Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
NLM (National Library of Medicine): ID: (101671502)
Index Copernicus Value 2023: 85.86
Volume 14, Issue 6; 2025, 6-17

ISSN (Online): 2279-0594
ISSN (Print): 2589-8752

Research Article

Evaluation of Hepatoprotective Activity of the Seed Extract of Madhuca Longifolia against Carbon Tetrachloride (CCl_4)-Induced Hepatotoxicity in Experimental Animal Models

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Article Info: Received: 10-10-2025 / Revised: 06-11-2025 / Accepted: 29-11-2025
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DOI: https://doi.org/10.32553/jbpr.v14i6.1382
Conflict of interest statement: No conflict of interest

Abstract:
The liver is the major organ responsible for the metabolism, detoxification, and biochemical regulation of various endogenous and exogenous substances. Hepatic damage caused by toxins, drugs, and oxidative stress remains a serious health concern worldwide. Conventional hepatoprotective drugs such as silymarin are effective but often limited by side effects and high cost, thereby encouraging the exploration of safer, natural alternatives. Madhuca longifolia (Family: Sapotaceae), commonly known as Mahua, has been traditionally used in Indian systems of medicine for the treatment of liver disorders, inflammation, and oxidative stress-related ailments.The present study was designed to evaluate the hepatoprotective potential of the ethanolic seed extract of Madhuca longifolia in experimental animal models. The crude extract was prepared by Soxhlet extraction using ethanol as the solvent and subjected to preliminary phytochemical screening, which revealed the presence of flavonoids, saponins, tannins, steroids, and glycosides. Acute oral toxicity studies were performed as per OECD guideline 423, confirming the safety of the extract up to 2000 mg/kg body weight. Wistar albino rats were divided into five groups: normal control, CCl_4-induced hepatotoxic control, standard (silymarin 100 mg/kg), and two test groups receiving Madhuca longifolia extract (200 and 400 mg/kg). The extract exhibited a significant ($p < 0.05$) reduction in serum hepatic marker enzymes (SGOT, SGPT, ALP, and bilirubin) and improved total protein and albumin levels when compared to the toxic control. Additionally, the extract enhanced antioxidant enzyme levels (SOD, CAT, GSH) and reduced lipid peroxidation (MDA). Histopathological studies of liver tissue supported the biochemical results by showing improved hepatic architecture in the treated groups. The results of the study clearly demonstrate that the ethanolic seed extract of Madhuca longifolia possesses significant hepatoprotective activity against carbon tetrachloride–induced liver damage in rats, which may be attributed to its antioxidant and free radical scavenging properties. The findings justify the traditional use of Madhuca longifolia in liver disorders and suggest further research for isolation, characterization, and mechanism elucidation of the active constituents.

Keywords: *Madhuca longifolia*; Hepatoprotective activity; Carbon tetrachloride (CCl_4); Hepatotoxicity; Antioxidant enzymes; Silymarin; Liver biomarkers; Oxidative stress.

Introduction

The liver is the most vital organ in the human body with regard to biochemical processes.

It possesses a remarkable ability to detoxicate hazardous substances and produces practical principles. Consequently, the detrimental effects of hepatotoxic agents resulting from liver impairment are severe. Hepatic damage is characterized by disruption of metabolic functions.

As a result, there is a growing demand for agents that can provide protection against this damage.[1]

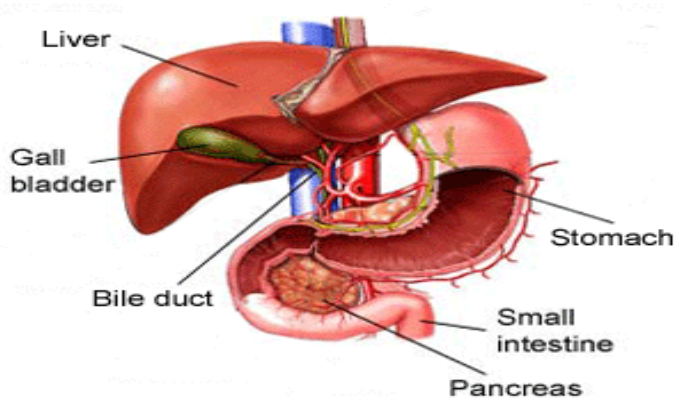

Figure No.1: Anatomy of Liver

Plant Profile:

Madhuca longifolia (Mahua) is a rich reservoir of bioactive phytochemicals that contribute to its diverse pharmacological properties, including hepatoprotective, antioxidant, anti-inflammatory, antimicrobial, and analgesic activities. Almost every part of the plant—leaves, bark, flowers, seeds, and seed oil—contains important secondary metabolites with potential therapeutic benefits.[2]

Taxonomy of Madhuca longifolia (KOENIG)

Systematic	Position
Class	Dicotyledons
Sub class	Gamopetalae
Series	Heteromerae
Order	Ebenales
Genous name	Madhuca
Species name	Longifolia
Family	Sapotaceae

Phamacological Activities

Madhuca longifolia has been extensively used in traditional Indian medicine for various purposes. The flowers are used to produce a fermented drink, while the seeds are a source of oil used for cooking and medicinal purposes.

