

Assessment of Hepatoprotective Potential of Moringa Concanensis Bark Extract in CCl₄-Induced Hepatotoxicity in Wistar Rats**Vijay Kumar Kumawat¹, Divya Singh², Mamta Sharma³, Lokesh Kumar Saini⁴, Aziz Ahmed⁵**¹Research Scholar, Department of Pharmacology, Jaipur College of Pharmacy, Jaipur, Rajasthan, India^{2,3,4}Department of Pharmacology, Jaipur College of Pharmacy, Jaipur, Rajasthan, India⁵Department of Pharmacognosy, Jaipur College of Pharmacy, Jaipur, Rajasthan, India

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Conflict of interest: Nil

Abstract

The present study was undertaken to evaluate the hepatoprotective and antioxidant activity of the plant extract against carbon tetrachloride (CCl₄)-induced hepatotoxicity in experimental animals. Preliminary phytochemical screening of the plant extract revealed the presence of alkaloids, flavonoids, tannins, phenolic compounds, saponins, glycosides, carbohydrates, and terpenoids. The total phenolic content and total flavonoid content of the plant extract were found to be 72.5mg GAE/g and 45.3mg QE/g extract, respectively. Acute toxicity evaluation showed no mortality or significant toxic manifestations at the tested dose. CCl₄ administration produced marked hepatic injury, evidenced by increased AST, ALT, ALP, and total bilirubin and decreased total protein and albumin levels. The CCl₄ Control Group also showed marked depletion of hepatic SOD, CAT, and GSH levels. Treatment with the plant extract improved these biochemical and antioxidant alterations in a dose-dependent manner. The high-dose plant extract showed greater protection, with AST, ALT, ALP, and total bilirubin reduced to 68.5U/L, 63.2 U/L, 129.4U/L, and 1.21mg/dL, respectively, while total protein and albumin increased to 6.34 and 3.76 g/dL. SOD, CAT, and GSH levels were restored to 6.93U/mg protein, 44.9μmol H₂O₂ decomposed/min/mg protein, and 5.92μg/mg protein, respectively. Histopathological examination further confirmed reduced hepatic necrosis, fatty degeneration, and inflammatory changes in animals treated with the plant extract. Overall, the findings indicate that the plant extract possesses promising hepatoprotective and antioxidant activity against CCl₄-induced hepatic injury, which may be associated with its phenolic, flavonoid, and other bioactive constituents.

Keywords: Hepatoprotective activity, Oxidative stress, Antioxidant enzymes, Phytochemicals, Medicinal plants, CCl₄-induced hepatotoxicity.

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Introduction

The liver is the largest internal organ and one of the most vital metabolic centers in the human body. It is located in the upper right quadrant of the abdominal cavity, just below the diaphragm, and weighs approximately 1.2–1.5 kg in adults. The liver performs a wide range of physiological

functions essential for maintaining homeostasis, including metabolism, detoxification, storage, and synthesis of various biomolecules. Structurally, the liver is composed of functional units called hepatic lobules, which consist of hepatocytes arranged in plates radiating from a central vein.

