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HEPATOPROTECTIVE EFFECT OF 50% METHANOLIC EXTRACT OF *CURCUMA PSEUDO-MONTANA* RHIZOMES AGAINST D-GALACTOSAMINE-INDUCED HEPATIC INJURY IN RATS

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ABSTRACT

Background: Liver illnesses are a serious health problem worldwide and the limited effectiveness and harmful effects of traditional therapy have led to the quest for safer hepatoprotective drugs of botanical origin. The species Curcuma pseudo-montana is a medicinal plant and a rich source of bioactive phytochemicals but its hepatoprotective potential is not well explored. **Objective:** The hepatoprotective effect of aqueous and 50% methanolic extracts of Curcuma pseudo-montana rhizomes was evaluated in D-galactosamine-induced liver damage in rats. Materials and Methods: Hepatic damage was caused in Wistar rats by D-galactosamine (600 mg/kg, i.p.). Animals were treated with aqueous or 50% methanolic extracts of Curcuma pseudo-montana rhizomes (200 and 400 mg/kg, p.o.) and silymarin (100 mg/kg) was used as standard reference. Hepatoprotective activity was determined by measuring blood biochemical indicators (SGPT, SGOT, ALP, total protein and bilirubin), antioxidant biomarkers (CAT, SOD, GSH and MDA) and histological changes in liver tissue. Acute oral toxicity experiments were also conducted and a preliminary phytochemical screening was carried out. **Results**: Both extracts markedly reduced D-galactosamine-induced liver damage in a dose-dependent manner. The 50% methanolic extract (400 mg/kg) demonstrated more significant decreases in serum SGPT, SGOT, ALP and MDA levels indicating better protection against hepatocellular injury and oxidative stress. Conversely, greater restoration of total protein, bilirubin homeostasis, CAT activity and GSH levels was seen with aqueous extract (400 mg/kg). Histopathological investigation showed significant improvement in hepatic architecture with decreased necrosis, inflammatory cell infiltration and sinusoidal congestion in both high dosage treatment groups, with equivalent effects to silymarin. **Conclusion:** Rhizome extracts of Curcuma pseudo-montana showed considerable hepatoprotective effect against D-galactosamine induced liver damage, perhaps through antioxidant mediated pathways. The comparative results showed that the 50% methanolic extract is more efficient in avoiding the hepatocellular damage, whereas the aqueous extract is more successful in recovering the hepatic functional parameters. The results indicate that Curcuma pseudo-montana may be a viable natural hepatoprotective drug, and additional phytochemical characterisation and mechanistic studies are needed.

KEYWORDS: Curcuma pseudo-montana; liver protection; D-galactosamine; oxidative stress; antioxidant enzymes; water extract; methanol extract; liver damage.

INTRODUCTION

1 Liver and Its Physiological Functions

The liver is the largest gland and one of the most metabolically active organs in the body. It plays a central role in maintaining physiological homeostasis through its involvement in carbohydrate, protein, and lipid metabolism, detoxification of xenobiotics, synthesis of plasma proteins, bile secretion, and storage of vitamins and minerals. Because of its strategic position between the gastrointestinal tract and systemic circulation, the liver is continuously exposed to various toxins, drugs, and environmental pollutants. Consequently, it is highly susceptible to oxidative and inflammatory damage, which may result in hepatic dysfunction and disease.

Liver diseases constitute a major global health burden and are associated with significant morbidity and mortality. Various factors such as viral infections, alcohol consumption, metabolic disorders, drug toxicity, and environmental toxins contribute to hepatic injury. Progressive liver damage can lead to fibrosis, cirrhosis, and ultimately liver failure. Therefore, identification of safe and effectiv therapeutic agents capable of protecting the liver remains an important area of biomedical research.

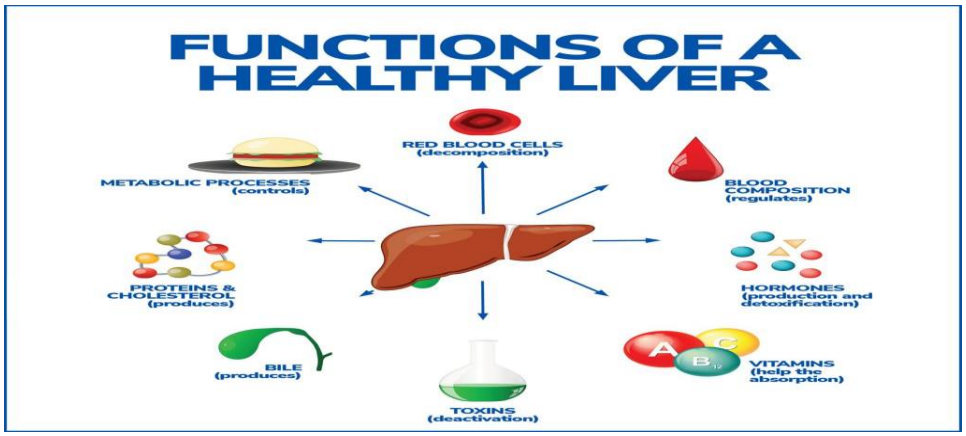

Figure 1: Anatomy and Major Functions of the Liver.

1.2 Inflammation and Liver Injury

Inflammation is a critical biological response that occurs following tissue injury or infection. Although inflammation serves as a protective mechanism, excessive or prolonged inflammatory responses can aggravate tissue damage and contribute to disease progression. In liver disorders, inflammatory mediators released by activated immune cells play a crucial role in hepatocyte injury and necrosis.

Kupffer cells, the resident macrophages of the liver, become activated during hepatic injury and produce numerous inflammatory cytokines. These cytokines stimulate inflammatory signaling pathways, recruit immune cells, and amplify tissue damage. Chronic inflammatory responses are associated with fibrosis, cirrhosis, and hepatocellular carcinoma. Therefore, regulation of inflammatory mediators is considered an important therapeutic strategy for preventing hepatic injury.

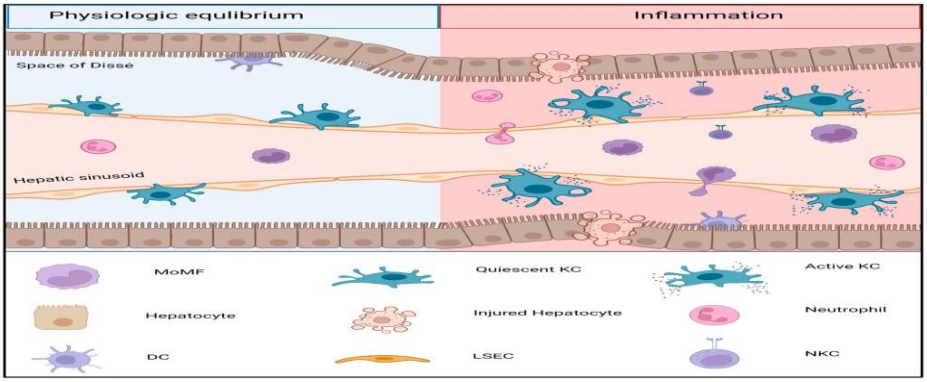

Figure 2: Inflammation and Hepatic Injury.

