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EVALUATION OF THE HEPATOPROTECTIVE ACTIVITY OF ANDROGRAPHIS PANICULATA AGAINST CARBON TETRACHLORIDE-INDUCED LIVER TOXICITY IN ALBINO RATS

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Article History	Abstract
Received: 11-06-2026 Revised: 03-07-2026 Accepted: 05-08-2026	Liver diseases represent a significant global health challenge and are among the leading causes of morbidity and mortality. Herbal medicines have attracted considerable attention as potential hepatoprotective agents because of their therapeutic efficacy and relatively low incidence of adverse effects. The present study was undertaken to investigate the hepatoprotective activity of the ethanolic leaf extract of <i>Andrographis paniculata</i> against carbon tetrachloride (CCl ₄)-induced hepatotoxicity in albino rats. Albino rats were orally administered the ethanolic leaf extract of <i>Andrographis paniculata</i> at a dose of 500 mg/kg body weight for five consecutive days. Hepatic injury was induced by subcutaneous administration of carbon tetrachloride (CCl ₄) on the second and third days of the experimental period. The hepatoprotective effect of the extract was compared with that of silymarin, a standard hepatoprotective drug. Liver function was evaluated by estimating serum biochemical markers, including serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), acid phosphatase (ACP), total bilirubin, and direct bilirubin, using standard biochemical kits and a semi-automatic biochemical analyzer. Treatment with the ethanolic leaf extract of <i>Andrographis paniculata</i> significantly attenuated CCl ₄ -induced hepatic damage. The extract markedly ($p < 0.01$) reduced the elevated levels of SGPT, SGOT, ALP, ACP, total bilirubin, and direct bilirubin when compared with the CCl ₄ -treated group. Histopathological examination of liver tissues further confirmed the hepatoprotective effect by demonstrating restoration of normal hepatic architecture and reduced cellular degeneration. The results of the present study indicate that the ethanolic leaf extract of <i>Andrographis paniculata</i> possesses significant hepatoprotective activity against CCl ₄ -induced liver injury in albino rats and may serve as a promising natural therapeutic agent for the management of liver disorders.
Keywords: <i>Andrographis paniculata</i> , hepatoprotective activity, ethanolic leaf extract, carbon tetrachloride (CCl ₄), hepatotoxicity, albino rats.	
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INTRODUCTION

The liver is the largest internal organ and a vital metabolic center responsible for regulating carbohydrate, protein, and lipid metabolism, detoxifying harmful substances, synthesizing essential biomolecules, and storing nutrients [1]. Anatomically, it is located mainly in the right upper abdomen and consists of distinct lobes, ligaments, and microscopic hepatic lobules supplied by a dual blood circulation. The liver also plays a crucial role in bile production, immune defense, drug metabolism, and antioxidant protection through enzymes and molecules that safeguard hepatocytes from oxidative stress and cellular damage [2]. The liver is highly susceptible to damage from drugs, xenobiotics, infections, alcohol, metabolic disorders, and autoimmune diseases because it is the primary site of detoxification and drug metabolism. Oxidative stress and reactive oxygen species play central roles in the development of liver injury, leading to fibrosis, cirrhosis, and hepatocellular carcinoma [3-4]. Although conventional therapies are available, their effectiveness is often limited by adverse effects. Natural hepatoprotective agents such as silymarin and Liv-52 have shown promising antioxidant and liver-protective properties,

while prompt withdrawal of the offending drug remains the cornerstone of managing drug-induced hepatotoxicity. Drug-induced hepatotoxicity is a significant cause of liver injury and may occur as predictable (intrinsic) or unpredictable (idiosyncratic) reactions. Hepatic damage results from mechanisms including oxidative stress, mitochondrial dysfunction, immune-mediated responses, and lipid peroxidation, leading to hepatocellular, cholestatic, or mixed liver injury. Risk factors include age, female sex, alcohol use, genetic predisposition, pre-existing liver disease, and concurrent medications. Clinical manifestations range from mild enzyme elevations to acute hepatitis, cholestasis, steatosis, cirrhosis, and fulminant liver failure, with antibiotics, NSAIDs, anticonvulsants, and antitubercular drugs among the most common hepatotoxic agents [5-9]. Drug-induced hepatotoxicity remains a major clinical challenge, with numerous medications, particularly NSAIDs, antibiotics, antitubercular drugs, and anticancer agents, capable of causing liver injury ranging from mild enzyme elevation to acute liver

failure. Early diagnosis using causality assessment tools such as RUCAM and prompt discontinuation of the offending drug are essential for effective management. Owing to the limitations and adverse effects of conventional drugs, medicinal plants and herbal medicines have gained significant attention as safer alternatives. Standardization, quality control, and toxicological evaluation are critical to ensure the safety, efficacy, and therapeutic reliability of herbal formulations for liver disorders [10-14].