The bark and leaves have been traditionally used to treat a range of ailments, including inflammation, fever, and diabetes.[3]

Traditional Medicinal Uses

- **Skin Diseases:** The bark and seed oil are traditionally applied for the treatment of eczema, psoriasis, and other dermatological

conditions due to their anti-inflammatory properties.

- **Gastrointestinal Issues:** Decoctions prepared from the bark and leaves are used to treat dysentery, diarrhea, and ulcers.
- **Rheumatism and Joint Pain:** Seed oil is used topically for massage in rheumatic pain, joint stiffness, and muscle inflammation.
- **Respiratory Conditions:** Mahua flowers are traditionally used to treat coughs, colds, and asthma due to their soothing and expectorant properties.
- **Reproductive Health:** Mahua is often incorporated in traditional medicine for improving reproductive health, including treatment for infertility and menstrual disorders.[4]

Collection of Plant Material

The mature dried seeds of *Madhuca longifolia* were collected during the month of Aug. from Local Area.

The seeds were free from fungal contamination and extraneous plant parts. The collected material was cleaned thoroughly, shade-dried at room temperature, and stored in an airtight container until further use.

Authentication of Plant Material

The collected plant material was taxonomically identified and authenticated by a qualified taxonomist at the Botanical department of A.P.S University, M.P.

Processing of Plant Material:

The authenticated seeds were dried under shade to avoid degradation of thermo-labile constituents and then coarsely powdered using a mechanical grinder. The powdered material was sieved (mesh size 40) and stored in an air-tight container for extraction and phytochemical analysis.[5]

Acute toxicity assay

The acute oral toxicity study were carried out as per Organization for Economic Co-operation and Development (OECD) Guidelines 425. The Ethanolic Extract were administered at the dose level of 400mg/kg and observed mortality after 24 hr. One-tenth of the median lethal dose (LD50) were taken as an effective dose.[6]

Induction of Hepatotoxicity (CCl_4 model)

For the induction of hepatotoxicity, a solution of CCl_4 and liquid paraffin in a 1:1 (v/v) ratio was freshly prepared before each administration. The animals in the toxic control and treatment groups received CCl_4 at a dose of 1 ml/kg body weight intraperitoneally (i.p.), twice weekly for 14 days**.** The vehicle (liquid paraffin) reduced the acute lethality of CCl_4 and ensured uniform dosing.[7]

Grouping of Animals

A total of 30 Wistar albino rats were randomly divided into six groups (n = 6 per group) as follows:[8]

Group	Treatment	Dose / Route
I – Normal Control	Received vehicle (0.5% Carboxymethylcellulose, CMC)	1 ml/kg, p.o.
II – Toxic Control	Received hepatotoxin (CCl_4 in liquid paraffin, 1:1 v/v)	1 ml/kg, i.p. (twice weekly for 14 days)
III – Standard	Received Silymarin + CCl_4	Silymarin 100 mg/kg, p.o. daily + CCl_4
IV – Test Group I	Received *M. longifolia* extract (low dose) + CCl_4	Extract 200 mg/kg, p.o. daily + CCl_4
V – Test Group II	Received *M. longifolia* extract (high dose) + CCl_4	Extract 400 mg/kg, p.o. daily + CCl_4

Duration of Treatment

The treatment was continued for 14 consecutive days**.** On day 15, animals were anesthetized, blood samples were collected via retro-orbital puncture for biochemical analysis, and the animals were sacrificed by cervical dislocation. The liver was excised, washed with ice-cold saline, weighed, and preserved for biochemical and histopathological studies.[9]

Parameters Evaluated: To assess the hepatoprotective activity of *Madhuca longifolia* seed extract, the following parameters were evaluated:[10-14]

- Body Weight Changes
- Serum Biochemical Parameters
- Serum Glutamate Oxaloacetate Transaminase (SGOT/AST)
- Serum Glutamate Pyruvate Transaminase (SGPT/ALT)
- Alkaline Phosphatase (ALP)
- Total Bilirubin (TB)
- Total Protein (TP)
- Albumin (ALB)

Oxidative Stress / Antioxidant Parameters: Liver tissue homogenates (10% w/v in phosphate buffer, pH 7.4) were prepared and centrifuged to obtain supernatants for assays:[15,16]

- Malondialdehyde (MDA)
- Reduced Glutathione (GSH)
- Superoxide Dismutase (SOD)

Histopathological Studies: Excised liver tissues were fixed in 10% formalin, processed through ascending grades of alcohol, cleared in xylene, and embedded in paraffin wax.