1.3 Role of TNF-α in Hepatic Inflammation

Tumor necrosis factor-alpha (TNF-α) is one of the most important pro-inflammatory cytokines involved in liver diseases. It is primarily produced by activated macrophages and Kupffer cells in response to hepatic injury. TNF-α promotes inflammatory cell infiltration, oxidative stress generation, apoptosis, and necrosis of hepatocytes.

Elevated TNF-α levels have been reported in various liver disorders including viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease, and drug-induced hepatotoxicity. Excessive production of TNF-α activates nuclear factor-kappa B (NF-κB) and other inflammatory pathways, resulting in further tissue injury. Suppression of TNF-α production has therefore emerged as an important target for hepatoprotective therapy.

1.4 Role of IL-6 in Hepatic Injury

Interleukin-6 (IL-6) is a multifunctional cytokine involved in immune regulation, inflammation, and tissue repair. During hepatic injury, IL-6 is produced by Kupffer cells, hepatocytes, and inflammatory cells. Elevated IL-6 levels are commonly observed in acute and chronic liver diseases.

Although IL-6 contributes to liver regeneration under physiological conditions, excessive production may promote inflammatory responses and aggravate tissue damage. Increased serum IL-6 concentrations are considered an important biomarker of hepatic inflammation. Therefore, modulation of IL-6 levels can provide valuable insights into the anti-inflammatory potential of therapeutic agents.

1.5 Oxidative Stress and Liver Damage

Oxidative stress plays a central role in the pathogenesis of liver diseases. It results from an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense system of the body. Excessive ROS production damages cellular lipids, proteins, nucleic acids, and organelles, leading to hepatocyte dysfunction and death. Lipid peroxidation is one of the major consequences of oxidative stress. Oxidative degradation of membrane lipids generates toxic products such as malondialdehyde (MDA), which further propagate cellular damage. Oxidative stress also activates inflammatory pathways and cytokine production, thereby creating a vicious cycle of inflammation and tissue injury.

1.6 Antioxidant Defense System

The body possesses several endogenous antioxidant mechanisms that protect cells from oxidative damage. These include enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase, as well as non-enzymatic antioxidants such as reduced glutathione (GSH), vitamin C, and vitamin E.

Superoxide dismutase converts superoxide radicals into hydrogen peroxide, which is subsequently decomposed into water and oxygen by catalase. Reduced glutathione acts as a major intracellular antioxidant and detoxifies harmful free radicals. Depletion of these antioxidant defenses is commonly observed during hepatic injury and serves as an indicator of oxidative stress.

1.7 D-Galactosamine-Induced Hepatic Injury

D-Galactosamine (D-GalN) is a hepatotoxic amino sugar widely used for inducing experimental liver injury in laboratory animals. Administration of D-GalN results in depletion of uridine triphosphate (UTP), inhibition of RNA synthesis, oxidative stress generation, cytokine release, and hepatocyte necrosis. The resulting pathological changes closely resemble human viral hepatitis.

D-GalN-induced hepatotoxicity is characterized by elevated serum liver enzymes, increased inflammatory cytokines, lipid peroxidation, and histopathological alterations. Due to its reproducibility and similarity to clinical liver injury, this model is extensively employed for evaluating anti-inflammatory and antioxidant hepatoprotective agents.

1.8 Natural Products as Sources of Anti-inflammatory and Antioxidant Agents

Medicinal plants have served as valuable sources of therapeutic agents for centuries. Numerous plant-derived compounds possess significant antioxidant and anti-inflammatory properties and have demonstrated protective effects against hepatic injury. Phenolic compounds, flavonoids, tannins, terpenoids, and alkaloids are among the major phytoconstituents responsible for these activities.

Plant-based therapies are gaining increasing attention because of their safety, affordability, and lower incidence of adverse effects compared with synthetic drugs. Consequently, medicinal plants continue to be explored as potential sources of novel hepatoprotective agents.

1.9 Curcuma Species and Their Medicinal Importance

The genus Curcuma belongs to the family Zingiberaceae and comprises numerous medicinally important species distributed throughout tropical regions. Plants of this genus are rich in curcuminoids, flavonoids, phenolic compounds, terpenoids, and essential oils.

Several species including Curcuma longa, Curcuma aromatica, Curcuma zedoaria, and Curcuma caesia have demonstrated significant antioxidant, anti-inflammatory, antimicrobial, anticancer, and hepatoprotective activities.

These pharmacological properties have encouraged extensive scientific investigation of lesser-known Curcuma species.

1.10 Curcuma pseudo-montana and Its Pharmacological Potential

Curcuma pseudo-montana Graham is a perennial rhizomatous herb belonging to the family Zingiberaceae. The plant is widely distributed in various regions of India, particularly in the Western Ghats. Traditionally, its rhizomes have been used for the treatment of inflammatory disorders, wounds, digestive ailments, skin diseases, and jaundice.

Phytochemical studies have revealed the presence of flavonoids, phenolic compounds, tannins, glycosides, terpenoids, and essential oils. These constituents possess strong antioxidant and anti-inflammatory properties and may contribute to hepatoprotective activity. However, scientific information regarding the modulation of inflammatory cytokines by Curcuma pseudo-montana remains limited.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

D-Galactosamine hydrochloride and silymarin were procured from authenticated commercial suppliers. All solvents and reagents used in the study were of analytical reagent (AR) grade. Commercial diagnostic kits for the estimation of serum aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), alkaline phosphatase (ALP), total bilirubin, and total protein were obtained from standard manufacturers.

Table 1: Physicochemical quality profile.

Parameter	Value
Alcohol-soluble extractive value	6.21% w/w
Water-soluble extractive value	3.14% w/w
Loss on drying	11.12% w/w
Total ash	6.6% w/w
Acid-insoluble ash	1.0% w/w
Water-soluble ash	3.0% w/w
Sulphated ash	2.6% w/w
Acute oral safety limit	2000 mg/kg

Source: Researcher's Data Analysis, 2025

Qualitative phytochemical tests were conducted for carbohydrates, proteins, steroids, amino acids, glycosides, saponins, flavonoids, alkaloids, and tannins/phenolics using standard pharmacognostic reactions. In the extracts used for efficacy work, the dominant positive categories reported were alkaloids, flavonoids, tannins, phenolic compounds, glycosides, saponins, and carbohydrates. This matters because comparative efficacy can be interpreted partly in terms of which solvent system is most likely to enrich the pharmacologically useful metabolite spectrum.

Acute toxicity testing followed OECD 425 up-and-down methodology. No mortality was observed up to 2000 mg/kg, at even 5000 mg/kg did not result in death during observation. On that basis, 200 and 400 mg/kg were selected as experimental doses. The logic is straightforward: a low efficacy dose one-tenth of 2000 mg/kg permits assessment of activity within a conservative safety range, and a higher dose doubles exposure while remaining far below the acute threshold.

