± 4.67 and 93.15 ± 2.02 IU/L, respectively, confirming hepatocellular damage. ALP also increased markedly from 143.11 ± 3.02 IU/L to 260.71 ± 4.41 IU/L, indicating impaired liver function. Treatment with MOEE significantly ameliorated these biochemical alterations in a dose-dependent manner. The 400 mg/kg dose produced the greatest hepatoprotective effect, reducing liver weight to 5.55 ± 0.14 g, AST

to 74.23 ± 1.05 IU/L, ALT to 64.05 ± 0.83 IU/L, and ALP to 152.24 ± 2.02 IU/L, with values approaching those of the silymarin-treated group. Similarly, total and direct bilirubin levels were markedly reduced, indicating recovery of hepatic excretory function. The hepatoprotective effect of MOEE is likely associated with the antioxidant activity of flavonoids, tannins, and triterpenoids identified during phytochemical screening. These phytoconstituents are known to reduce oxidative stress and stabilize hepatocellular membranes, thereby limiting paracetamol-induced liver injury.

Table 3. Effect of MOEE on liver weight and serum biochemical parameters in paracetamol-induced hepatotoxicity

Treatment	Liver weight (g)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total bilirubin (mg/dL)	Direct bilirubin (mg/dL)
Normal Control	5.65 ± 0.14	58.78 ± 2.64	51.70 ± 0.55	143.11 ± 3.02	3.60 ± 0.01	0.73 ± 0.05
Paracetamol Control	6.85 ± 0.16	109.43 ± 4.67	93.15 ± 2.02	260.71 ± 4.41	4.61 ± 0.04	2.34 ± 0.63
Silymarin + PCM	5.58 ± 0.18	77.65 ± 0.23	68.15 ± 1.02	153.24 ± 1.01	2.85 ± 0.02	0.78 ± 0.02
MOEE 100 mg/kg + PCM	5.85 ± 0.11	82.40 ± 2.10	78.41 ± 0.84	185.35 ± 2.32	3.52 ± 0.03	1.89 ± 0.08
MOEE 200 mg/kg + PCM	5.70 ± 0.13	81.15 ± 2.11	71.11 ± 2.11	171.44 ± 1.01	3.18 ± 0.02	0.95 ± 0.05
MOEE 400 mg/kg + PCM	5.55 ± 0.14	74.23 ± 1.05	64.05 ± 0.83	152.24 ± 2.02	2.91 ± 0.30	0.81 ± 0.01

3.4 Effect of *Moringa oleifera* Ethanolic Extract on Endogenous Antioxidant Status in Isoniazid-Induced Hepatotoxicity

Isoniazid administration markedly impaired the endogenous antioxidant defense system, as evidenced by significant reductions in total protein, GSH, total thiol, CAT, and SOD, together with a marked increase in lipid peroxidation (LPO) compared with the normal control group (Table 4). For example, GSH decreased from 27.54 ± 0.94 to 12.64 ± 0.97 nmol/mg protein, whereas LPO increased markedly from 3.36 ± 0.32 to 59.65 ± 1.02 U/mg protein, indicating severe oxidative stress. Treatment with MOEE significantly improved all antioxidant parameters and reduced lipid peroxidation. Among the tested doses, the 100 mg/kg group exhibited the greatest antioxidant response, restoring GSH to 28.65 ± 0.97 nmol/mg protein, CAT to 190.6 ± 1.04 U/mg protein, SOD to 43.56 ± 1.00 U/mg protein, and reducing LPO to 5.24 ± 0.15 U/mg protein. These findings indicate that the hepatoprotective effect of MOEE is closely associated with restoration of endogenous antioxidant defenses and attenuation of oxidative stress.

Table 4. Effect of MOEE on endogenous antioxidant parameters in isoniazid-induced hepatotoxicity

Treatment	Total protein (mg/100 mg tissue)	GSH (nmol/mg protein)	Total thiol (nmol/mg protein)	CAT (U/mg protein)	LPO (U/mg protein)	SOD (U/mg protein)
Normal Control	45.64 ± 0.94	27.54 ± 0.94	86.99 ± 0.69	196.6 ± 0.93	3.36 ± 0.32	45.65 ± 0.96
Isoniazid Control	12.50 ± 0.96	12.64 ± 0.97	44.63 ± 0.99	55.63 ± 0.96	59.65 ± 1.02	13.51 ± 0.98
Silymarin + INH	39.51 ± 1.01	24.68 ± 0.99	79.37 ± 1.01	152.5 ± 0.95	8.51 ± 0.28	41.42 ± 0.96
MOEE 100 mg/kg + INH	43.47 ± 0.93	28.65 ± 0.97	80.65 ± 1.04	190.6 ± 1.04	5.24 ± 0.15	43.56 ± 1.00
MOEE 200 mg/kg + INH	27.44 ± 1.03	20.48 ± 1.02	70.59 ± 0.89	111.6 ± 0.98	10.18 ± 0.62	33.63 ± 0.92
MOEE 400 mg/kg + INH	35.26 ± 1.13	25.50 ± 0.91	78.81 ± 0.96	147.7 ± 0.97	9.68 ± 1.18	39.40 ± 0.98