Sections of 5 µm thickness were cut using a microtome and stained with Hematoxylin and Eosin (H&E).[16-20]

Slides were examined under a light microscope for histological changes such as:

- Hepatocellular necrosis
- Fatty degeneration
- Ballooning of hepatocytes
- Inflammatory cell infiltration
- Regeneration of hepatocytes

Statistical Analysis

All results were expressed as Mean ± SEM (n=6). Data were analyzed using One-Way ANOVA followed by Tukey's multiple comparison test. A value of $p < 0.05$ was considered statistically significant.

Result and Discussion:

Phytochemical Screening

These findings suggest that the hepatoprotective activity of Madhuca longifolia may be attributed to the synergistic action of multiple phytoconstituents, particularly flavonoids, tannins, and saponins, which are well-documented for their antioxidant and hepatoprotective roles.

Table 1: Phytochemical Screening of *Madhuca longifolia* Extract

S.No.	Phytoconstituents	Test Used	Result
1.	Carbohydrates	Molish's test	Present (+)
2.	Alkaloids	Dragendroff's test Mayer's test	Present (+)
3	Phenolic Compounds	Ferric Chloride Test	Present (+)
4	Flavonoids	Sulphuric acid test	Present (+)
5	Glycosides	Keller- Killiani test	Present (+)
6	Saponins	Froth Test	Present (+)
7	Tannins	Ferric Chloride Test	Present (+)
8	Steroids	LeibermannBurchardreaction	Present (+)
9	Proteins & Amino Acids	Ninhydrin test	Trace(+/-)
10	Fixed Oils & Fats	Spot test	Absent(-)

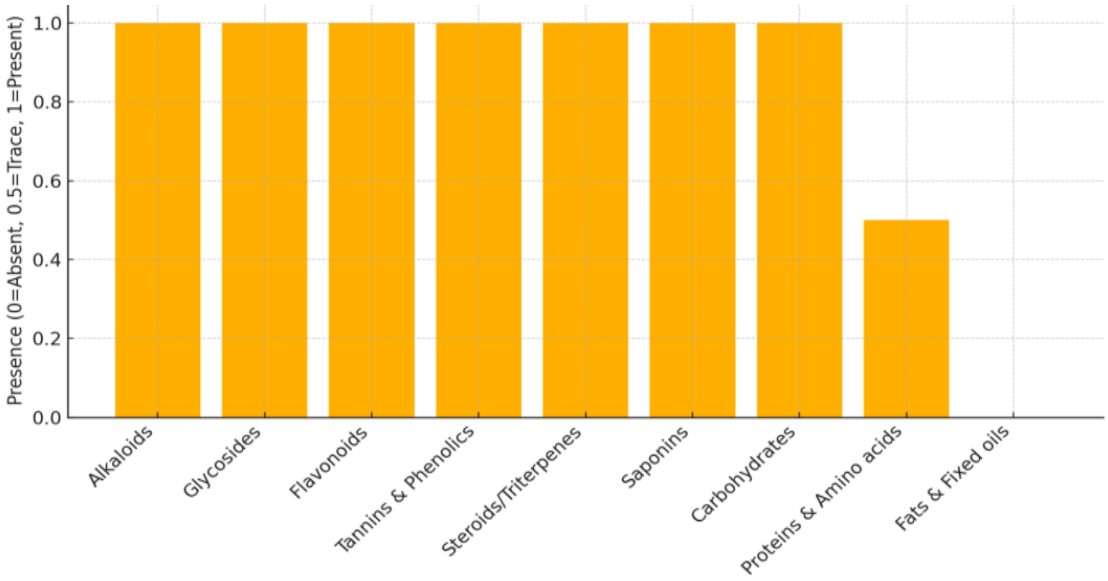

Figure 2: Phytochemical Screening of Madhuca longifolia

Body Weight Changes:

The effect of Madhuca longifolia seed extract on body weight of Wistar albino rats was evaluated at the beginning and end of the experimental period.

Animals in the toxic control group (CCl_4-treated) showed a significant reduction in body weight, indicating general toxicity and poor health status due to hepatic injury. In contrast, rats treated with silymarin (standard) and M. longifolia extract (200 and 400 mg/kg) demonstrated a marked improvement in body weight compared to the toxic control, suggesting restoration of normal metabolic activity and protective effect against hepatotoxicity.

Table 2: Effect of *Madhuca longifolia* Extract on Body Weight of Rats

Group	Initial Body Weight (g)	Final Body Weight (g)	% Change
Normal Control	165.3 ± 3.2	185.4 ± 3.6	+12.1%
Toxic Control (CCl_4)	168.1 ± 2.9	150.2 ± 3.1	–10.6%
Standard(Silymarin100 mg/kg)	166.7 ± 3.1	182.9 ± 3.4	+9.7%
Test Group I (Extract 200 mg/kg + CCl_4)	167.2 ± 2.8	176.8 ± 3.0	+5.7%
Test Group II (Extract 400 mg/kg + CCl_4)	165.9 ± 3.0	180.4 ± 2.9	+8.7%

Serum Glutamate Oxaloacetate Transaminase (SGOT/AST)

The toxic control group exhibited a significant elevation in SGOT levels (162.8 U/L) compared to the normal control group (72.4 U/L), indicating severe hepatocellular damage.