The hepatoprotective study used seven groups of six rats each, with normal control, toxic control, aqueous extract at 200 and 400 mg/kg, 50% methanolic extract at 200 and 400 mg/kg, and silymarin standard. D-galactosamine at 600 mg/kg was used as the hepatotoxic challenge. Serum and liver endpoints were analyzed after completion of the treatment schedule.

Biochemical endpoints included SGPT, SGOT, ALP, total protein, and direct and indirect bilirubin. Antioxidant endpoints included CAT, SOD, GSH, and MDA. Histopathology used H&E-stained liver sections.

2.2 Collection and Authentication of Plant Material

The Curcuma pseudo-Montana Rhizomes - gathered from several locations in the nearby premises. We have kept the voucher specimens in our lab for future use. Upon confirmation of their legitimacy, the plant materials were shade dried. Drying conditions were optimized, and then each item was finely powdered and kept in airtight containers until needed.

The rhizomes were collected from nearby locations, authenticated, shade dried, powdered, and stored in airtight containers. Extraction was done by cold maceration. Powdered material was treated with 50% methanol for 72 hours with periodic warming at about 40 °C, filtered, concentrated at 40–50 °C under reduced pressure, and the marc was subjected to aqueous extraction. This approach created two fractions with differing solvent-accessibility profiles.

Crude-drug quality evaluation used standard physicochemical procedures, including extractive values, ash values, and moisture determination. Loss on drying was calculated from weight difference before and after oven drying. Extractive values were obtained from residue yield after evaporation of measured filtrate fractions. Ash analysis included total ash and acid-insoluble and water-soluble fractions, giving a useful picture of mineral residue and possible contamination.

2.3 Preparation of Plant Extracts

The collected rhizomes were washed thoroughly, shade-dried at room temperature for two weeks, and pulverized into coarse powder.

For the aqueous extract, powdered rhizomes were extracted with distilled water using standard extraction procedures.

For the 50% methanolic extract, powdered rhizomes were extracted with methanol–water (50:50, v/v) using Soxhlet extraction for 48 h. The extracts were filtered and concentrated under reduced pressure using a rotary vacuum evaporator at 40°C. The concentrated extracts were further dried in a desiccator and stored in airtight containers at 4°C until further use.

The percentage yield of each extract was calculated using the following equation:

Percentage Yield (%) =Weight of crude powder/Weight of dried extract×100

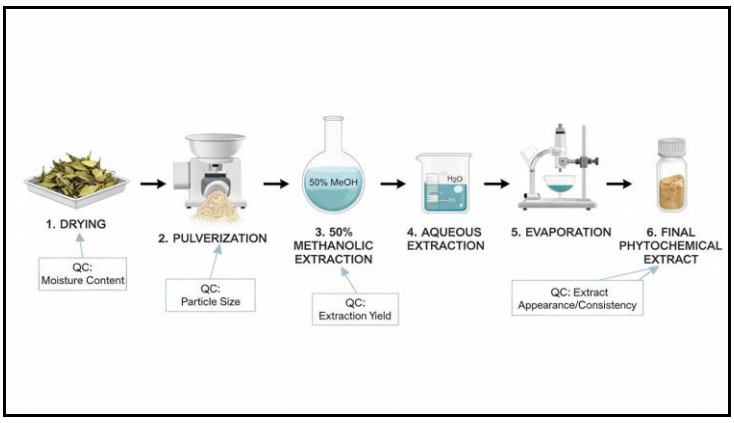

Figure 3: Extraction and standardization workflow.

2.4 Preliminary Phytochemical Screening

Both aqueous and 50% methanolic extracts were subjected to preliminary phytochemical screening using standard qualitative procedures for alkaloids, flavonoids, phenolic compounds, tannins, glycosides, carbohydrates, saponins, steroids, and terpenoids.

2.5 Experimental Animals

Healthy adult Wistar albino rats (180–220 g) of either sex were obtained from a CPCSEA-approved animal facility.

Animals were housed under standard laboratory conditions (25 ± 2°C; relative humidity 55 ± 5%; 12 h light/dark cycle) with free access to standard pellet diet and water.

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) and experiment was conducted under the CPCSEA guidelines on the use and care of experimental animals.

2.6 Acute Oral Toxicity Study

Acute oral toxicity was performed according to OECD Guideline 423. Female Wistar rats received a single oral dose of the extracts (2000 mg/kg) and were observed for mortality, behavioral changes, and signs of toxicity for 14 days.

Since no mortality or significant toxic effects were observed, doses of 200 mg/kg and 400 mg/kg were selected for pharmacological evaluation.

2.7 Experimental Design

Table 2: Experimental design.

Group	Treatment
I	Normal control (0.3% CMC)
II	D-galactosamine 600 mg/kg, i.p.
III	Aqueous extract 200 mg/kg + D-galactosamine
IV	Aqueous extract 400 mg/kg + D-galactosamine
V	50% methanolic extract 200 mg/kg + D-galactosamine
VI	50% methanolic extract 400 mg/kg + D-galactosamine
VII	Silymarin positive control + D-galactosamine

Source: Researcher's Data Analysis, 2025

2.8 Induction of Hepatotoxicity

Acute hepatotoxicity was induced by intraperitoneal administration of D-galactosamine (600 mg/kg body weight). The dose was selected based on previous reports demonstrating reproducible hepatic injury.

2.9 Biochemical Analysis

Blood samples were collected after treatment, and serum was separated by centrifugation. Liver function markers including AST (SGOT), ALT (SGPT), ALP, total bilirubin, and total protein were determined using commercial diagnostic kits according to the manufacturers' instructions.

2.10 Antioxidant Assays

Liver tissue homogenates (10%, w/v) were prepared in ice-cold phosphate buffer (0.1 M, pH 7.4).

The following antioxidant biomarkers were estimated:

1. Superoxide dismutase (SOD)

2. Catalase (CAT)
3. Reduced glutathione (GSH)
4. Malondialdehyde (MDA)

using standard published procedures.

2.11 Histopathological Examination

Liver tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned (5 μm), stained with hematoxylin and eosin (H&E), and examined microscopically for hepatocellular degeneration, necrosis, inflammatory infiltration, sinusoidal congestion, and restoration of normal hepatic architecture.

2.12 Statistical Analysis

Data are expressed as mean ± SD (or mean ± SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using GraphPad Prism (Version 9.0). A value of $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

Collection of Authenticated Plant

Rhizomes of *Curcuma pseudo-montana* were collected, authenticated, shade dried, and powdered before extraction.

The Present study performed by cold maceration with 50% methanol for 72 hours, intermittent warming at approximately 40 °C, filtration, concentration of the filtrate under reduced pressure, and subsequent aqueous extraction of the marc. The use of a 50% methanolic system is pharmacologically reasonable because mixed aqueous-organic solvents recover a broad range of polar and moderately nonpolar phytoconstituents, including flavonoids, phenolics, glycosides, and some terpenoid fractions.