METHODOLOGY

Methodology

Plant Material Collection and Authentication

Fresh leaves of *Andrographis paniculata* were collected from the surrounding gardens of Hyderabad and authenticated by a senior botanist. The collected leaves were shade-dried, powdered using a mechanical grinder, and stored in airtight containers until extraction.

Preparation of Plant Extract

The powdered leaves were first defatted with petroleum ether using a Soxhlet apparatus. The defatted material was then extracted with 60% ethanol (hydroalcoholic extract). The extract was concentrated under reduced pressure and stored in airtight containers until further use [15].

Phytochemical Screening

The ethanolic extract was subjected to preliminary phytochemical screening using standard qualitative tests to identify the presence of major secondary metabolites:

- **Alkaloids:** Mayer's, Dragendorff's, and Wagner's tests
- **Flavonoids:** Shinoda test and alkaline reagent test
- **Cardiac glycosides:** Keller–Killiani test
- **Phlobatannins:** Hydrochloric acid test
- **Saponins:** Frothing test
- **Steroids:** Liebermann–Burchard test
- **Tannins:** Ferric chloride test
- **Triterpenoids:** Sulfuric acid test

Experimental Animals

Healthy adult Wistar albino rats of either sex weighing **150–200 g** were obtained from Synzyn Laboratories. The animals were acclimatized for one week under standard laboratory conditions (temperature **27 ± 2°C**, **12 h light/12 h dark cycle**) with free access to standard pellet diet and water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA guidelines.

Acute Toxicity Study

The acute oral toxicity of the ethanolic extract of *A. paniculata* was evaluated according to standard guidelines. The extract was found to be safe up to **5000 mg/kg body weight**. Therefore, **1/10th of the maximum safe dose (500 mg/kg, p.o.)** was selected for the hepatoprotective study.

Carbon Tetrachloride (CCl₄)-Induced Hepatotoxicity Model

Rats were randomly divided into five groups (n = 6 per group):

Table 01: Experimental grouping and treatment regimen for CCl₄-induced hepatotoxicity

Group	Treatment
Group I (Normal Control)	Vehicle only (oral) for 5 days + liquid paraffin (1 mL/kg, s.c.) on Days 2 and 3
Group II (Hepatotoxic Control)	Vehicle (oral) + CCl ₄ :liquid paraffin (1:1, 2 mL/kg, s.c.) on Days 2 and 3
Group III (Test Group)	<i>A. paniculata</i> extract (500 mg/kg, p.o.) for 5 days + CCl ₄ on Days 2 and 3
Group IV (Standard Group)	Silymarin (25 mg/kg, p.o.) for 5 days + CCl ₄ on Days 2 and 3
Group V	(If included in the original protocol, specify the treatment; otherwise omit or clarify, as the provided text contains inconsistencies.)

The extract or silymarin was administered orally once daily for five consecutive days. Carbon tetrachloride (CCl₄) was administered subcutaneously 30 minutes after the respective treatments on Days 2 and 3 to induce liver injury.

Blood Collection and Biochemical Analysis

At the end of the treatment period, animals were sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture and allowed to clot for 45 minutes at room temperature. Serum was separated by centrifugation at **3000 rpm for 15 minutes** and stored at **4°C** until analysis.

Serum biochemical parameters measured included:

- Serum glutamate oxaloacetate transaminase (SGOT/AST)
- Serum glutamate pyruvate transaminase (SGPT/ALT)
- Alkaline phosphatase (ALP)
- Acid phosphatase (ACP)
- Total bilirubin
- Direct bilirubin

Commercial diagnostic kits (Giri Diagnostic Kit Pvt. Ltd.) were used according to the manufacturer's instructions.

Histopathological Examination

Liver tissues were excised immediately after sacrifice and fixed in **10% neutral buffered formalin**. The tissues were processed, embedded in paraffin wax, sectioned at **5–6 µm**, and stained with hematoxylin and eosin (H&E). The stained sections were examined microscopically to evaluate hepatic architecture, necrosis, fatty degeneration, inflammation, and other histopathological changes.