Administration of silymarin (100 mg/kg) markedly reduced SGOT activity (85.2 U/L), demonstrating hepatoprotection. Treatment with *M. longifolia* extract at 200 mg/kg and 400 mg/kg significantly lowered SGOT levels (101.3 and 90.7 U/L, respectively) compared to the toxic control, with the higher dose showing near-normalization. The extract control group (400 mg/kg alone) maintained values (75.5 U/L) comparable to the normal control, confirming the safety of the extract.

Table 3: Effect of *Madhuca longifolia* Extract on SGOT Levels in Rats

Group	SGOT (U/L) (Mean ± SEM)
Normal Control	72.4 ± 2.3
Toxic Control (CCl_4)	162.8 ± 4.5
Standard (Silymarin 100 mg/kg)	85.2 ± 2.8
Test Group I (Extract 200 mg/kg + CCl_4)	101.3 ± 3.2
Test Group II (Extract 400 mg/kg + CCl_4)	90.7 ± 2.7

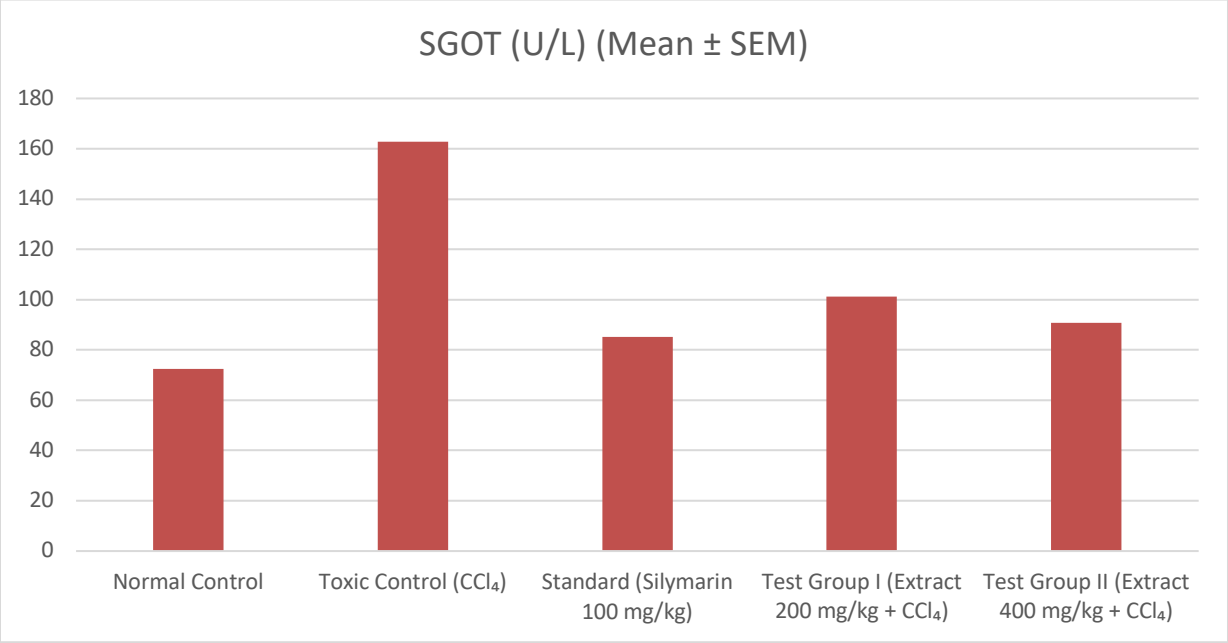

Figure 3: Effect of *Madhuca longifolia* Extract on SGOT Levels in Rats

Serum Glutamate Pyruvate Transaminase (SGPT/ALT)

The toxic control group showed a marked increase in SGPT levels (155.6 U/L) compared to the normal control group (68.5 U/L), indicating severe hepatocellular injury. Treatment with silymarin (100 mg/kg) significantly reduced SGPT values (79.3 U/L), approaching near-normal levels. Similarly, rats treated with *M. longifolia* extract at 200 mg/kg and 400 mg/kg showed reduced SGPT levels (95.7 U/L and 86.2 U/L, respectively) when compared to the toxic control, with the higher dose being more effective.