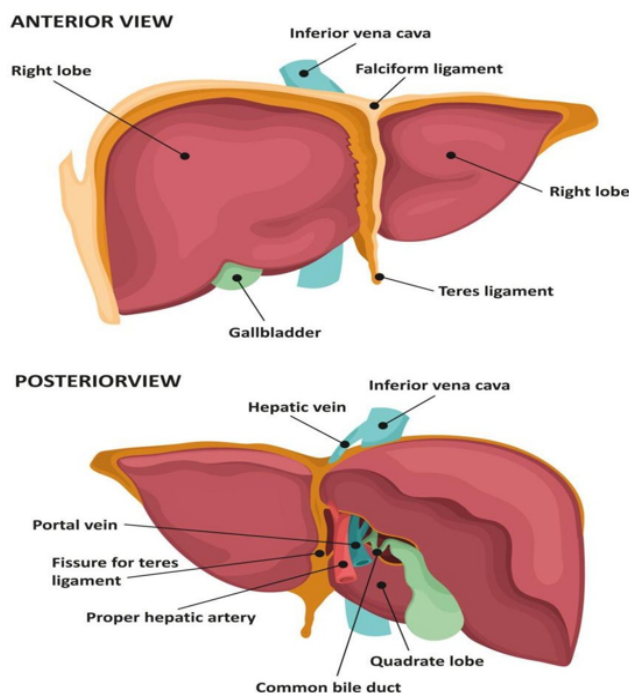


Figure 1: Anatomy of liver

Hepatotoxicity: Hepatotoxicity refers to liver damage caused by exposure to chemical substances such as drugs, toxins, alcohol, and environmental pollutants.

The liver, being the primary organ for metabolism and detoxification, is highly vulnerable to injury from xenobiotics and their reactive metabolites. Hepatic injury can manifest as acute or chronic conditions, ranging from mild biochemical alterations to severe liver failure.

CCl₄-Induced Hepatotoxicity Model: Carbon tetrachloride-induced hepatotoxicity is one of the most widely used experimental models for studying liver injury and evaluating hepatoprotective agents.

Due to its reproducibility and well-established mechanism, the CCl₄ model is extensively used in pharmacological research to assess hepatoprotective activity.

Plant Profile: *Moringa concanensis* Nimmo, a member of the family Moringaceae, is an underexplored medicinal species closely allied to *Moringa oleifera*. The bark is a rich source of polyphenols and other secondary metabolites that contribute to antioxidant and hepatoprotective effects. In recent years, interest has grown in validating these traditional claims through phytochemical and pharmacological studies, especially using toxin-induced liver injury models.



Figure 2: Plant Profile of *Moringa concanensis*

Pharmacological Activities of Bark

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Antioxidant Activity: The bark extract exhibits strong antioxidant activity by scavenging free radicals and inhibiting lipid peroxidation. This activity is mainly due to the presence of phenolic and flavonoid compounds, which neutralize reactive oxygen species and protect cellular components from oxidative damage.

Anti-inflammatory Activity: The bark demonstrates anti-inflammatory effects by inhibiting the synthesis and release of inflammatory mediators such as prostaglandins and cytokines. This helps in reducing tissue inflammation and associated damage.

Antimicrobial Activity: The bark extract shows antibacterial and antifungal activity against various pathogens. This supports its traditional use in treating infections and wounds.

Other Activities: Additional pharmacological activities of the bark include antidiabetic, analgesic, and antiulcer effects, which further broaden its therapeutic applications.

Collection and Authentication of Plant Material:

The bark of *Moringa concanensis* Nimmo were collected from a suitable natural habitat during the appropriate season to ensure the availability of fresh and healthy plant material. The collected plant material were authenticated by a qualified botanist in a recognized herbarium center.

Preparation of Extract: Extraction is the process of separating bioactive constituents from plant material using suitable solvents. The efficiency of extraction depends on the polarity of the solvent, temperature, duration, and nature of the plant material. Soxhlet extraction is a widely used method for obtaining phytoconstituents from crude drugs, as it allows continuous extraction with fresh solvent, ensuring maximum yield.

Preliminary Phytochemical Screening: Preliminary phytochemical screening is an essential step in the evaluation of medicinal plants, which helps in identifying the presence of various bioactive constituents responsible for pharmacological activity.

Quantitative Phytochemical Analysis:

Quantitative phytochemical analysis were carried out to estimate the total phenolic content (TPC) and total flavonoid content (TFC) present in the ethanolic extract of *Moringa concanensis* bark.

These estimations are important because phenolic and flavonoid compounds are known to contribute significantly to antioxidant and hepatoprotective activity.

Acute Toxicity Study: The study may be carried out according to OECD Test Guideline 423, Acute Oral Toxicity. Under this method, small groups of animals are dosed stepwise to assess acute toxicity classification while minimizing animal use.

Ethical Approval: The experimental protocol were approved by the Institutional Animal Ethics Committee (IAEC) and conducted in accordance with CCSEA guidelines, Government of India. All procedures was performed ensuring minimal animal suffering under standard laboratory conditions.

