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Original Article

Hepatoprotective Effect of an Herbal Compound in Carbon Tetrachloride–Induced Liver Injury in Wistar Rats: An Experimental Study

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ABSTRACT

Background: Liver disease is a major and rising contributor to global morbidity and mortality, and the therapeutic options for many forms of hepatocellular injury remain limited. Plant-derived compounds have long been used in traditional systems of medicine for liver disorders, and several have shown promise as hepatoprotective agents in experimental models.

Objective: To evaluate the hepatoprotective activity of [HERBAL COMPOUND — insert botanical name] against carbon tetrachloride (CCl_4)–induced liver injury in Wistar rats, using silymarin as the reference standard.

Methods: Thirty adult Wistar rats were randomly allocated to five groups (n = 6): normal control, toxicant control (CCl_4), standard (silymarin 100 mg/kg), and two test groups receiving the herbal extract at 200 and 400 mg/kg orally for 14 days. Hepatic injury was induced with CCl_4 (1 mL/kg i.p.). Serum AST, ALT, ALP, total bilirubin, total protein and albumin were estimated, together with hepatic antioxidant markers (SOD, catalase, reduced glutathione, malondialdehyde) and histopathology.

Results: CCl_4 produced a marked rise in serum transaminases, ALP and bilirubin, depletion of hepatic antioxidants, elevated lipid peroxidation, and severe centrizonal necrosis. Pre/co-treatment with the herbal extract restored these parameters towards normal in a dose-dependent manner, with the 400 mg/kg dose approaching the protection afforded by silymarin (illustrative data — replace with actual values).

Conclusion: The herbal compound exhibited significant, dose-dependent hepatoprotective activity against CCl_4-induced liver injury, plausibly mediated through antioxidant and membrane-stabilising mechanisms. These findings support further phytochemical and clinical investigation.

Keywords: Hepatoprotection; herbal medicine; carbon tetrachloride; liver injury; antioxidant; silymarin; Wistar rats.

INTRODUCTION

The liver is the principal organ of metabolism, detoxification and biotransformation, and is therefore continuously exposed to xenobiotics, drugs, environmental toxins and oxidative insult. Because of this central role, it is also highly vulnerable to injury, and liver disease has emerged as one of the leading causes of morbidity and mortality worldwide.[11] Hepatocellular damage from drugs, alcohol, viral infection and metabolic dysfunction frequently progresses through stages of inflammation, fibrosis and cirrhosis when left unchecked.

Despite the magnitude of this burden, the pharmacological armamentarium for hepatoprotection remains limited. Many conventional agents offer only symptomatic benefit, may carry their own hepatotoxic potential, and are often expensive or inaccessible in resource-constrained settings. This therapeutic gap has sustained long-standing interest in plant-derived remedies, several of which have been used empirically for centuries in Ayurveda and other traditional systems for the management of jaundice and liver disorders.

Carbon tetrachloride (CCl_4) is among the most widely used and best-characterised hepatotoxicants in experimental hepatology. It is metabolised by hepatic cytochrome P450 (chiefly CYP2E1) to the highly reactive trichloromethyl and trichloromethyl-peroxyl radicals, which initiate lipid peroxidation of membrane phospholipids, disrupt calcium homeostasis, and ultimately cause centrizonal necrosis.[1,8] The resulting biochemical and histological changes closely mimic human toxic and oxidative liver injury, making CCl_4 an established model for screening candidate hepatoprotective agents.[8] Many medicinal plants exert hepatoprotective effects largely through their antioxidant constituents — flavonoids, polyphenols, triterpenoids and related phytochemicals — which scavenge free radicals, augment endogenous antioxidant defences and stabilise hepatocyte membranes. Silymarin, a flavonolignan complex from Silybum marianum, is the most extensively validated example and is routinely used as the positive control in such studies.[7]

Against this background, the present study was undertaken to evaluate the hepatoprotective potential of [HERBAL COMPOUND — insert botanical name and part used] against CCl_4-induced liver injury in Wistar rats, by assessing serum biochemical markers of hepatocellular integrity, hepatic antioxidant status and histopathological changes, with silymarin as the reference standard.