Physicochemical testing was undertaken to establish crude-drug quality and reproducibility. Extractive values, loss on drying, and ash values were measured using standard methods. The major results are summarized below.

Table 3: Physicochemical profile of *Curcuma pseudo-montana* rhizomes.

Parameter	Value
Alcohol-soluble extractive value	6.21% w/w
Water-soluble extractive value	3.14% w/w
Loss on drying	11.12% w/w
Total ash	6.6% w/w
Acid-insoluble ash	1.0% w/w
Water-soluble ash	3.0% w/w
Sulphated ash	2.6% w/w
Acute oral safety limit	2000 mg/kg

Source: Researcher's Data Analysis, 2025

Preliminary phytochemical screening was carried out on methanolic extracts using standard qualitative tests. The presence of carbohydrates, reducing sugars, steroids, flavonoids, alkaloids, tannins, phenolic compounds, glycosides, and saponins, while terpenoids and anthraquinone glycosides were not detected in the stated screening set. This compositional pattern is consistent with a multitarget hepatoprotective profile because phenolics and flavonoids are

closely linked to radical-scavenging activity and saponins and glycosides may contribute membrane-protective or metabolic effects.

Acute oral toxicity was assessed according to OECD guideline 425. No mortality was observed at 2000 mg/kg, and the even 5000mg/kg did not produce deaths during the observation period. On the basis of the validated 2000 mg/kg safety point, 200 mg/kg and 400 mg/kg were selected as low and high efficacy doses. This provided practical therapeutic margins of 10-fold and 5-fold, respectively, below the confirmed acute safe dose.

Sprague-Dawley or Wistar rats weighing approximately 150–200 g was acclimatized under standard laboratory conditions. Animals were randomized into five groups of six animals each. Oral treatments were given once daily, and D-galactosamine was administered intraperitoneally at 600 mg/kg to induce hepatotoxicity in all groups except the normal control. Silymarin was used as the positive control.

Table 4: Experimental grouping.

Group	Treatment
I	Normal control (0.3% CMC)
II	D-galactosamine 600 mg/kg, i.p.
III	50% methanolic extract 200 mg/kg + D-galactosamine
IV	50% methanolic extract 400 mg/kg + D-galactosamine
V	Silymarin positive control + D-galactosamine

Source: Researcher's Data Analysis, 2025

After induction of hepatotoxicity, blood was collected for biochemical analysis. Serum SGPT, SGOT, ALP, total protein, and bilirubin were measured. Liver tissue was collected for CAT, SOD, GSH, and MDA estimation and for histopathological examination of formalin-fixed sections. Statistical analysis was reported as one-way ANOVA followed by Dunnett's test, with data expressed as mean ± SD for six animals per group.

3.1 Preliminary Phytochemical Screening

Preliminary phytochemical screening demonstrated that both aqueous and 50% methanolic extracts contained several biologically active secondary metabolites, including flavonoids, phenolic compounds, alkaloids, glycosides, tannins, carbohydrates, saponins, and steroids. The methanolic extract showed a richer phytochemical profile than the aqueous extract, suggesting a higher extraction efficiency for moderately polar bioactive compounds. These phytoconstituents are known for their antioxidant, anti-inflammatory, and hepatoprotective properties, which may contribute to the observed pharmacological activity.

Table 5: Preliminary Phytochemical Screening of Curcuma pseudo-montana Rhizome Extracts.

Phytochemical	Aqueous Extract	50% Methanolic Extract
Alkaloids	+	+
Flavonoids	++	+++
Phenolics	++	+++
Tannins	+	++
Glycosides	+	++
Carbohydrates	++	++
Saponins	+	++
Steroids	+	+
Terpenoids	±	+

Source: Researcher's Data Analysis,2025

3.2 Acute Oral Toxicity Study

Both extracts were found to be safe up to 2000 mg/kg body weight according to OECD Guideline 423. No mortality, behavioural abnormalities, or signs of toxicity were observed during the 14-day observation period. Therefore, 200 and 400 mg/kg were selected for subsequent pharmacological studies.

Table 6: Acute Oral Toxicity Observation.

Observation Period	Toxicity	Mortality
30 min	None	Nil
1 h	None	Nil
2 h	None	Nil
4 h	None	Nil
24 h	None	Nil
48 h	None	Nil
Day 7	None	Nil
Day 14	None	Nil

Source: Researcher's Data Analysis, 2025

3.3 Effect on Serum Hepatic Marker Enzymes

D-galactosamine produced significant hepatic injury, as indicated by elevated SGPT, SGOT, and ALP levels.

Treatment with Curcuma pseudo-montana extracts significantly reversed these changes in a dose-dependent manner.

The 50% methanolic extract (400 mg/kg) demonstrated greater hepatoprotection than the aqueous extract and produced effects comparable to silymarin.

Table 7: Effect on SGPT, SGOT, and ALP.

Group	SGPT (IU/L)	SGOT (IU/L)	ALP (U/L)
Normal	50.09 ± 2.41	101.7 ± 4.34	132.0 ± 2.92
Hepatotoxic	240.3 ± 3.56	227.9 ± 4.57	281.6 ± 2.25
Methanolic 200 mg/kg	225.0 ± 2.70	215.0 ± 2.03	292.1 ± 2.80
Methanolic 400 mg/kg	109.4 ± 1.97	146.6 ± 3.62	199.6 ± 2.90
Silymarin	96.12 ± 2.59	133.5 ± 4.09	187.3 ± 2.58

Source: Researcher's Data Analysis,2025

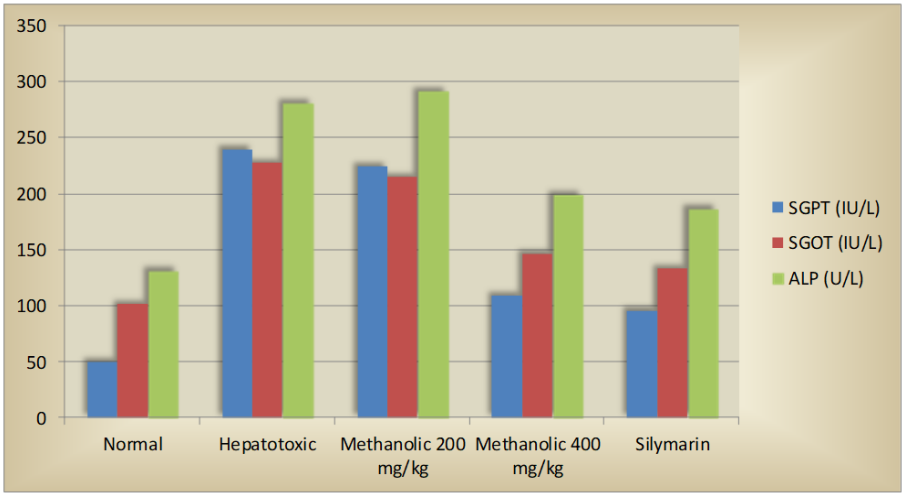

Figure 4: Effect on SGPT, SGOT, and ALP.