Induction of Hepatotoxicity and Experimental Grouping: Hepatotoxicity were induced experimentally by using carbon tetrachloride (CCl₄), a well-established hepatotoxic agent widely used in preclinical screening of hepatoprotective drugs.

CCl₄ is commonly administered after dilution with olive oil because this improves handling and supports reproducible delivery in animal studies.

Table 1: Experimental Grouping for Hepatoprotective Study

Group	Group Name	Treatment	Dose	Route
Group I	Normal Control	Normal saline / vehicle only	1ml/kg or as required	Oral
Group II	Toxic Control	CCl ₄ in olive oil (1:1 v/v)	1ml/kg	Intraperitoneal
Group III	Standard Control	Silymarin + CCl ₄	Silymarin 100 mg/kg + CCl ₄ 1 ml/kg	Oral
Group IV	Test Low Dose	<i>Moringa concanensis</i> bark extract + CCl ₄	Extract 100mg/kg + CCl ₄ 1 ml/kg	Oral
Group V	Test High Dose	<i>Moringa concanensis</i> bark extract + CCl ₄	Extract 200 mg/kg + CCl ₄ 1 ml/kg	Oral

Collection of Blood Samples and Serum Separation: Collection of blood samples and proper separation of serum are very important steps in the evaluation of hepatoprotective activity. Therefore, in the present study, blood collection and serum separation was carried out carefully under standard laboratory procedures.

Biochemical Estimation: Biochemical estimation of serum parameters were carried out to assess the extent of liver damage and the protective effect of the ethanolic extract of *Moringa concanensis* bark. In hepatotoxicity studies, serum enzymes and related markers as well as Histopathology study are considered highly sensitive indicators of

hepatocellular injury because damaged hepatocytes release intracellular enzymes into circulation.

Statistical Analysis: All experimental data obtained from biochemical and antioxidant studies was expressed as Mean $\pm$ Standard Error of Mean (SEM). Statistical analysis were performed using appropriate statistical software.

Results

Table 2: Preliminary Phytochemical Constituents of Plant Extract

S. No.	Phytochemical Constituent	Result
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Phenolic Compounds	+
5	Saponins	+
6	Glycosides	+
7	Carbohydrates	+
8	Terpenoids	+
9	Steroids	–
10	Proteins	–