Table 4: Effect of *Madhuca longifolia* Extract on SGPT Levels in Rats

Group	SGPT (U/L) (Mean ± SEM)
Normal Control	68.5 ± 2.4
Toxic Control (CCl_4)	155.6 ± 4.2
Standard (Silymarin 100 mg/kg)	79.3 ± 2.6
Test Group I (Extract 200 mg/kg + CCl_4)	95.7 ± 3.0
Test Group II (Extract 400 mg/kg + CCl_4)	86.2 ± 2.8

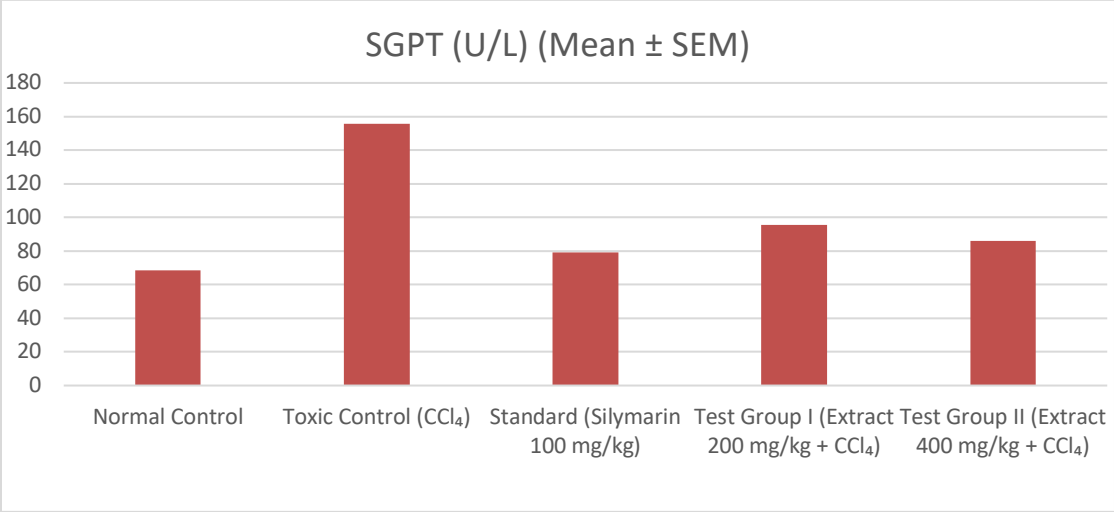

Figure 4: Effect of *Madhuca longifolia* Extract on SGPT Levels in Rats

Alkaline Phosphatase (ALP):

The toxic control group exhibited a marked increase in ALP levels (245.5 U/L) compared to the normal control group (118.2 U/L), indicating hepatobiliary dysfunction and membrane damage. Administration of silymarin (100 mg/kg) significantly reduced ALP values (132.6 U/L), suggesting improvement in liver function. Treatment with *M. longifolia* extract at 200 mg/kg and 400 mg/kg resulted in a significant reduction in ALP levels (168.3 and 140.5 U/L, respectively) compared to the toxic control, with the higher dose showing better protection.

Table 5: Effect of *Madhuca longifolia* Extract on ALP Levels in Rats

Group	**ALP (U/L) (Mean ± SEM)**
Normal Control	118.2 ± 3.4
Toxic Control (CCl_4)	245.5 ± 5.2
Standard (Silymarin 100 mg/kg)	132.6 ± 3.7
Test Group I (Extract 200 mg/kg + CCl_4)	168.3 ± 4.1
Test Group II (Extract 400 mg/kg + CCl_4)	140.5 ± 3.5

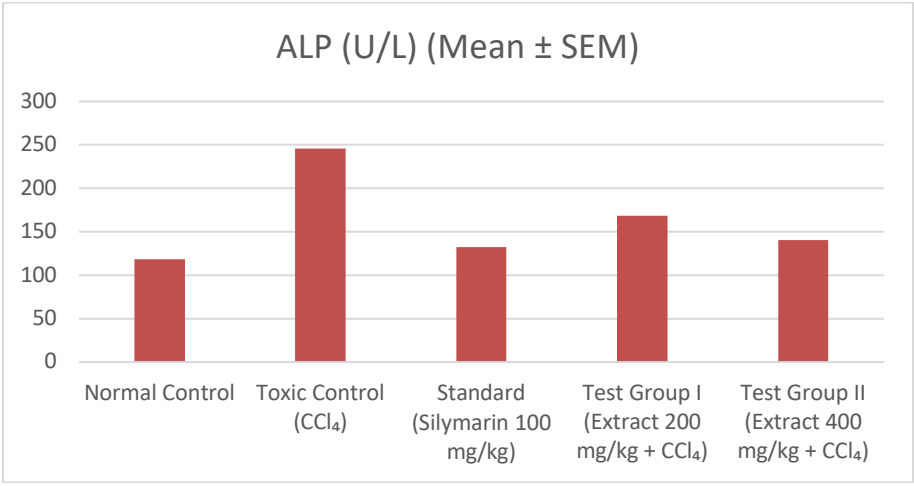

Figure 5: Effect of *Madhuca longifolia* Extract on ALPLevels in Rats

Total Bilirubin

The toxic control group exhibited a significant elevation in serum bilirubin concentration (2.85 mg/dl) compared to the normal control group (0.62 mg/dl), reflecting impaired bilirubin metabolism and excretory dysfunction due to hepatocellular injury. Treatment with silymarin (100 mg/kg) markedly reduced bilirubin levels (0.91 mg/dl), confirming its hepatoprotective effect.