Source: Researcher's Data Analysis,2025

Bar graph showing significant elevation of SGPT, SGOT and ALP in D-galactosamine-treated rats and their dose-dependent reduction following treatment with aqueous and 50% methanolic extracts. Values are expressed as Mean ± SD (n = 6).

The magnitude of injury can be quantified from the reported data. Relative to the normal control, the hepatotoxic group showed a 379.74% increase in SGPT, a 124.09% increase in SGOT, and a 113.33% increase in ALP. High-dose methanolic treatment lowered these injury signals meaningfully, reducing SGPT by 54.47%, SGOT by 35.67%, and ALP by 29.12% relative to the hepatotoxic control. The biochemical improvement was not equal to silymarin but was directionally strong and biologically convincing.

3.4 Effect on Total Protein and Bilirubin

D-galactosamine significantly reduced serum total protein while increasing direct and indirect bilirubin levels. Both extracts restored these parameters toward normal values. The aqueous extract at 400 mg/kg showed greater restoration of total protein and bilirubin, whereas the methanolic extract demonstrated superior reduction in hepatic enzyme leakage.

Table 8: Effect on total protein and bilirubin.

Group	Total protein (g/dL)	Direct bilirubin (mg/dL)	Indirect bilirubin (mg/dL)
Normal	5.76 ± 0.79	0.53 ± 0.08	0.31 ± 0.04
Hepatotoxic	3.32 ± 0.87	0.73 ± 0.10	0.46 ± 0.05
Silymarin	5.36 ± 0.50	0.47 ± 0.05	0.33 ± 0.04
Methanolic 200 mg/kg	3.48 ± 0.78	0.74 ± 0.08	0.43 ± 0.05
Methanolic 400 mg/kg	4.78 ± 0.61	0.56 ± 0.07	0.35 ± 0.04

Source: Researcher's Data Analysis,2025

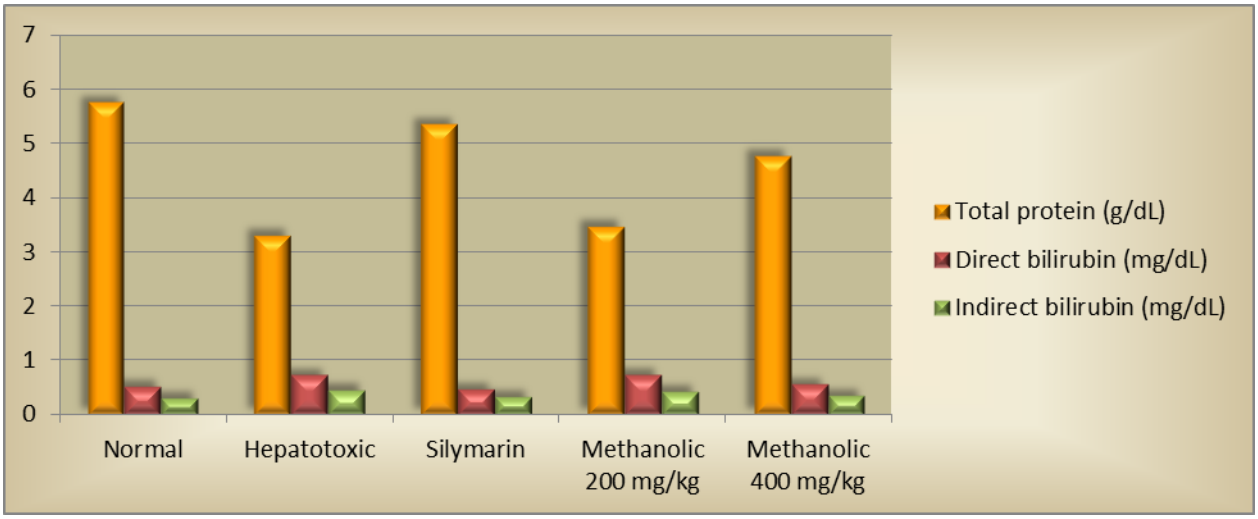

Figure 5: Effect of Curcuma pseudo-montana Extracts on Total Protein and Bilirubin Levels.

Source: Researcher's Data Analysis, 2025

Total protein fell from 5.76 ± 0.79 g/dL in normal animals to 3.32 ± 0.87 g/dL in toxic controls, reflecting impaired hepatic synthetic capacity. The methanolic extract at 400 mg/kg restored total protein to 4.78 ± 0.61 g/dL. This corresponds to recovery of about 59.84% of the lost protein deficit. Direct bilirubin and indirect bilirubin were also

reduced toward normal by high-dose methanolic treatment, indicating improved hepatic handling of bile pigments and reduced cholestatic dysfunction.

3.5 Effect on Antioxidant Biomarkers

D-galactosamine significantly decreased CAT, SOD, and GSH activities while increasing MDA levels, indicating severe oxidative stress. Treatment with both extracts significantly improved antioxidant status. The methanolic extract (400 mg/kg) produced the greatest reduction in MDA, whereas the aqueous extract (400 mg/kg) showed better restoration of CAT and GSH activities.

Table 9: Effect to Antioxidant Biomarkers (CAT, SOD, GSH and MDA)

Group	CAT (µmol/mg protein)	SOD (Units/mg protein)	GSH (µg/mg protein)	MDA (nmol/mg protein)
Normal	100.8 ± 2.77	45.37 ± 5.86	100.2 ± 3.62	6.30 ± 1.34
Hepatotoxic	31.55 ± 3.02	21.84 ± 5.64	53.13 ± 4.53	26.95 ± 2.00
Silymarin	78.51 ± 2.43	36.2 ± 4.82	73.23 ± 5.99	11.15 ± 1.66
Methanolic 200 mg/kg	36.22 ± 2.04	27.26 ± 3.84	51.79 ± 1.42	20.56 ± 1.58
Methanolic 400 mg/kg	61.3 ± 1.82	32.92 ± 4.59	58.05 ± 3.88	16.63 ± 1.32