Aim and objectives

Aim: To investigate the hepatoprotective effect of the test herbal compound in an experimental model of induced liver injury.

- To establish CCl_4-induced hepatotoxicity in Wistar rats as the injury model.
- To assess the effect of two graded doses of the herbal extract on serum markers of liver function (AST, ALT, ALP, bilirubin, total protein, albumin).
- To evaluate hepatic oxidative-stress and antioxidant parameters (SOD, catalase, reduced glutathione, malondialdehyde).
- To compare the histopathological protection conferred by the extract with that of standard silymarin.

MATERIALS AND METHODS

Study design and setting

This was an experimental, laboratory-based animal study conducted in the Department of Pharmacology, Narayan Medical College and Hospital, Jamuhar, Sasaram, Bihar, in collaboration with the Department of General Medicine, over a one-year period from April 2025 to January 2026.

Plant material and preparation of the extract

[Describe the plant: botanical name, family, authentication by a qualified botanist with herbarium/voucher specimen number, the part used (leaf/root/whole plant), collection site and month.] The plant material was shade-dried, coarsely powdered and subjected to extraction (e.g., cold maceration / Soxhlet extraction) using [solvent — e.g., 70% hydroalcoholic solvent]. The extract was concentrated under reduced pressure, the percentage yield recorded, and the dried extract stored at 4 °C until use. Working suspensions were freshly prepared in [vehicle — e.g., 0.5% carboxymethyl cellulose / normal saline] before each administration.

Preliminary phytochemical screening

The extract was subjected to standard qualitative phytochemical tests for the presence of alkaloids, flavonoids, tannins, saponins, glycosides, steroids, triterpenoids and phenolic compounds. [Report findings.]

Drugs and chemicals

Carbon tetrachloride and silymarin were procured from [supplier]. All other reagents and chemicals used were of analytical grade. Standard diagnostic kits were used for serum biochemical estimations [supplier/kit details].

Experimental animals

Healthy adult Wistar albino rats of either sex, weighing 150–200 g, were obtained from [registered animal supplier]. Animals were housed in polypropylene cages under standard laboratory conditions (temperature 22 ± 2 °C, relative humidity 50–60%, 12-h light/dark cycle) with free access to standard pellet diet and water ad libitum. They were acclimatised for seven days before the experiment.

Acute oral toxicity study
Acute oral toxicity was evaluated as per OECD Guideline 423.[9] The extract was found to be safe up to [dose] mg/kg with no mortality or signs of toxicity; one-tenth and one-fifth of this dose (or the doses selected from the literature) were chosen as the test doses (200 and 400 mg/kg).

Induction of hepatotoxicity
Hepatic injury was induced by intraperitoneal administration of carbon tetrachloride (1 mL/kg, prepared as a 1:1 v/v mixture in olive oil) on days 7 and 14 of the study to all groups except the normal control.

Experimental design and grouping
Thirty rats were randomly allocated into five groups of six animals each. Treatments were administered orally once daily for 14 consecutive days as shown in Table 1.

Table 1. Experimental groups and treatment schedule

Group	Treatment	Dose / Schedule
I (Normal control)	Vehicle only	Olive oil / normal saline p.o. daily for 14 days
II (Toxicant control)	CCl4 only	CCl4 1 mL/kg (1:1 in olive oil) i.p. on days 7 and 14
III (Standard)	Silymarin + CCl4	Silymarin 100 mg/kg p.o. daily + CCl4 as above
IV (Test low dose)	Extract + CCl4	Extract 200 mg/kg p.o. daily + CCl4 as above
V (Test high dose)	Extract + CCl4	Extract 400 mg/kg p.o. daily + CCl4 as above

CCl_4, carbon tetrachloride; i.p., intraperitoneal; p.o., per oral. Doses are indicative and should match the study protocol.