Statistical Analysis

All experimental data were expressed as **mean ± standard error of the mean (SEM)**. Statistical analysis was performed using **one-way analysis of variance (ANOVA)** followed by **Dunnett's multiple comparison test**. Differences were considered statistically significant at **P < 0.05**, while **P < 0.01** indicated a highly significant difference.

RESULTS AND DISCUSSION

Preliminary Phytochemical Analysis

Preliminary qualitative phytochemical screening of the ethanolic leaf extract of *Andrographis paniculata* revealed the presence of various bioactive phytoconstituents. The extract showed positive reactions for alkaloids, flavonoids, cardiac glycosides, saponins, steroids, tannins, and triterpenes, while phlobatannins and Mayer's test for alkaloids showed negative results. The presence of these phytochemicals suggests that the extract may possess antioxidant and hepatoprotective properties. The results of the phytochemical screening are presented in Table 02.

Table 02: Preliminary qualitative phytochemical analysis of ethanolic extract of *Andrographis paniculata*

Phytochemical constituents	Test	Ethanolic extract
Alkaloids	Dragendorff's test	+
	Mayer's test	-
	Wagner's test	+
Flavonoids	Shinoda test	+
	Alkaline reagent test	+
Cardiac glycosides	Keller–Killiani test	+
Phlobatannins	HCl test	-
Saponins	Frothing test	+
Steroids	Liebermann–Burchard test	+
Tannins	Ferric chloride (FeCl ₃) test	+
Triterpenes	H ₂ SO ₄ test	+

Note: (+) Presence; (-) Absence.

Effect of *Andrographis paniculata* Extract on CCl₄-Induced Hepatotoxicity

Carbon tetrachloride administration significantly ($P < 0.01$) elevated serum hepatic marker enzymes and bilirubin levels compared with the normal control group, confirming the induction of hepatotoxicity. Treatment with the ethanolic leaf extract of *A. paniculata* (500 mg/kg, p.o.) significantly reduced SGPT ($P < 0.01$), SGOT ($P < 0.05$), and direct bilirubin ($P < 0.05$) levels, indicating protection against CCl₄-induced hepatic damage. The extract also produced reductions in ALP, ACP, and total bilirubin levels, although these changes were statistically non-significant. The standard hepatoprotective drug silymarin (25 mg/kg, p.o.) showed significant improvement in all biochemical parameters compared with the CCl₄ control group. The effect of *A. paniculata* extract on serum biochemical markers is summarized in Table 03.

Table 03: Effect of ethanolic leaf extract of *Andrographis paniculata* on CCl₄-induced hepatotoxicity in rats

Groups	Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	ACP (IU/L)	Direct bilirubin (mg/dL)	Total bilirubin (mg/dL)
I	Vehicle control (1 mL/kg, s.c.)	42.10 ± 1.20**	65.26 ± 1.50**	150.12 ± 3.24**	12.63 ± 0.214**	0.28 ± 0.09**	0.50 ± 0.01*
II	CCl ₄ + liquid paraffin (2 mL/kg, s.c.)	175.02 ± 1.03**	275.3 ± 10.1**	240.12 ± 1.50**	40.01 ± 1.20**	1.50 ± 0.02**	2.12 ± 0.05**
III	Ethanolic extract of <i>A. paniculata</i> (500 mg/kg, p.o.)	121.39 ± 1.50**	165.12 ± 2.20*	212.2 ± 1.15 ns	30.9 ± 1.10 ns	0.85 ± 0.06*	2.01 ± 0.05 ns
IV	Silymarin (25 mg/kg, p.o.)	102.02 ± 2.66**	105 ± 1.60**	182.25 ± 1.22**	20.09 ± 3.11*	0.70 ± 0.01**	1.20 ± 0.01**

Values are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test.

* $P < 0.05$, ** $P < 0.01$, ns = non-significant compared with CCl₄ control group.

Histopathological Evaluation

Histopathological examination further confirmed the hepatoprotective activity of the ethanolic extract of *A. paniculata* (Figure 01). The liver sections of the normal control group showed normal hepatic architecture with intact hepatocytes (Figure 01A). In contrast, the CCl₄-treated hepatotoxic group exhibited severe hepatocellular damage, including degeneration, necrosis, and distortion of hepatic architecture (Figure 01B). Treatment with *A. paniculata* extract (500 mg/kg) showed marked improvement, with reduced necrotic changes and regeneration of hepatocytes (Figure 01C). The silymarin-treated group showed restoration of normal hepatic structure with minimal pathological alterations (Figure 01D).