Note: (+) Present, (–) Absent

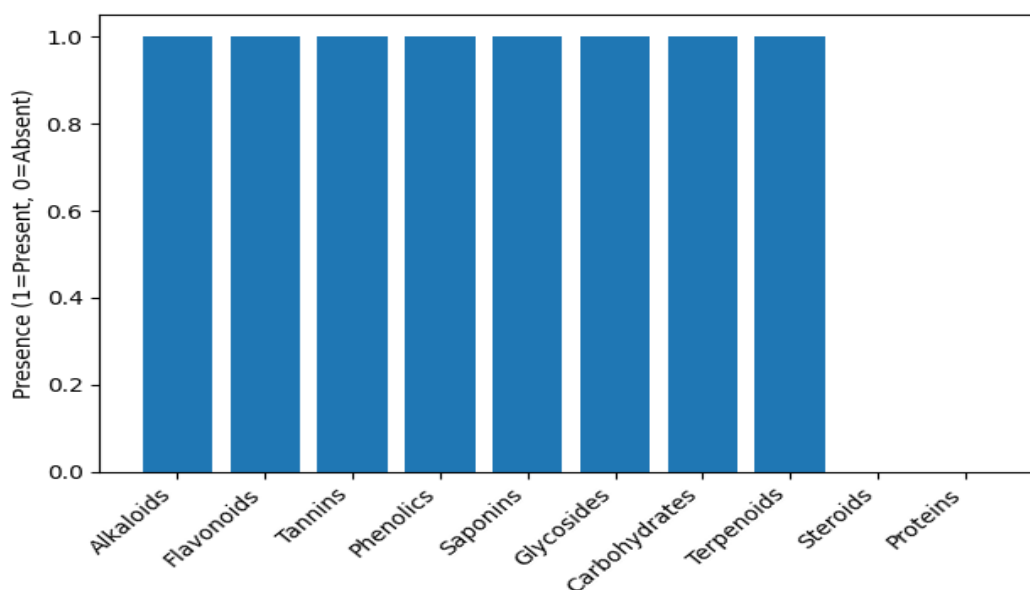


Figure 3: Preliminary Phytochemical Constituents of Plant Extract

The higher number of present phytoconstituents suggests that the plant extract is rich in secondary metabolites, which are responsible for various pharmacological activities such as antioxidant, anti-inflammatory, and protective effects.

Quantitative Phytochemical Analysis: The results are expressed as mg Gallic Acid Equivalent per gram of extract (mg GAE/g extract) for TPC and mg Quercetin Equivalent per gram of extract (mg QE/g extract) for TFC.

Table 3: Quantitative Phytochemical Constituents of Plant Extract

S. No.	Parameter	Result
1	Total Phenolic Content (TPC)	72.5mg GAE/g extract
2	Total Flavonoid Content (TFC)	45.3mg QE/g extract

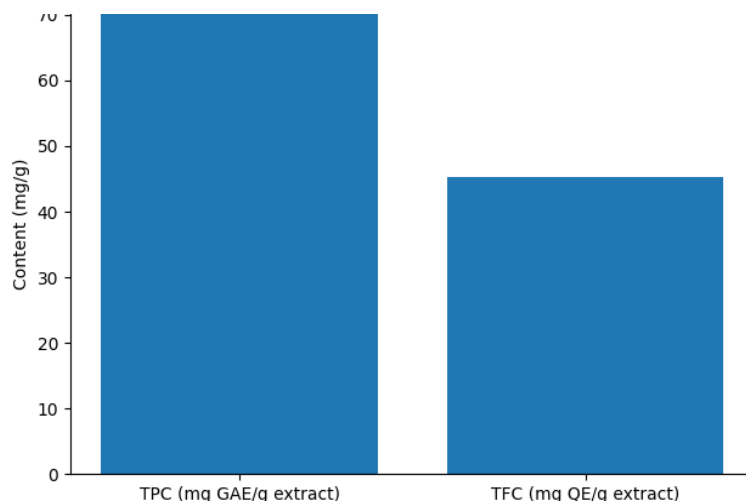


Figure 4: Quantitative Phytochemical Constituents of Plant Extract

The presence of a significant amount of flavonoids further supports the medicinal potential of the extract. Flavonoids are reported to exhibit antioxidant, anti-inflammatory, antimicrobial, and cytoprotective activities.

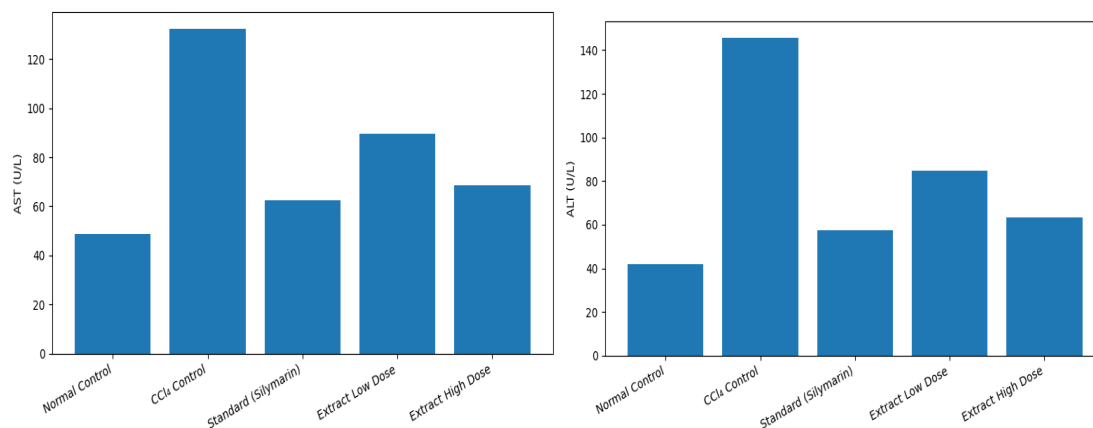
Acute Toxicity Study: The acute toxicity study of the plant extract was performed as per OECD guideline 423. The extract did not produce any mortality or noticeable toxic symptoms at the dose of 2000 mg/kg body weight administered orally.