3.5 Effect of *Moringa oleifera* Ethanolic Extract on Endogenous Antioxidant Status in Paracetamol-Induced Hepatotoxicity

Paracetamol administration markedly impaired the endogenous antioxidant defense system, as evidenced by significant reductions in total protein, GSH, total thiol, CAT, and SOD levels, together with a marked increase in lipid peroxidation (LPO) compared with the normal control group (Table 5).

For instance, GSH decreased from 24.42 ± 0.85 to 11.53 ± 0.85 nmol/mg protein, whereas LPO increased from 3.26 ± 0.26 to 54.33 ± 1.02 U/mg protein, indicating severe oxidative stress.

Treatment with MOEE significantly restored the antioxidant defense system and reduced oxidative damage. Among the tested doses, the 100 mg/kg group exhibited the greatest antioxidant response, increasing GSH to 25.43 ± 0.92 nmol/mg protein, CAT to 186.3 ± 1.03 U/mg protein, and SOD to 40.62 ± 1.01 U/mg protein, while reducing LPO to 4.88 ± 0.14 U/mg protein. These findings suggest that the hepatoprotective effect of MOEE is closely associated with restoration of endogenous antioxidant defenses and suppression of lipid peroxidation.

Table 5. Effect of MOEE on endogenous antioxidant parameters in paracetamol-induced hepatotoxicity

Treatment	Total protein (mg/100 mg tissue)	GSH (nmol/mg protein)	Total thiol (nmol/mg protein)	CAT (U/mg protein)	LPO (U/mg protein)	SOD (U/mg protein)
Normal Control	42.52 ± 0.83	24.42 ± 0.85	83.86 ± 0.62	187.5 ± 0.88	3.26 ± 0.26	42.53 ± 0.88
Paracetamol Control	10.30 ± 0.88	11.53 ± 0.85	38.47 ± 0.95	51.52 ± 0.96	54.33 ± 1.02	11.48 ± 0.88
Silymarin + PCM	33.48 ± 1.01	22.44 ± 0.91	74.35 ± 1.00	143.4 ± 0.95	7.49 ± 0.26	37.33 ± 0.89
MOEE 100 mg/kg + PCM	37.42 ± 0.87	25.43 ± 0.92	76.63 ± 1.02	186.3 ± 1.03	4.88 ± 0.14	40.62 ± 1.01
MOEE 200 mg/kg + PCM	24.22 ± 1.02	18.17 ± 1.00	66.55 ± 0.79	107.2 ± 0.89	9.13 ± 0.42	28.41 ± 0.99
MOEE 400 mg/kg + PCM	33.23 ± 1.11	21.40 ± 0.87	72.72 ± 0.87	136.3 ± 0.93	8.55 ± 1.15	36.20 ± 0.94

3.6 Histopathological Evaluation

Histopathological examination supported the biochemical findings and confirmed the hepatoprotective effect of the ethanolic extract of *Moringa oleifera* in both isoniazid- and paracetamol-induced hepatotoxicity models. Liver sections from the disease control groups showed marked hepatocellular degeneration, inflammatory cell infiltration, sinusoidal congestion, fatty changes, and focal necrosis, whereas the normal control group exhibited intact hepatic architecture. Treatment with MOEE progressively improved liver histology, with the 400 mg/kg group showing near-normal hepatic architecture and minimal pathological alterations, comparable to the silymarin-treated group. These findings correlate well with the improvements observed in serum biochemical and antioxidant parameters, confirming the hepatoprotective potential of MOEE. Figures 2 and 3. Representative histopathological photomicrographs of liver sections from the isoniazid-induced (Figure 2) and paracetamol-induced (Figure 3) hepatotoxicity models. In both models, the normal control (A) exhibited intact hepatic architecture with normal hepatocytes, central vein, and hepatic sinusoids, whereas the disease control groups (B) showed marked hepatocellular degeneration, inflammatory cell infiltration, sinusoidal congestion, fatty changes, ballooning degeneration, and focal necrosis. The silymarin-treated groups (C) demonstrated marked restoration of normal hepatic architecture. Treatment with MOEE resulted in progressive histological improvement, with the 100 mg/kg groups (D) showing moderate hepatoprotection and reduced inflammatory changes, the 200 mg/kg groups (E) exhibiting improved hepatic organization with reduced hepatocellular degeneration, and the 400 mg/kg groups (F) displaying near-normal hepatic architecture with minimal pathological alterations, indicating substantial protection against chemically induced liver injury.