Overall, the biochemical and histopathological findings demonstrate that the ethanolic leaf extract of *Andrographis paniculata* exhibits significant hepatoprotective activity against CCl₄-induced liver injury, which may be attributed to the presence of flavonoids, alkaloids, tannins, saponins, and triterpenoids identified in the phytochemical analysis (Table 02).

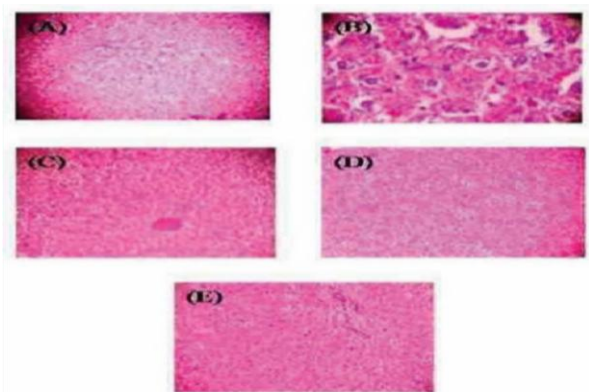


Figure 01: Histopathological evaluation of rat liver sections showing normal hepatocyte architecture in control group (A), severe hepatocellular damage in CCl₄-induced hepatotoxic group (B), reduced necrotic changes with hepatocyte regeneration in extract-treated groups (C and D), and restoration of normal hepatic architecture in the silymarin-treated group (E). Sections were stained with hematoxylin and eosin (H&E) and examined at 10× magnification.

Carbon tetrachloride (CCl₄) is a well-established experimental hepatotoxic agent that induces acute liver injury through oxidative stress, resulting in hepatocellular necrosis, apoptosis, and impaired liver function. The metabolism of CCl₄ by hepatic cytochrome P450 enzymes produces reactive metabolites, particularly trichloromethyl radicals (CCl₃•) [15], which initiate lipid peroxidation, disrupt cellular membranes, increase intracellular calcium accumulation, and activate inflammatory pathways, ultimately leading to hepatocyte damage [16-17].

In the present study, CCl₄ administration significantly increased serum liver marker enzymes (SGPT, SGOT, ALP, and bilirubin), indicating hepatic injury [18]. Treatment with the ethanolic extract of *Andrographis paniculata* significantly reduced these elevated biochemical parameters, demonstrating its protective effect against CCl₄-induced hepatotoxicity. The hepatoprotective activity may be attributed to the presence of bioactive phytoconstituents such as flavonoids, alkaloids, tannins, saponins, and triterpenoids, which are known for their antioxidant and free radical scavenging properties [19-20].

Histopathological findings further supported the biochemical results, showing reduced hepatocellular degeneration and restoration of normal hepatic architecture in extract-treated animals. The protective effect of *A. paniculata* may involve inhibition of lipid peroxidation, enhancement of antioxidant defense mechanisms, and stabilization of hepatocyte membranes [20-25].

Overall, the findings demonstrate that the ethanolic leaf extract of *Andrographis paniculata* exhibits significant hepatoprotective activity against CCl₄-induced liver damage by improving biochemical parameters and preserving hepatic structure. These results suggest that *A. paniculata* could be a promising natural source for the management of oxidative stress-mediated liver disorders.

CONCLUSION

The present study demonstrated the hepatoprotective potential of the ethanolic leaf extract of *Andrographis paniculata* against CCl₄-induced hepatic injury in rats. Preliminary phytochemical analysis confirmed the presence of flavonoids, alkaloids, tannins, saponins, triterpenoids, steroids, and cardiac glycosides, which may contribute to the observed biological activity. The extract significantly attenuated the elevation of serum hepatic biomarkers, including SGPT, SGOT, and bilirubin, and improved the biochemical alterations associated with CCl₄-induced hepatotoxicity. Histopathological observations further supported the protective effect by showing reduced hepatocellular degeneration and restoration of normal liver architecture. The hepatoprotective activity of *A. paniculata* may be attributed to its antioxidant and free radical scavenging properties, which help in preventing oxidative stress-mediated hepatic damage. These findings suggest that *Andrographis paniculata* may serve as a promising natural hepatoprotective agent; however, further studies are required to elucidate its active constituents and underlying molecular mechanisms.

AUTHOR CONTRIBUTIONS

Both authors contributed to equally.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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