Similarly, administration of *M. longifolia* extract at 200 mg/kg and 400 mg/kg significantly decreased bilirubin levels (1.45 and 1.10 mg/dl, respectively) compared to the toxic control, with the higher dose producing results closer to normal.

Table 6: Effect of *Madhuca longifolia* Extract on Total Bilirubin Levels in Rats

Group	Total Bilirubin (mg/dl) (Mean ± SEM)
Normal Control	0.62 ± 0.04
Toxic Control (CCl_4)	2.85 ± 0.11
Standard (Silymarin 100 mg/kg)	0.91 ± 0.05
Test Group I (Extract 200 mg/kg + CCl_4)	1.45 ± 0.07
Test Group II (Extract 400 mg/kg + CCl_4)	1.10 ± 0.06

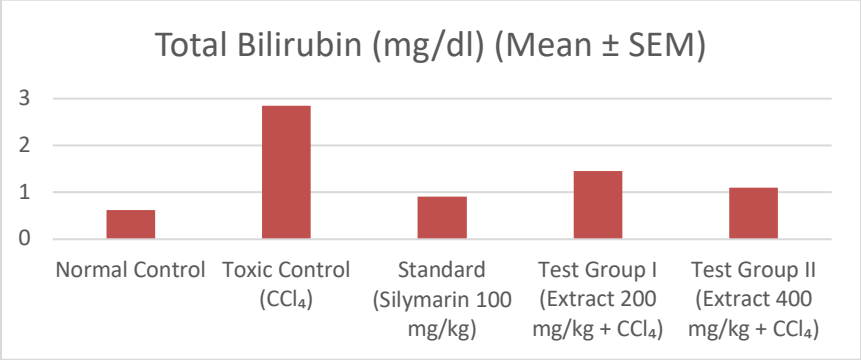

Figure 6: Effect of *Madhuca longifolia* Extract on Total Bilirubin in Rats

Total Protein

The toxic control group exhibited a significant reduction in total protein concentration (4.28 g/dl) compared to the normal control group (6.95 g/dl), reflecting impaired protein synthesis due to hepatic dysfunction. Administration of silymarin (100 mg/kg) significantly restored protein levels (6.72 g/dl), indicating hepatoprotective activity.

Similarly, treatment with *M. longifolia* extract at 200 mg/kg and 400 mg/kg improved total protein values (5.81 and 6.32 g/dl, respectively) compared to the toxic control, with the higher dose showing better efficacy.

Table 7: Effect of *Madhuca longifolia* Extract on Total Protein Levels in Rats

Group	Total Protein (g/dl) (Mean ± SEM)
Normal Control	6.95 ± 0.15
Toxic Control (CCl_4)	4.28 ± 0.12
Standard (Silymarin 100 mg/kg)	6.72 ± 0.14
Test Group I (Extract 200 mg/kg + CCl_4)	5.81 ± 0.13
Test Group II (Extract 400 mg/kg + CCl_4)	6.32 ± 0.12

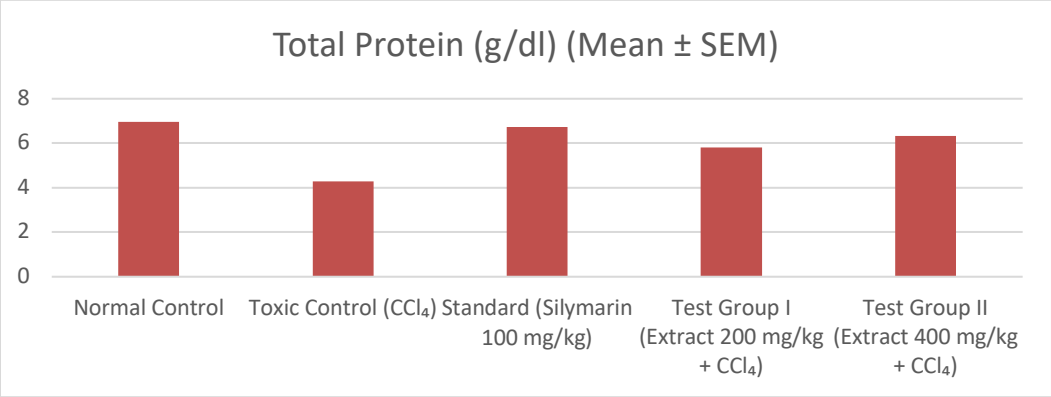

Figure 7: Effect of *Madhuca longifolia* Extract on Total Protein Levels in Rats

Albumin: The toxic control group demonstrated a significant decline in serum albumin concentration (2.15 g/dl) compared to the normal control group (4.12 g/dl), indicating impaired hepatic synthetic function due to liver injury. Administration of silymarin (100 mg/kg) significantly improved albumin levels (3.95 g/dl), suggesting effective hepatoprotection. Similarly, treatment with *M. longifolia* extract at 200 mg/kg and 400 mg/kg increased albumin levels (3.41 and 3.78 g/dl, respectively) compared to the toxic control, with the higher dose showing better restoration of normal values.