Hence, the LD₅₀ of the extract was considered to be greater than 2000 mg/kg, indicating that the extract is relatively safe.

Biochemical Results: The levels of AST, ALT, ALP, and total bilirubin were markedly elevated in the toxic control group, indicating severe hepatocellular damage and impaired liver function. In contrast, total protein and albumin levels were significantly reduced, reflecting decreased synthetic capacity of the liver.

Table 4: Effect of Plant Extract on Serum Biochemical Parameters in CCl₄-Induced Hepatotoxicity

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)
Normal Control	48.6	42.1	96.5	0.72	7.12	4.32
CCl ₄ Control	132.4	145.8	218.6	2.84	4.18	2.31
Standard (Silymarin)	62.3	57.4	118.3	1.05	6.61	3.98
Extract Low Dose	89.7	84.6	156.7	1.74	5.72	3.24
Extract High Dose	68.5	63.2	129.4	1.21	6.34	3.76



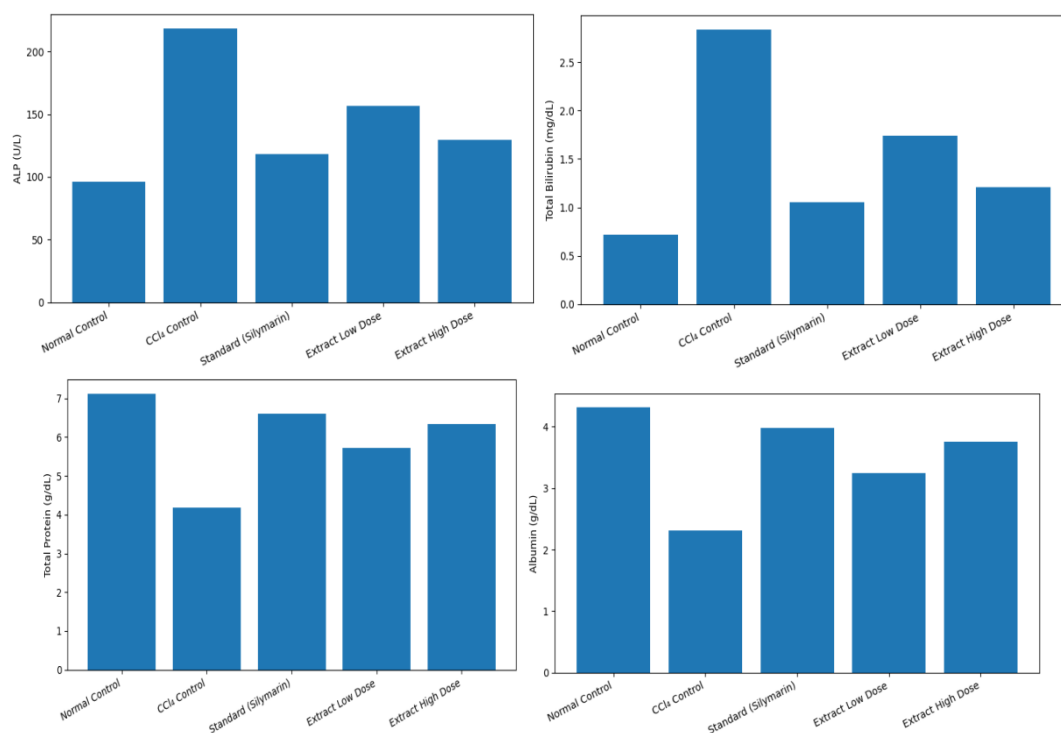


Figure 5: Effect of Plant Extract on All Serum Biochemical Parameters in CCl₄-Induced Hepatotoxicity