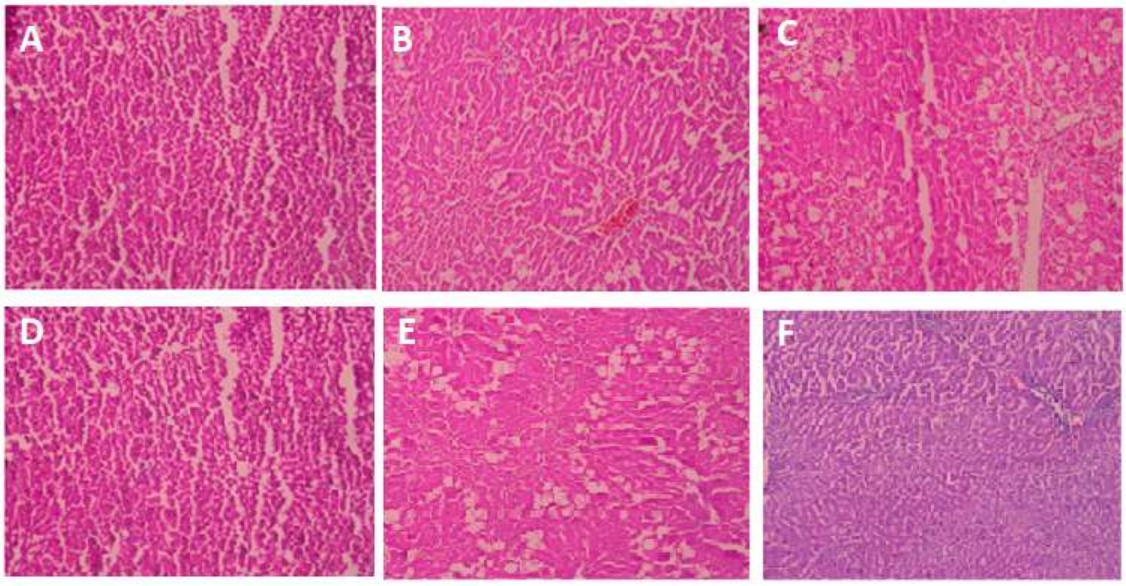

Figure 2. Histopathological photomicrographs of liver sections from the isoniazid-induced hepatotoxicity model.

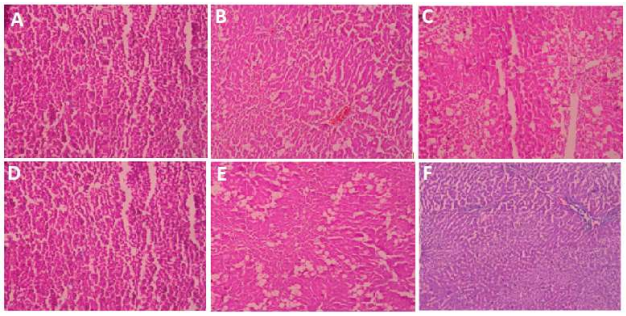

Figure 3. Representative histopathological photomicrographs of liver sections from the paracetamol-induced hepatotoxicity model.

3.7 In Vitro Antioxidant Activity of *Moringa oleifera* Ethanolic Extract

The ethanolic extract of *Moringa* oleifera exhibited concentration-dependent free radical scavenging activity in all in vitro antioxidant assays (Table 6; Figures 4-6). Among the evaluated assays, the hydroxyl radical scavenging assay showed the lowest IC_{50} value (62.8 µg/mL), followed by lipid peroxidation inhibition (65.0 µg/mL), indicating strong antioxidant potential. The extract also demonstrated DPPH and nitric oxide radical scavenging activities with IC_{50} values of 116 µg/mL and 362 µg/mL, respectively. These findings support the in vivo antioxidant and hepatoprotective effects of M. oleifera, suggesting that its bioactive constituents effectively scavenge reactive oxygen species and attenuate oxidative stress.