Collection of samples
Twenty-four hours after the last dose, animals were anaesthetised and blood was collected by retro-orbital puncture/cardiac puncture. Serum was separated by centrifugation for biochemical analysis. Animals were then humanely euthanised; the liver was excised, weighed, and portions were homogenised for antioxidant assays while representative sections were fixed in 10% neutral buffered formalin for histopathology.

Biochemical estimations
Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, total protein and albumin were estimated using standard kit-based colorimetric/enzymatic methods on an autoanalyser.[2]

Antioxidant and oxidative-stress markers
Liver homogenates were assayed for superoxide dismutase (SOD)[3], catalase (CAT)[4], reduced glutathione (GSH)[5] and the lipid-peroxidation product malondialdehyde (MDA)[6] using established spectrophotometric methods.

Histopathological examination
Formalin-fixed liver tissues were processed, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin. Sections were examined under a light microscope by a pathologist blinded to group allocation and graded for necrosis, fatty change, inflammatory infiltration and architectural distortion.

Statistical analysis
Data are expressed as mean ± standard error of the mean (SEM). Differences between groups were analysed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test using [SPSS / GraphPad Prism version __]. A p-value < 0.05 was considered statistically significant.

RESULTS
Effect on serum biochemical markers
Administration of CCl_4 (Group II) produced a marked elevation of serum AST, ALT, ALP and total bilirubin and a fall in total protein and albumin compared with the normal control (Group I), confirming hepatocellular injury. Treatment with the herbal extract reduced these enzyme levels in a dose-dependent manner; the higher dose (Group V) produced protection comparable to standard silymarin (Group III) (Table 2).

Table 2. Effect of the herbal compound on serum liver-function markers (illustrative)

Parameter	Gr. I	Gr. II	Gr. III	Gr. IV	Gr. V
AST (IU/L)	82.4 ± 5.1	248.6 ± 12.3	108.5 ± 7.2	176.3 ± 9.8	121.7 ± 8.0
ALT (IU/L)	44.1 ± 3.6	212.9 ± 10.7	71.4 ± 5.5	148.2 ± 8.9	86.3 ± 6.1
ALP (IU/L)	128.5 ± 6.9	356.7 ± 15.4	162.3 ± 9.1	268.4 ± 12.0	184.6 ± 10.2
Total bilirubin (mg/dL)	0.62 ± 0.05	2.18 ± 0.16	0.94 ± 0.08	1.56 ± 0.12	1.05 ± 0.09
Total protein (g/dL)	7.4 ± 0.3	4.6 ± 0.3	6.8 ± 0.3	5.5 ± 0.3	6.5 ± 0.3
Albumin (g/dL)	4.1 ± 0.2	2.5 ± 0.2	3.7 ± 0.2	3.0 ± 0.2	3.6 ± 0.2

*Values are mean ± SEM (n = 6). Add significance symbols once analysed, e.g., *p < 0.05 vs Group I; †p < 0.05 vs Group II. Gr., Group.*

Effect on hepatic antioxidant status

CCl_4 intoxication significantly depleted hepatic SOD, catalase and reduced glutathione and increased MDA, indicating enhanced oxidative stress and lipid peroxidation. The extract restored antioxidant enzyme activity and lowered MDA dose-dependently, supporting an antioxidant mechanism of protection (Table 3).

Table 3. Effect of the herbal compound on hepatic antioxidant and lipid-peroxidation markers (illustrative)

Parameter (hepatic tissue)	Gr. I	Gr. II	Gr. III	Gr. IV	Gr. V
SOD (U/mg protein)	12.8 ± 0.9	5.1 ± 0.5	10.6 ± 0.8	7.4 ± 0.6	9.8 ± 0.7
Catalase (U/mg protein)	48.6 ± 3.1	19.4 ± 2.0	40.2 ± 2.8	27.9 ± 2.3	37.1 ± 2.6
Reduced GSH (nmol/mg)	32.4 ± 2.2	13.1 ± 1.4	27.8 ± 1.9	18.6 ± 1.6	25.3 ± 1.8
MDA (nmol/mg protein)	1.42 ± 0.11	4.86 ± 0.32	2.05 ± 0.17	3.41 ± 0.25	2.38 ± 0.19

Values are mean ± SEM (n = 6). SOD, superoxide dismutase; GSH, reduced glutathione; MDA, malondialdehyde.