Table 8: Effect of *Madhuca longifolia* Extract on Albumin Levels in Rats

Group	**Albumin (g/dl) (Mean ± SEM)**
Normal Control	4.12 ± 0.10
Toxic Control (CCl_4)	2.15 ± 0.08
Standard (Silymarin 100 mg/kg)	3.95 ± 0.09
Test Group I (Extract 200 mg/kg + CCl_4)	3.41 ± 0.10
Test Group II (Extract 400 mg/kg + CCl_4)	3.78 ± 0.09

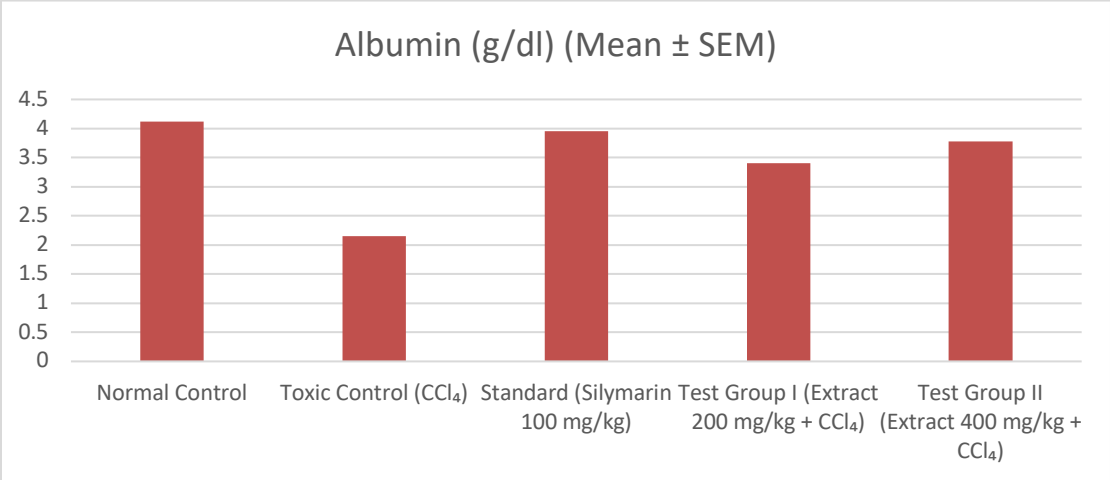

Figure 8: Effect of *Madhuca longifolia* Extract on Albumin Levels in Rats

Oxidative Stress / Antioxidant Markers:
The effect of Madhuca longifolia seed extract on oxidative stress and antioxidant defense markers (MDA, GSH, SOD, and CAT) in CCl_4-induced hepatotoxic rats is summarized in Table 9 and illustrated in Figures 9**.**

Table 9: Effect of *Madhuca longifolia* Extract on Oxidative Stress / Antioxidant Parameters

Group	MDA (nmol/mg protein)	GSH (µmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
Normal Control	2.15 ± 0.08	6.82 ± 0.14	8.25 ± 0.21	42.5 ± 1.1
Toxic Control (CCl_4)	7.82 ± 0.22	2.45 ± 0.10	3.14 ± 0.12	18.3 ± 0.9
Standard (Silymarin 100 mg/kg)	2.76 ± 0.11	6.35 ± 0.12	7.84 ± 0.20	39.8 ± 1.0
Test Group I (Extract 200 mg/kg + CCl_4)	4.21 ± 0.15	4.95 ± 0.11	6.12 ± 0.17	32.6 ± 0.8
Test Group II (Extract 400 mg/kg + CCl_4)	2.92 ± 0.12	6.41 ± 0.13	7.65 ± 0.19	38.7 ± 1.2