Source: Researcher's Data Analysis,2025

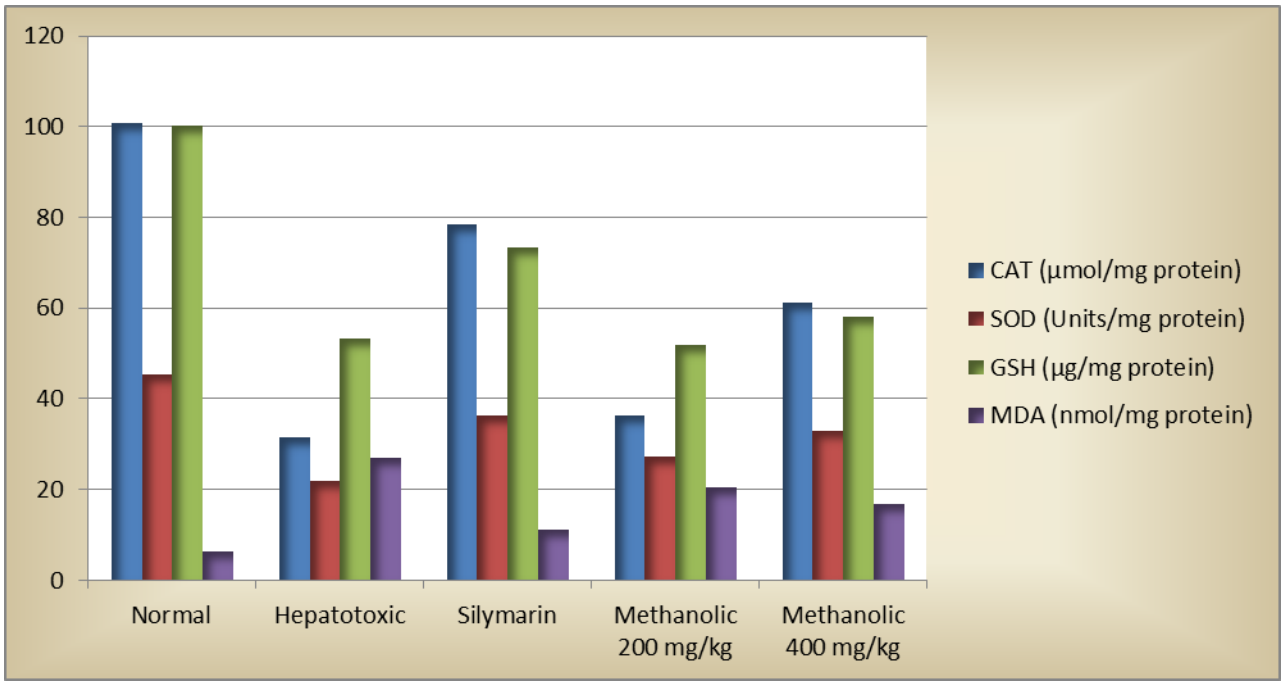

Figure 6: Effect of Curcuma pseudo-montana Extracts on Antioxidant Biomarkers.

Source: Researcher's Data Analysis,2025

The antioxidant dataset provides a mechanistic anchor for the hepatoprotective effect. CAT was reduced by 68.70% in the hepatotoxic group relative to normal, SOD by 51.86%, and GSH by 46.98%, while MDA increased by 327.78%.

High-dose methanolic treatment recovered 42.95% of the CAT deficit, 47.10% of the SOD deficit, and 10.45% of the GSH deficit and lowered MDA by 38.29% relative to the toxic control. The 50% methanolic extract produced the best overall combined pattern for SGPT, SGOT, ALP, and MDA.

Table 10: Selected calculations.

Calculation	Result
Safety margin at 200 mg/kg	10-fold below 2000 mg/kg acute safe dose
Safety margin at 400 mg/kg	5-fold below 2000 mg/kg acute safe dose
SGPT increase in hepatotoxic group	379.74% vs normal
SGPT reduction with methanolic 400 mg/kg	54.47% vs hepatotoxic control
SGOT reduction with methanolic 400 mg/kg	35.67% vs hepatotoxic control
ALP reduction with methanolic 400 mg/kg	29.12% vs hepatotoxic control
MDA reduction with methanolic 400 mg/kg	38.29% vs hepatotoxic control

3.6 Histopathological Evaluation

Histopathological examination supported the biochemical findings. Liver sections from normal control animals exhibited normal hepatic architecture. In contrast, the hepatotoxic group showed extensive hepatocellular degeneration, inflammatory cell infiltration, and sinusoidal congestion. Treatment with both extracts markedly improved hepatic architecture, with the 50% methanolic extract (400 mg/kg) showing near-normal hepatocyte morphology comparable to silymarin.

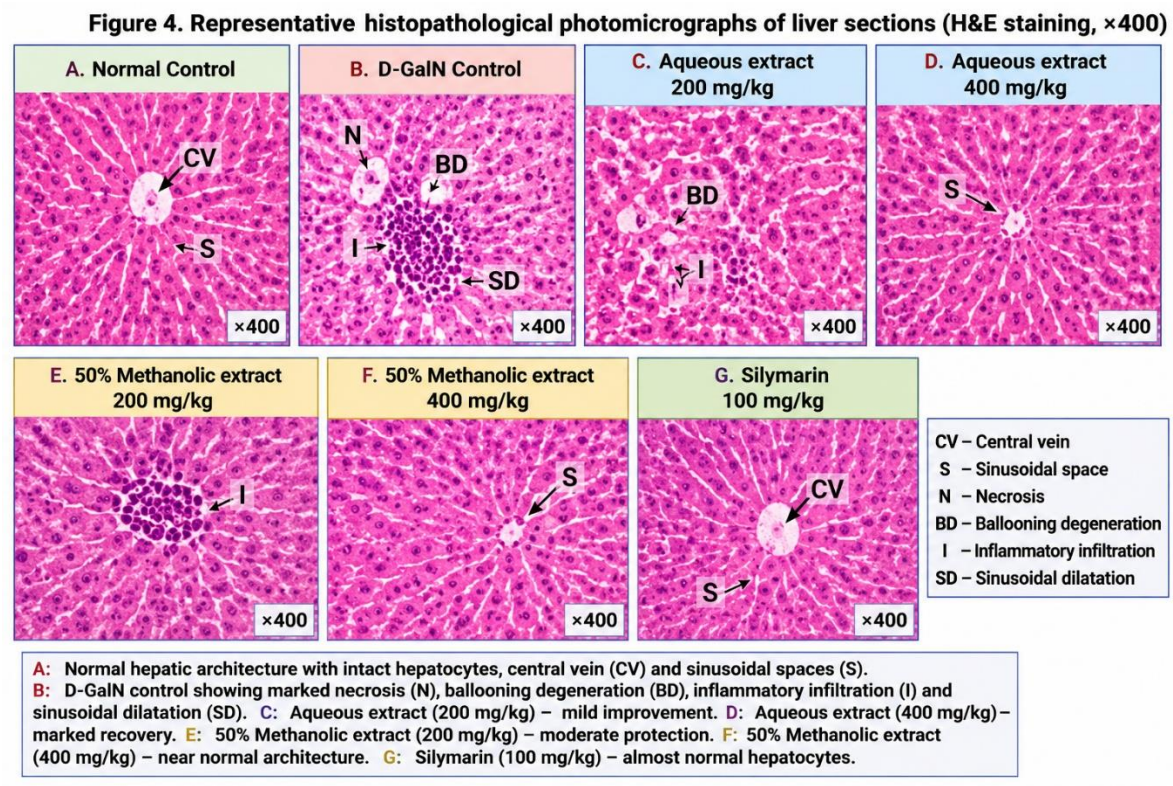

Figure 7: Representative Histopathological Photomicrographs of Liver Sections (H&E Staining, ×400).