Histopathological findings

Liver sections from the normal control showed intact lobular architecture. The toxicant control showed severe centrizonal necrosis, ballooning degeneration, fatty change and dense inflammatory infiltration. Sections from silymarin- and extract-treated groups showed graded preservation of architecture, with the high-dose extract approaching the standard (Table 4). [Insert representative photomicrographs as Figure 1 (a–e) with magnification and scale bars.]

Table 4. Histopathological grading of liver sections (illustrative)

Group	Histological observation	Injury grade
I	Normal lobular architecture, intact central vein, well-preserved hepatocytes with distinct nuclei.	0 (Nil)
II	Extensive centrizonal necrosis, ballooning degeneration, fatty change, marked inflammatory infiltration.	3+ (Severe)
III	Near-normal architecture, minimal focal necrosis and inflammation.	1+ (Mild)
IV	Moderate reduction in necrosis and fatty change, scattered inflammatory foci.	2+ (Moderate)
V	Largely preserved architecture, occasional degenerated hepatocytes, minimal infiltration.	1+ (Mild)

Injury graded 0 (nil) to 3+ (severe) based on necrosis, fatty change, inflammation and architectural distortion.

DISCUSSION

The present study evaluated the hepatoprotective activity of the test herbal compound against CCl_4-induced liver injury, a model that reproduces the oxidative and necro-inflammatory features of human toxic hepatitis. The marked rise in serum transaminases, ALP and bilirubin in the toxicant group reflects loss of hepatocyte membrane integrity and leakage of

cytosolic enzymes, while the fall in total protein and albumin indicates impaired hepatic synthetic function — a constellation consistent with established descriptions of CCl_4 toxicity.[1,8]

Co-administration of the herbal extract attenuated these changes in a dose-dependent fashion, with the higher dose producing protection approaching that of silymarin. Restoration of serum enzyme levels towards normal indicates stabilisation of hepatocyte membranes and preservation of functional hepatic mass.

The biochemical pattern of injury in this model is driven largely by free-radical generation and lipid peroxidation. The observed depletion of SOD, catalase and reduced glutathione, alongside elevated MDA, confirms a state of oxidative stress in the toxicant group. The capacity of the extract to replenish these antioxidant defences and to lower lipid peroxidation strongly suggests that its hepatoprotection is mediated, at least in part, through antioxidant and free-radical-scavenging activity — an effect commonly attributed to flavonoid, polyphenolic and triterpenoid constituents of medicinal plants.[7]
The histopathological findings corroborate the biochemical results: the graded reduction in necrosis, fatty change and inflammatory infiltration with increasing dose mirrors the dose-dependent biochemical recovery and reinforces the conclusion that the extract limits structural hepatocellular damage.

Limitations

- This is a preclinical study in a single rodent model; extrapolation to human disease requires caution.
- A single hepatotoxicant (CCl_4) was used; confirmation in other models (e.g., paracetamol, alcohol) would strengthen the findings.
- Bioactivity-guided isolation of the responsible phytoconstituent(s) and mechanistic/molecular studies were beyond the present scope.

CONCLUSION

Within the limits of this experimental study, the test herbal compound demonstrated significant, dose-dependent hepatoprotective activity against carbon tetrachloride–induced liver injury in Wistar rats, as evidenced by improvement in serum liver-function markers, restoration of hepatic antioxidant status and preservation of hepatic architecture. The protection at the higher dose approached that of standard silymarin, supporting an antioxidant and membrane-stabilising mechanism of action. These findings justify further phytochemical characterisation, mechanistic evaluation and, ultimately, clinical investigation of the compound as a potential hepatoprotective agent.

Declarations

Conflict of interest: The authors declare no conflict of interest.
Author contributions: SK conceived and designed the study, conducted the experiments and drafted the manuscript; AK contributed to study design, data interpretation and critical revision. Both authors read and approved the final manuscript.
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