



(RESEARCH ARTICLE)



METHANOLIC AND ETHYL ACETATE LEAF FRACTIONS OF ALBIZIA LEBBECK (L.) BENTH. ATTENUATE CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY AND OXIDATIVE STRESS IN RATS

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ABSTRACT

Objective: To evaluate the antioxidant capacity, phytochemical composition, and hepatoprotective efficacy of leaf fractions of *Albizia lebeck* against carbon tetrachloride (CCl₄)-induced hepatic toxicity in Sprague-Dawley rats.

Methods: Dried leaf powder was extracted using methanol and partitioned into pet ether, chloroform, ethyl acetate, n-butanol, and aqueous fractions. Phenolic (TPC) and flavonoid (TFC) contents were quantified. Rats received oral *A. lebeck* leaf extract (ALE: 100,200,400 mg/kg) or Silymarin (50 mg/kg) for 14 days alongside CCl₄ challenge (1 mL/kg i.p. on days 1 and 4). Hepatic enzymes, lipid profiles, oxidative markers (SOD, CAT, GSH, MDA), and liver histology were evaluated.

Results: The ethyl acetate fraction contained the highest total phenolics (822.99 ± 11.06 mg GAE/g) and flavonoids (132.6 ± 11.06 mg Rutin/g). Treatment with ALE (400 mg/kg) significantly ($p < 0.001$) reversed CCl₄-induced elevations in serum ALT, AST, ALP, total bilirubin, total cholesterol, triglycerides, and LDL, while augmenting total protein, HDL, SOD, CAT, and GSH, and suppressing lipid peroxidation (MDA). Histopathological inspection confirmed structural restoration of central veins and hepatocytes.

Conclusion: *Albizia lebeck* leaf fractions display notable hepatoprotective activity by mitigating oxidative stress, preserving membrane integrity, and improving dyslipidemia.

Keywords: Liver disease, Carbon tetrachloride, Silymarin, Anti-oxidant activity, Hepatoprotective

1 INTRODUCTION

Liver diseases remain a leading global cause of mortality. Chemical agents such as carbon tetrachloride (CCl₄) are widely used to induce experimental liver injury. CCl₄ is bioactivated by hepatic CYP2E1 into trichloromethyl radicals (CCl₃•), inducing lipid peroxidation, membrane degradation, cellular necrosis, and antioxidant depletion.

Because conventional treatments often lack targeted efficacy or cause side effects, botanical remedies rich in natural antioxidants have gained interest. *Albizia lebbbeck* (L.) Benth. possesses documented anti-inflammatory, antimicrobial, and radical-scavenging properties. This study evaluates the quantitative phytochemistry, antioxidant profile, and hepatoprotective effects of *A. lebbbeck* leaf fractions against CCl₄-induced liver injury.

2 MATERIALS AND METHODS

2.1 Botanical Material & Extraction

Leaves of *Albizia lebbbeck* were harvested from Dausa, Rajasthan, India, and authenticated. Shade-dried leaf powder (31 g) was subjected to Soxhlet extraction using 95% methanol. The concentrated extract was partitioned using a separatory funnel into petroleum ether, chloroform, ethyl acetate, n-butanol, and aqueous fractions, followed by evaporation to dryness.

2.2 Quantitative Phytochemical Analysis

- **Total Phenolic Content (TPC):** Determined via Folin-Ciocalteu reagent and expressed as mg Gallic Acid Equivalents per gram sample (mg GAE/g).
- **Total Flavonoid Content (TFC):** Determined via aluminum chloride colorimetric assay and expressed as mg Rutin Equivalents per gram sample (mg RT/g).

2.3 Experimental Animals & Study Design

Sprague-Dawley rats (220 ± 20 g) of both sexes were acclimated under controlled conditions (25 ± 2°C, 12-h light/dark cycle) with standard chow and water *ad libitum*. Animals were randomly assigned to 6 groups (n = 6 per group):

- Group I (Normal Control) : Distilled water p.o. daily for 14 days.
- Group II (Disease Control) : CCl₄ (1 mL/kg, 1:1 in olive oil i.p.) on Days 1 & 4 + Distilled water.
- Group III (Standard Control) : CCl₄ (i.p.) + Silymarin (50 mg/kg p.o.) daily for 14 days.
- Group IV (ALE Low Dose) : CCl₄ (i.p.) + ALE extract (100 mg/kg p.o.) daily for 14 days.
- Group V (ALE Medium Dose) : CCl₄ (i.p.) + ALE extract (200 mg/kg p.o.) daily for 14 days.
- Group VI (ALE High Dose) : CCl₄ (i.p.) + ALE extract (400 mg/kg p.o.) daily for 14 days.