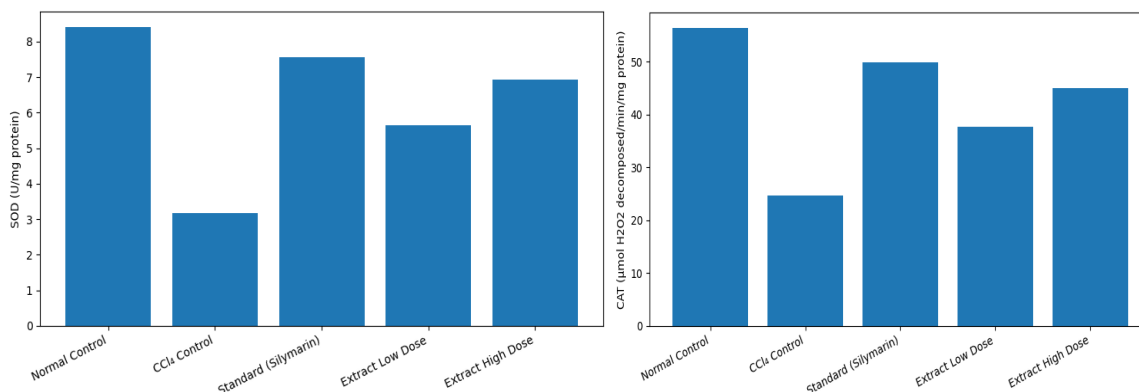
The biochemical findings clearly demonstrate that CCl₄ produced severe liver damage, as evidenced by elevated serum enzyme markers and reduced protein synthesis markers. Treatment with the plant extract significantly reversed these changes. The high-dose extract showed better hepatoprotective activity than the low-dose extract, though Silymarin remained the most effective treatment. These results suggest that the plant extract possesses significant hepatoprotective potential against CCl₄-induced hepatotoxicity.

Antioxidant Results: The antioxidant analysis of liver tissue revealed a marked reduction in endogenous antioxidant enzymes in the CCl₄ control group when compared with the normal control group.

The levels of superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were significantly decreased in the toxic control group, indicating severe oxidative stress induced by CCl₄ administration.

Table 5: Effect of Plant Extract on Antioxidant Parameters in Liver Tissue of Experimental Animals

Group	SOD (U/mg protein)	CAT (μmol H ₂ O ₂ decomposed/min/mg protein)	GSH (μg/mg protein)
Normal Control	8.42	56.4	7.18
CCl ₄ Control	3.18	24.7	2.64
Standard (Silymarin)	7.56	49.8	6.45
Extract Low Dose	5.64	37.6	4.83
Extract High Dose	6.93	44.9	5.92



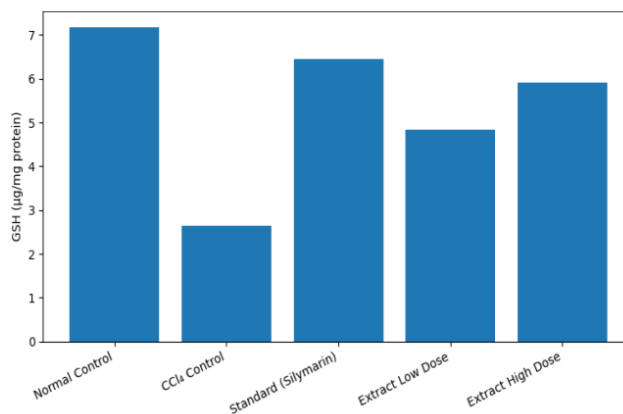


Figure 6: Effect of Plant Extract on All Antioxidant Parameters in Liver Tissue of Experimental Animals

The antioxidant results clearly show that CCl₄-induced hepatotoxicity caused severe oxidative stress, as evidenced by the marked reduction in SOD, CAT, and GSH levels.