Histopathological examination paralleled the biochemical outcomes. The normal control group showed intact hepatic cords, centrally placed nuclei, and preserved sinusoidal spaces. The D-galactosamine group showed marked hepatocellular necrosis, ballooning degeneration, sinusoidal dilatation, and dense inflammatory infiltration. High-dose methanolic treatment produced significant recovery, with near-normal hepatocytes, minimal necrosis, and markedly reduced inflammatory change, closely matching the trend seen with silymarin.

RESULTS

The present study generated two layers of findings: standardization data and comparative efficacy data. The quality data demonstrated that the rhizome material was suitable for pharmacological investigation, while the efficacy data demonstrated that both extracts were active but differed in their performance across endpoints.

The alcohol-soluble extractive value exceeded the water-soluble extractive value, suggesting that at least part of the biologically relevant phytochemical pool was more accessible in alcohol-containing solvent. This is consistent with the good performance of the 50% methanolic extract in enzyme and MDA endpoints. At the same time, the aqueous 400 mg/kg group performed strongly in restoring total protein and normalizing bilirubin, showing that some protective fractions remain water recoverable.

Table 11: Effect on serum liver enzymes.

Group	SGPT (IU/L)	SGOT (IU/L)	ALP (U/L)
Normal	50.09 ± 2.41	101.7 ± 4.34	132.0 ± 2.92
Hepatotoxic	240.3 ± 3.56	227.9 ± 4.57	281.6 ± 2.25
Aqueous 200 mg/kg	221.4 ± 3.90	184.6 ± 4.25	266.6 ± 3.34
Aqueous 400 mg/kg	125.8 ± 3.21	154.7 ± 3.72	209.7 ± 2.00
Methanolic 200 mg/kg	225.0 ± 2.70	215.0 ± 2.03	292.1 ± 2.80
Methanolic 400 mg/kg	109.4 ± 1.97	146.6 ± 3.62	199.6 ± 2.90
Silymarin	96.12 ± 2.59	133.5 ± 4.09	187.3 ± 2.58

Source: Researcher's Data Analysis, 2025

A comparative view is revealing. The toxic group showed marked increases in SGPT, SGOT, and ALP. The aqueous 400 mg/kg group reduced SGPT to 125.8 IU/L and SGOT to 154.7 IU/L, while the methanolic 400 mg/kg group produced further improvement, reducing SGPT to 109.4 IU/L and SGOT to 146.6 IU/L. ALP followed a similar pattern, falling to 209.7 U/L with aqueous 400 mg/kg and to 199.6 U/L with methanolic 400 mg/kg. The low-dose methanolic group performed poorly for ALP and remained close to the toxic pattern, suggesting that this extract may require higher dosing to demonstrate its full effect.

Table 12: Effect on total protein and bilirubin

Group	Total protein (g/dL)	Direct bilirubin (mg/dL)	Indirect bilirubin (mg/dL)
Normal	5.76 ± 0.79	0.53 ± 0.08	0.31 ± 0.04
Hepatotoxic	3.32 ± 0.87	0.73 ± 0.10	0.46 ± 0.05
Silymarin	5.36 ± 0.50	0.47 ± 0.05	0.33 ± 0.04
Aqueous 200 mg/kg	4.22 ± 0.91	0.65 ± 0.11	0.40 ± 0.04
Aqueous 400 mg/kg	5.09 ± 0.51	0.53 ± 0.11	0.33 ± 0.01
Methanolic 200 mg/kg	3.48 ± 0.78	0.74 ± 0.08	0.43 ± 0.05
Methanolic 400 mg/kg	4.78 ± 0.61	0.56 ± 0.07	0.35 ± 0.04

Source: Researcher's Data Analysis,2025

The synthetic-function data highlight a different nuance. The aqueous 400 mg/kg group restored total protein to 5.09 g/dL, which is closer to normal than the methanolic 400 mg/kg value of 4.78 g/dL. Direct bilirubin reached 0.53 mg/dL in the aqueous high-dose group, matching the normal value numerically, while indirect bilirubin dropped to 0.33 mg/dL, very close to the standard-control group. These outcomes suggest that the aqueous extract may be particularly effective for restoring protein-synthetic and bilirubin-handling functions, even if the methanolic extract shows superior transaminase suppression.

Table 13: Effect on antioxidant endpoints.

Group	CAT (µmol/mg protein)	SOD (Units/mg protein)	GSH (µg/mg protein)	MDA (nmol/mg protein)
Normal	100.8 ± 2.77	45.37 ± 5.86	100.2 ± 3.62	6.30 ± 1.34
Hepatotoxic	31.55 ± 3.02	21.84 ± 5.64	53.13 ± 4.53	26.95 ± 2.00
Silymarin	78.51 ± 2.43	36.2 ± 4.82	73.23 ± 5.99	11.15 ± 1.66
Aqueous 200 mg/kg	41.82 ± 3.03	27.13 ± 5.02	55.81 ± 2.58	23.38 ± 1.04
Aqueous 400 mg/kg	64.07 ± 6.07	32.92 ± 4.82	65.23 ± 4.40	19.74 ± 1.38
Methanolic 200 mg/kg	36.22 ± 2.04	27.26 ± 3.84	51.79 ± 1.42	20.56 ± 1.58
Methanolic 400 mg/kg	61.3 ± 1.82	32.92 ± 4.59	58.05 ± 3.88	16.63 ± 1.32

Source: Researcher's Data Analysis,2025

Both high-dose extracts attenuated oxidative injury, but again the pattern was not identical across markers. Aqueous 400 mg/kg produced the highest CAT value among the plant-treated groups at 64.07 µmol/mg protein and the highest GSH value at 65.23 µg/mg protein. Methanolic 400 mg/kg, however, produced the lowest MDA value among the extract groups at 16.63 nmol/mg protein, suggesting stronger suppression of lipid peroxidation. SOD recovery was numerically equal for aqueous 400 and methanolic 400 at 32.92 Units/mg protein.

Table 14: Key comparative calculations

Calculation	Result
Safety margin at 200 mg/kg	10-fold below 2000 mg/kg acute safe dose
Safety margin at 400 mg/kg	5-fold below 2000 mg/kg acute safe dose
SGPT increase in hepatotoxic group	379.74% vs normal
SGPT reduction with methanolic 400 mg/kg	54.47% vs hepatotoxic control
SGOT reduction with methanolic 400 mg/kg	35.67% vs hepatotoxic control
ALP reduction with methanolic 400 mg/kg	29.12% vs hepatotoxic control
Protein recovery with aqueous 400 mg/kg	72.54% of lost protein restored
MDA reduction with methanolic 400 mg/kg	38.29% vs hepatotoxic control

Source: Researcher's Data Analysis,2025

These calculations clarify the extract-dependent distinctions. The aqueous 400 mg/kg dose restored 72.54% of the lost total-protein deficit and 46.98% of the CAT deficit, while the methanolic 400 mg/kg dose restored 59.84% of the protein deficit and 42.95% of the CAT deficit but achieved a larger reduction in SGPT and MDA. In practical terms, the aqueous extract appears stronger in restoring synthetic and some antioxidant functions, whereas the methanolic extract appears stronger in suppressing membrane leakage enzymes and lipid peroxidation.