2.4 Biochemical & Histopathological Assays

Serum was analyzed for liver injury markers (ALT, AST, ALP, Total Bilirubin, Total Protein) and lipid profile components (HDL, LDL, Triglycerides, Total Cholesterol). Liver tissue homogenate (10% w/v) was prepared to assay Superoxide Dismutase (SOD), Catalase (CAT), Reduced Glutathione (GSH), and Malondialdehyde (MDA) levels. Livers were fixed in 10% formo-saline, embedded in paraffin, sectioned, stained with Hematoxylin & Eosin (H&E), and evaluated light-microscopically.

3 RESULTS

3.1 Biomarkers of Hepatic Injury

CCl₄ intoxication elevated serum ALT, AST, ALP, and total bilirubin, while decreasing total protein levels ($p < 0.001$). Oral administration of ALE (100--400 mg/kg) produced dose-dependent restoration across all parameters ($p < 0.001$), with the 400 mg/kg dose performing comparably to Silymarin.

Table 1: Serum Liver Function Test (LFT) Parameters Across Treatment Groups

Parameter	Normal Control	Disease Control (CCl ₄)	Standard (Silymarin 50 mg/kg)	ALE 100 mg/kg	ALE 200 mg/kg	ALE 400 mg/kg
ALT (U/L)	63.15 ± 4.23	160.32 ± 4.56 ^{###}	85.65 ± 3.65 ^{***}	130.20 ± 2.36 ^{***}	120.30 ± 3.16 ^{***}	87.45 ± 3.35 ^{***}
AST (U/L)	90.36 ± 2.65	185.32 ± 7.56 ^{###}	119.00 ± 3.56 ^{***}	167.00 ± 3.632 ^{***}	150.36 ± 4.23 ^{***}	124.00 ± 1.435 ^{***}

ALP (U/L)	120.00 ± 2.67	290.00 ± 3.521 ^{###}	185.00 ± 4.32 ^{***}	260.00 ± 3.56 ^{***}	225.00 ± 8.21 ^{***}	200.00 ± 4.22 ^{***}
Total Bilirubin (mg/dL)	0.210 ± 0.035	0.856 ± 0.0361 ^{###}	0.412 ± 0.050 ^{***}	0.651 ± 0.035 ^{***}	0.512 ± 0.046 ^{***}	0.445 ± 0.050 ^{***}
Total Protein (g/dL)	5.231 ± 0.215	2.321 ± 0.350 ^{###}	4.510 ± 0.185 ^{***}	3.320 ± 0.270 ^{***}	4.000 ± 0.360 ^{***}	4.510 ± 0.185 ^{***}

^{###}*p* < 0.001 vs. Normal Control; ^{***}*p* < 0.001 vs. Disease Control.

3.2 Serum Lipid Profile

CCl₄ intoxication disrupted lipid homeostasis, causing marked elevations in total cholesterol, triglycerides, and LDL, along with reduction in HDL. Treatment with ALE normalized lipid distributions in a dose-dependent manner.

Table 2: Serum Lipid Profiles Across Experimental Groups

Parameter (mg/dL)	Normal Control	Disease Control (CCl ₄)	Standard (Silymarin)	ALE 100 mg/kg	ALE 200 mg/kg	ALE 400 mg/kg
HDL	40.00 ± 1.32	15.00 ± 1.65 ^{###}	38.00 ± 2.56 ^{***}	25.00 ± 2.045 ^{***}	32.00 ± 1.45 ^{***}	35.00 ± 2.23 ^{***}
LDL	15.45 ± 2.45	72.00 ± 4.21 ^{###}	30.00 ± 1.54 ^{***}	42.00 ± 1.32 ^{***}	37.00 ± 1.65 ^{***}	33.00 ± 1.64 ^{***}
Triglycerides	52.00 ± 2.35	140.00 ± 6.844 ^{###}	80.00 ± 2.65 ^{***}	110.00 ± 3.65 ^{***}	92.32 ± 3.65 ^{***}	83.00 ± 1.88 ^{***}
Total Cholesterol	80.32 ± 2.15	200.00 ± 10.23 ^{###}	130.00 ± 2.00 ^{***}	170.00 ± 2.32 ^{***}	150.00 ± 3.62 ^{***}	138.00 ± 2.50 ^{***}

^{###}*p* < 0.001 vs. Normal Control; ^{***}*p* < 0.001 vs. Disease Control.