Treatment with the plant extract significantly reversed these changes and improved the antioxidant defense system of the liver. The effect was dose-dependent, with the high-dose extract producing greater protection than the low dose. These findings support the antioxidant-mediated hepatoprotective activity of the plant extract.

Histopathological Study: Histopathological examination of liver tissue sections was carried out

to evaluate the structural changes induced by CCl₄ and the protective effect of the plant extract and standard drug treatment. The liver sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope.

Normal Control Group: The liver section of the normal control group showed normal hepatic architecture. Hepatocytes were arranged in cords around the central vein with clear cytoplasm and distinct nuclei. The sinusoidal spaces were normal, and no pathological abnormalities such as necrosis, fatty change, or inflammatory infiltration were observed.



Figure 7: Image of Normal Control Group

CCl₄ Control Group: The liver section of the CCl₄ control group showed severe pathological changes, including centrilobular necrosis, fatty degeneration, hepatocellular ballooning, inflammatory cell infiltration, and sinusoidal congestion. The normal hepatic architecture was markedly disturbed, confirming successful induction of hepatotoxicity.

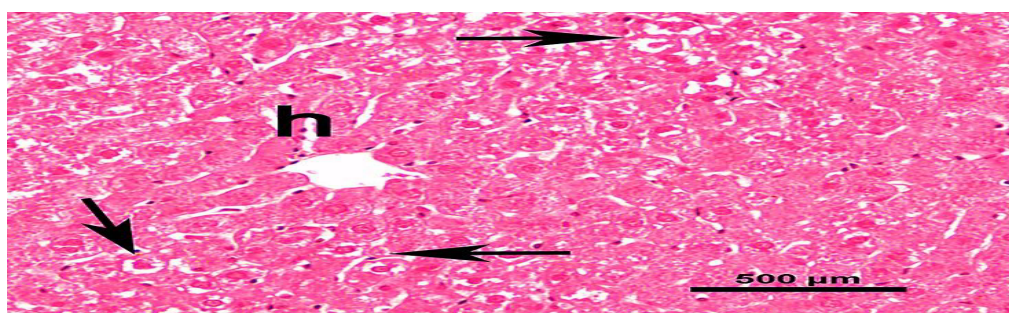


Figure 8: Image of CCl₄ Control Group

Standard (Silymarin) Group: The liver section of the standard-treated group showed remarkable protection against CCl₄-induced damage. Most hepatocytes appeared normal, with only mild sinusoidal dilation and slight inflammatory infiltration. Necrotic and fatty changes were markedly reduced, indicating the strong hepatoprotective effect of Silymarin.

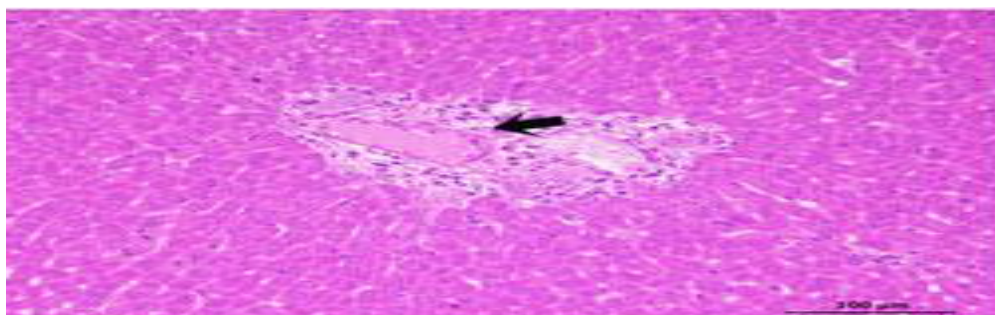


Figure 9: Image of Standard (Silymarin) Group

Extract Low Dose Group: The liver section of the extract low-dose group showed moderate recovery of liver tissue. Compared with the CCl₄ control group, there was a clear reduction in necrosis and fatty degeneration. However, some mild inflammatory infiltration and focal hepatocellular damage were still observed.