Table 6. In vitro antioxidant activity of *Moringa oleifera* ethanolic extract

Assay	IC_{50} (µg/mL)
Hydroxyl radical scavenging	62.8
Lipid peroxidation inhibition	65.0
DPPH radical scavenging	116
Nitric oxide scavenging	362

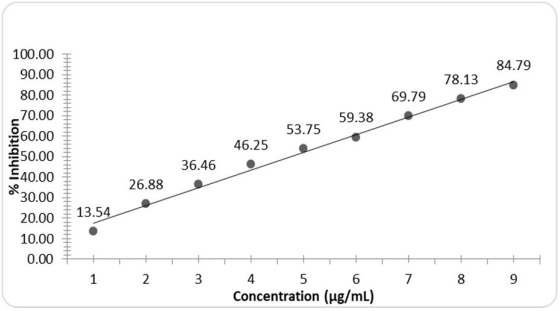

Figure 4 – Hydroxyl radical scavenging activity

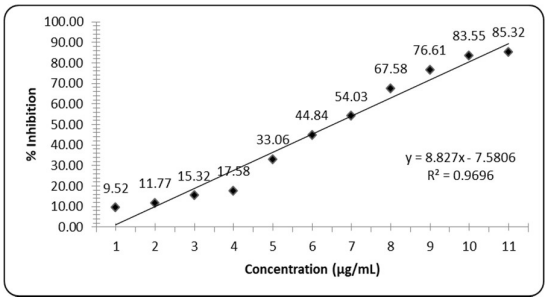

Figure 5 – Lipid peroxidation scavenging activity

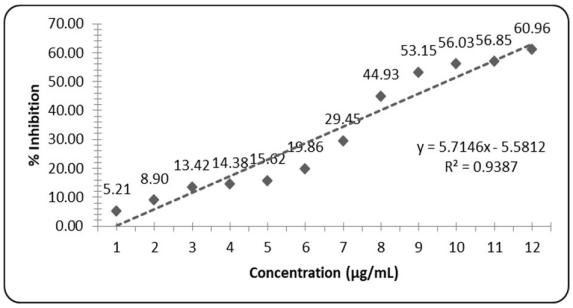

Figure 6 – Nitric oxide scavenging activity

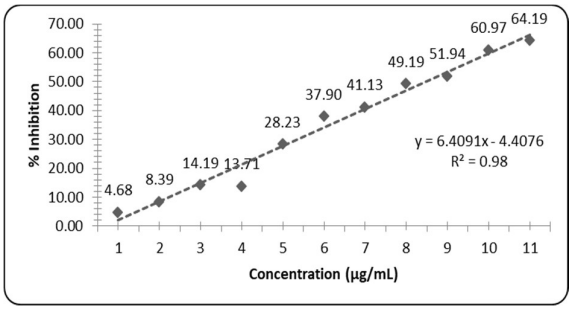

Figure 7 – DPPH scavenging activity

4. Conclusion

The present study demonstrated that the ethanolic extract of *Moringa oleifera* possesses significant hepatoprotective and antioxidant activities against isoniazid- and paracetamol-induced hepatotoxicity in experimental rats. Treatment with the extract effectively ameliorated drug-induced liver damage, as evidenced by reductions in serum AST, ALT, ALP, total bilirubin, and direct bilirubin levels, together with restoration of endogenous antioxidant defenses, including glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), total thiol, and total protein, while suppressing lipid peroxidation. Histopathological examination further confirmed the protective effect of the extract by demonstrating marked improvement in hepatic architecture and attenuation of hepatocellular degeneration, inflammatory infiltration, and necrosis. In addition, the extract exhibited potent in vitro free radical scavenging activity against hydroxyl, DPPH, nitric oxide radicals, and lipid peroxidation, supporting its antioxidant potential. The hepatoprotective activity of *M. oleifera* is likely attributable to its bioactive phytoconstituents, particularly flavonoids, tannins, and triterpenoids, which effectively reduce oxidative stress and preserve hepatocellular integrity. Overall, these findings suggest that the ethanolic extract of *Moringa oleifera* represents a promising natural therapeutic candidate for the prevention and management of drug-induced liver injury. Nevertheless, further studies focusing on the isolation of active constituents, molecular mechanisms, pharmacokinetics, and well-designed clinical trials are warranted to validate its therapeutic potential in humans.

Abbreviations: ALP-alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; CAT, catalase; DILI, drug-induced liver injury; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); GSH, reduced glutathione; H&E, hematoxylin and eosin; IC_{50}, half-maximal inhibitory concentration; INH, isoniazid; IU/L, international units per liter; LPO, lipid peroxidation; MDA, malondialdehyde; MOEE, *Moringa oleifera* ethanolic extract; NAPQI, *N*-acetyl-*p*-benzoquinone imine; NO, nitric oxide; PCM, paracetamol; ROS, reactive oxygen species; SEM, standard error of the mean; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TP, total protein; TT, total thiol; and UV, ultraviolet.

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