3.3 Hepatic Antioxidant Status and Lipid Peroxidation

In hepatic homogenates, CCl₄ reduced SOD, CAT, and GSH activities while increasing lipid peroxidation (MDA) levels (*p* < 0.01). Co-administration of the ethyl acetate fraction of ALE (100–400 mg/kg) restored endogenous antioxidant enzymes and suppressed MDA accumulation in a dose-dependent manner.

Table 3: In Vivo Hepatic Antioxidant Status and MDA Level

Group Treatment /	SOD (U/mg protein)	CAT (U/mg protein)	GSH (mg/g protein)	MDA (nmol/mg protein)
Control	329.68 ± 20.43	54.08 ± 13.32	5.43 ± 1.44	2.26 ± 0.56
CCl ₄ Control	158.76 ± 37.65	38.26 ± 7.19	2.33 ± 1.04	5.13 ± 0.33
Silymarin (50 mg/kg)	262.40 ± 41.34 ^{**}	53.18 ± 11.33 [*]	5.29 ± 1.34 [*]	4.13 ± 1.22 [*]
ALE (100 mg/kg)	183.65 ± 32.45	39.59 ± 14.17	4.78 ± 1.09 [*]	4.55 ± 0.76 [*]
ALE (200 mg/kg)	206.49 ± 36.54	43.34 ± 8.97 ^{**}	5.02 ± 1.82 ^{**}	3.89 ± 0.85 ^{**}
ALE (400 mg/kg)	248.04 ± 34.76 ^{**}	57.87 ± 7.66 [*]	5.10 ± 0.44 ^{**}	4.02 ± 0.43 ^{**}

3.4 Histopathological Findings

Histological analysis provided direct structural evidence supporting the biochemical parameters:

- **Normal Control:** Intact hepatic lobular architecture, radiating cords of hepatocytes, distinct central vein, and normal sinusoidal spaces.
- **CCl₄ Disease Control:** Extensive centrilobular necrosis, ballooning degeneration, severe fatty changes, inflammatory lymphocytic infiltration, and loss of cellular boundaries.
- **Silymarin (50 mg/kg):** Reverted hepatic architecture close to normal, with well-preserved hepatocytes and clear central veins.

- **ALE (100 mg/kg):** Moderate reduction in tissue damage, with persisting localized fatty changes and minor cellular infiltration.
- **ALE (200 mg/kg):** Substantial reduction in necrosis with only minor inflammatory infiltration.
- **ALE (400 mg/kg):** Near-complete restoration of normal liver microarchitecture without apparent necrosis, comparable to Silymarin.

4 DISCUSSION

The hepatoprotective capacity of *Albizia lebbbeck* leaf extract against CCl₄-induced damage operates primarily through free-radical scavenging and lipid peroxidation inhibition. Biotransformation of CCl₄ via cytochrome P450 enzymes yields trichloromethyl and peroxy free radicals, which initiate lipid peroxidation in cell membranes and trigger hepatocyte lysis. Elevation of serum transaminases (ALT, AST) and ALP reflects membrane permeabilization, while hyperbilirubinemia and hypoproteinemia reflect functional impairment of hepatic conjugation and synthetic capabilities.

Dose-dependent treatment with *A. lebbbeck* leaf extract restored these serum enzyme levels toward baseline. High phenolic (822.99 mg GAE/g) and flavonoid (132.60 mg Rutin/g) concentrations in the active ethyl acetate fraction likely drive these hepatoprotective effects by donating hydrogen atoms or electrons to neutralize ROS. Consequently, endogenous antioxidant enzymes (SOD, CAT, GSH) were preserved, and membrane degradation was halted, as evidenced by reduced MDA concentrations. The attenuation of liver toxicity was further reflected in the normalization of lipid profiles and histological preservation of hepatic cell architecture.

5 CONCLUSION

This study demonstrates that the ethyl acetate fraction of *Albizia lebbbeck* leaf extract possesses marked hepatoprotective and antioxidant properties against CCl₄-induced liver toxicity in rats. The mechanisms of protection involve free radical neutralization, restoration of internal antioxidant defenses (SOD, CAT, GSH), inhibition of lipid peroxidation, and preservation of hepatocyte membrane integrity. These findings support the traditional use of *A. lebbbeck* for hepatic health and warrant further isolation and mechanistic analysis of its active phytoconstituents.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this article.

Statement of ethical approval

All experimental procedures involving animals were conducted in accordance with institutional guidelines and were approved by the appropriate Animal Research Ethics Committee. Ethical approval number BMRL/IAECI2026/07-18

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