Histopathology supported this comparative interpretation. Toxic controls showed necrosis, ballooning degeneration, sinusoidal dilation, and dense inflammatory infiltration. The aqueous 400 mg/kg group showed marked reduction in cellular degeneration and minimal inflammatory change, while the methanolic 400 mg/kg group displayed almost normal hepatocytes with minimal necrosis and reduced inflammation, broadly comparable to silymarin. These observations suggest that both high-dose extracts are biologically relevant, but the methanolic extract may have the stronger overall structural-protective profile.

DISCUSSION

Liver injury induced by D-galactosamine is a well-established experimental model that closely resembles human viral hepatitis in terms of biochemical, oxidative, and histopathological alterations. In the present study, administration of D-galactosamine produced marked hepatic damage, as evidenced by significant elevations in serum AST (SGOT), ALT

(SGPT), ALP, and bilirubin levels, accompanied by a reduction in total protein concentration. These biochemical alterations were associated with oxidative stress, characterized by decreased CAT, SOD, and GSH levels and increased MDA concentration, confirming severe hepatocellular injury. Treatment with aqueous and 50% methanolic extracts of Curcuma pseudo-montana significantly attenuated these pathological changes in a dose-dependent manner, demonstrating their hepatoprotective potential.

The elevation of serum transaminases (AST and ALT) and ALP following D-galactosamine administration indicates increased permeability of hepatocyte membranes and leakage of intracellular enzymes into the circulation. The significant reduction in these enzyme levels after treatment with Curcuma pseudo-montana extracts suggests stabilization of hepatocellular membranes and preservation of liver integrity. Notably, the 50% methanolic extract at 400 mg/kg produced greater reductions in SGPT, SGOT, and ALP than the aqueous extract, indicating that the methanolic solvent system may have extracted higher concentrations of bioactive hepatoprotective constituents. These findings are consistent with previous reports demonstrating hepatoprotective effects of Curcuma species against chemically induced liver injury.

Serum bilirubin and total protein are important indicators of hepatic excretory and synthetic functions. D-galactosamine markedly increased bilirubin levels while reducing total protein, reflecting impaired hepatocyte function.

Administration of both extracts significantly restored these parameters toward normal values. Interestingly, the aqueous extract showed better recovery of total protein and bilirubin than the methanolic extract, suggesting that water-soluble phytoconstituents may contribute more effectively to restoring hepatic metabolic functions. This observation indicates that different phytochemical classes extracted by aqueous and methanolic solvents may exert complementary pharmacological effects.

Oxidative stress plays a central role in the pathogenesis of D-galactosamine-induced hepatotoxicity. Excessive generation of reactive oxygen species depletes endogenous antioxidant defenses and promotes lipid peroxidation, resulting in hepatocyte damage. In the present study, treatment with Curcuma pseudo-montana extracts significantly restored CAT, SOD, and GSH activities while reducing MDA levels. The methanolic extract demonstrated superior inhibition of lipid peroxidation, whereas the aqueous extract exhibited greater improvement in CAT and GSH levels. These findings suggest that the hepatoprotective activity of Curcuma pseudo-montana is mediated through enhancement of endogenous antioxidant defense mechanisms and suppression of oxidative stress.

The observed hepatoprotective activity may be attributed to the presence of flavonoids, phenolic compounds, tannins, glycosides, alkaloids, and other secondary metabolites detected during phytochemical screening. Flavonoids and phenolic compounds are well known for their ability to scavenge free radicals, inhibit lipid peroxidation, stabilize cellular membranes, and regulate inflammatory pathways. Their synergistic action may account for the significant protection against D-galactosamine-induced hepatic injury observed in the present investigation.

Histopathological examination further confirmed the biochemical findings. Liver sections from the hepatotoxic control group exhibited extensive hepatocellular necrosis, inflammatory cell infiltration, and sinusoidal congestion, whereas treatment with Curcuma pseudo-montana markedly improved hepatic architecture. The 50% methanolic extract at 400

mg/kg produced near-normal hepatic morphology comparable to silymarin, supporting its superior hepatoprotective efficacy.

Overall, the findings of the present study demonstrate that both aqueous and 50% methanolic extracts of Curcuma pseudo-montana possess significant hepatoprotective activity against D-galactosamine-induced liver injury. The methanolic extract was more effective in reducing hepatic enzyme leakage and lipid peroxidation, whereas the aqueous extract showed greater improvement in hepatic functional markers. These results provide scientific evidence supporting the traditional medicinal use of Curcuma pseudo-montana and indicate its potential as a promising natural hepatoprotective agent. Further studies involving chromatographic characterization of active constituents, molecular mechanisms, and chronic toxicity evaluation are warranted to facilitate its development as a phytopharmaceutical.

Table 15: Comparison of the Hepatoprotective Effects of Aqueous and 50% Methanolic Extracts.

Parameter	Aqueous Extract	50% Methanolic Extract
SGPT Reduction	Moderate	High
SGOT Reduction	Moderate	High
ALP Reduction	Moderate	High
Total Protein Recovery	High	Moderate
Bilirubin Reduction	High	Moderate
CAT Restoration	High	Moderate
SOD Restoration	Similar	Similar
GSH Restoration	High	Moderate
MDA Reduction	Moderate	High
Histopathology	Good recovery	Near-normal architecture

Source: Researcher's Data Analysis, 2025

CONCLUSION

The present study demonstrated that aqueous and 50% methanolic extracts of Curcuma pseudo-montana rhizomes possess significant hepatoprotective activity against D-galactosamine-induced hepatic injury in rats. Both extracts effectively ameliorated liver damage by restoring serum biochemical markers, improving endogenous antioxidant defense systems, reducing lipid peroxidation, and preserving normal hepatic architecture. Comparative evaluation revealed that the 50% methanolic extract exhibited superior efficacy in reducing serum transaminases (SGPT and SGOT), alkaline phosphatase (ALP), and malondialdehyde (MDA), indicating enhanced protection against hepatocellular damage and oxidative stress. In contrast, the aqueous extract demonstrated greater restoration of total protein, bilirubin homeostasis, catalase (CAT), and reduced glutathione (GSH), suggesting improved recovery of hepatic functional capacity.

The hepatoprotective effects observed are likely attributable to the synergistic antioxidant and cytoprotective activities of phytoconstituents such as flavonoids, phenolic compounds, tannins, glycosides, alkaloids, and saponins present in Curcuma pseudo-montana. These findings provide scientific evidence supporting the traditional medicinal use of this plant for the management of liver disorders. Furthermore, highlighting the therapeutic potential of Curcuma pseudo-montana as a promising natural hepatoprotective agent. Future investigations involving chromatographic characterization of bioactive constituents, molecular mechanism studies, pharmacokinetic evaluation, and long-term toxicity assessment are warranted to facilitate its development as a standardized phytopharmaceutical.

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