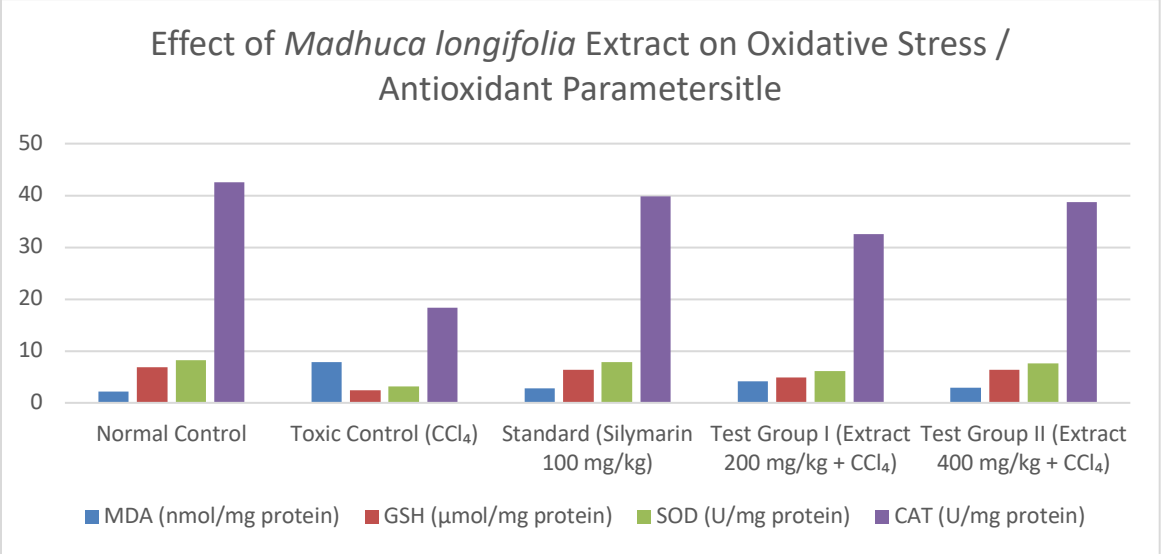

Figure 9: **Effect of *Madhuca longifolia* Extract on Oxidative Stress / Antioxidant Parameters**

Histopathology Results: Histopathological examination of liver tissues from different groups revealed marked differences in architecture, as summarized below and shown in Figures**.**

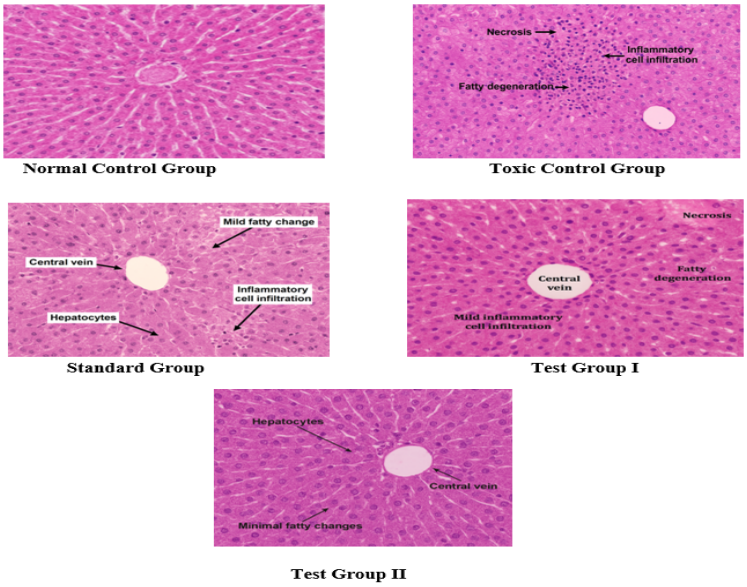

Figure 10:

Liver sections from the normal control group showed well-preserved hepatic architecture with intact cell morphology. Liver sections from the toxic control group (CCl_4-treated) revealed severe hepatic damage**.** The architecture was distorted with centrilobular necrosis, fatty (vacuolar) degeneration, cytoplasmic vacuolation, loss of cellular boundaries, sinusoidal dilation, and inflammatory cell infiltration**.** Liver sections from the standard group (Silymarin 100 mg/kg + CCl_4) revealed a marked restoration of hepatic architecture compared to the toxic control. Hepatocytes appeared normal with well-preserved cytoplasm and centrally placed nuclei. Only mild fatty changes and occasional inflammatory cell infiltration were observed.

Liver sections from Test Group I (treated with *Madhuca longifolia* extract at 200 mg/kg along with CCl_4) showed moderate improvement in hepatic architecture compared to the toxic control. Liver sections from Test Group II (treated with *Madhuca longifolia* extract at 400 mg/kg along with CCl_4) showed a marked improvement in hepatic histology, indicating strong hepatoprotective effects. Hepatocytes appeared mostly normal, with well-preserved cytoplasm and centrally located nuclei. Only minimal fatty changes and very mild inflammatory infiltration were observed.

Conclusion

The extract significantly reduced serum liver enzymes, bilirubin, and MDA levels, while restoring total protein, albumin, and endogenous antioxidants. Histological studies further confirmed the biochemical findings, showing restoration of hepatocyte architecture and reduced necrosis in extract-treated groups.

Phytochemical screening revealed the presence of flavonoids, tannins, triterpenoids, saponins, and glycosides, which likely contributed to the hepatoprotective activity through antioxidant, free radical scavenging, membrane-stabilizing, and protein synthesis-enhancing mechanisms. The extract control group showed no evidence of hepatotoxicity, confirming the safety of the extract at the tested dose. Thus, it can be concluded that the ethanolic seed extract of Madhuca longifolia possesses significant hepatoprotective activity, validating its traditional use in the management of liver disorders

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