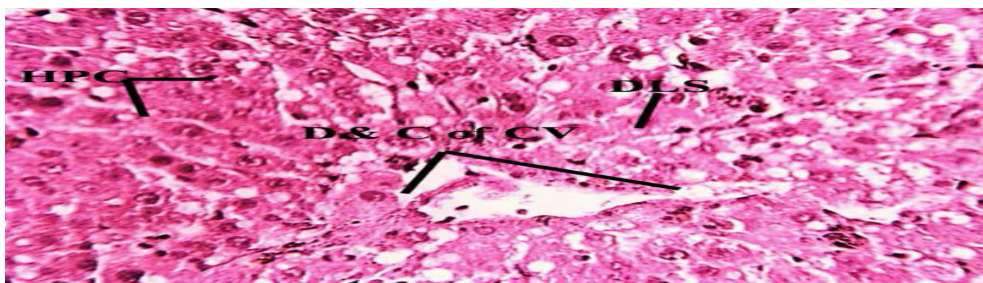


Figure 10: Image of Extract Low Dose Group

Extract High Dose Group: The liver section of the extract high-dose group showed significant restoration of liver architecture. Hepatocytes appeared nearly normal with minimal degenerative changes. Inflammatory infiltration and sinusoidal congestion were greatly reduced. The high dose produced better histological protection than the low dose and approached the effect of the standard drug.

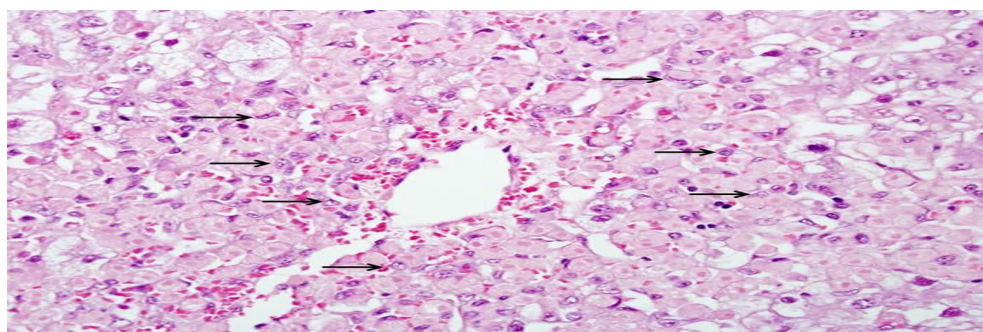


Figure 11: Image of Extract High Dose Group

Histopathological examination confirmed the biochemical findings.

The CCl₄ control group showed severe hepatic injury with necrosis, fatty change, and inflammation, whereas treatment with Silymarin and the plant extract protected the liver tissue against such damage.

The extract exhibited dose-dependent hepatoprotective activity, with the high-dose group

showing greater restoration of normal hepatic structure than the low-dose group. These results indicate that the plant extract possesses significant hepatoprotective potential, possibly due to its antioxidant and membrane-stabilizing properties.

Discussion

The present study was undertaken to evaluate the hepatoprotective potential of *Moringa concanensis* bark extract against CCl₄-induced hepatotoxicity in

Wistar rats. CCl₄ produces hepatic injury mainly through oxidative stress, lipid peroxidation, and depletion of endogenous antioxidant defenses. The phytoconstituents present in *M. concanensis*, particularly phenolic and flavonoid compounds, may contribute to antioxidant-mediated protection of hepatic tissue. Evaluation of biochemical, antioxidant, and histopathological parameters provides a comprehensive assessment of the protective effect of the extract. Overall, the study provides a scientific basis for further investigation of *Moringa concanensis* bark as a potential herbal hepatoprotective agent.

Conclusion

Moringa concanensis bark extract showed promising hepatoprotective activity against CCl₄-induced liver injury in Wistar rats. The extract improved biochemical and antioxidant parameters and reduced

The protective effect may be attributed to its antioxidant phytoconstituents, particularly phenolics and flavonoids. Thus, *M. concanensis* bark may serve as a potential natural hepatoprotective agent and warrants further investigation.

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