RESEARCH PAPER

EVALUATION OF THE HEPATOPROTECTIVE POTENTIAL OF HYDROALCOHOLIC EXTRACT OF *SCAPHIUM AFFINE* IN ETHANOL-INDUCED LIVER INJURY IN RATS

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Abstract
The present study was undertaken to evaluate the hepatoprotective activity of the hydroalcoholic extract of *Scaphium affine* against ethanol-induced hepatotoxicity in rats. Hepatotoxicity was induced by administering ethanol (5 mL/kg body weight), and the protective effect of the extract was assessed at doses of 100 and 200 mg/kg. Silymarin (10 mg/kg) was used as the standard hepatoprotective drug. Various biochemical parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, albumin, total protein, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), and tumor necrosis factor-alpha (TNF-α) were evaluated. Histopathological examination of liver tissues was also performed to assess structural changes. Ethanol administration significantly increased serum ALT, AST, ALP, bilirubin, MDA, and TNF-α levels while decreasing albumin, total protein, SOD, GSH, and CAT levels, indicating severe hepatic injury, oxidative stress, and inflammation. Treatment with the hydroalcoholic extract of *Scaphium affine* significantly ameliorated these biochemical alterations in a dose-dependent manner. The extract also improved body weight and restored antioxidant enzyme activities. Histopathological studies revealed that the extract markedly reduced hepatic necrosis, fatty degeneration, and inflammatory cell infiltration, thereby preserving normal liver architecture. The hepatoprotective effect of the extract at 200 mg/kg was comparable to that of silymarin. The findings suggest that the hydroalcoholic extract of *Scaphium affine* possesses significant hepatoprotective activity, which may be attributed to its antioxidant and anti-inflammatory properties. Therefore, *Scaphium affine* may serve as a promising natural therapeutic agent for the prevention and management of liver disorders.

Keywords: *Scaphium affine*; Hepatoprotective activity; Ethanol-induced hepatotoxicity; Oxidative stress; Antioxidant activity; Liver function enzymes; TNF-α; Silymarin; Histopathology; Phytochemicals
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Introduction

The liver is one of the most vital organs in the human body and plays a central role in metabolism, detoxification, protein synthesis, and maintenance of physiological homeostasis (Mohajan, 2025). Due to its involvement in the biotransformation of xenobiotics and toxic substances, the liver is highly susceptible to damage caused by drugs, alcohol, environmental pollutants, and infectious agents (Mahanayak, 2024). Liver diseases remain a major global health concern, contributing significantly to morbidity and mortality worldwide. Despite advances in modern medicine, effective therapies for liver disorders are limited, and many currently available drugs are associated with adverse effects (Williams, 2006).

Alcohol consumption is one of the leading causes of liver injury and is responsible for a spectrum of hepatic disorders ranging from fatty liver and alcoholic hepatitis to fibrosis, cirrhosis, and hepatocellular carcinoma (Mitra et al., 2020). Ethanol metabolism generates excessive reactive oxygen species (ROS), resulting in oxidative stress, lipid peroxidation, inflammation, and hepatocellular necrosis. Elevated levels of serum liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin are commonly associated with ethanol-induced hepatic damage (Zima et al., 2001). Furthermore, alcohol-induced oxidative stress impairs endogenous antioxidant defense systems, including superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT), thereby exacerbating liver injury (Zima et al., 2001).

Medicinal plants have been extensively explored as potential sources of hepatoprotective agents because of their rich phytochemical composition and relatively low toxicity. Plant-derived bioactive compounds, particularly flavonoids, phenolics, tannins, and alkaloids, possess potent antioxidant and anti-inflammatory properties that can protect hepatic tissues from toxic insults. Consequently, there is growing interest in identifying novel herbal remedies capable of preventing or treating liver diseases (Gonfa et al., 2025).

Scaphium affine (Mast.) Pierre, belonging to the family Malvaceae, is a medicinal plant traditionally used in several Asian countries for the management of various ailments (Phonsena and Wilkie, 2008). Its seeds are reported to contain a variety of bioactive phytoconstituents, including phenolic compounds and flavonoids, which exhibit antioxidant, anti-inflammatory, and free radical scavenging activities. However, scientific evidence regarding its hepatoprotective potential remains limited.

Therefore, the present study was designed to evaluate the hepatoprotective activity of the hydroalcoholic extract of *Scaphium affine* seeds against ethanol-induced liver injury in Wistar rats. The study assessed liver function biomarkers, oxidative stress parameters, inflammatory mediators, and histopathological alterations to elucidate the protective effects of the extract and its possible mechanisms of action.

Material and Methods

Material

The seeds of *Scaphium affine* were collected and authenticated prior to the study. Ethanol and petroleum ether used for extraction were of analytical grade. Silymarin was procured and used as the standard hepatoprotective drug. Commercial diagnostic kits for the estimation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, albumin, and total protein were obtained from Roche Diagnostics. Chemicals and reagents required for the estimation of oxidative stress markers, including malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT), were of analytical grade and purchased from reputed suppliers. The ELISA kit used for determination of tumor necrosis factor-alpha (TNF-α) was obtained from a standard commercial source. Formalin, hematoxylin, eosin, paraffin wax, xylene, and other histopathological reagents were used for tissue processing and microscopic evaluation. All other chemicals and solvents used throughout the study were of analytical reagent grade and were used without further purification.

Methods

Extraction with Hydroalcoholic Solvent Using Maceration Method

Fifty grams of shade-dried *Scaphium affine* seed powder were initially defatted with petroleum ether by the maceration method. The extraction process was continued until complete removal of fatty constituents. The defatted powder was then extracted using a hydroalcoholic solvent system (ethanol:water, 80:20 v/v) by maceration. The mixture was kept in a sterile environment for 48 h with occasional shaking. Subsequently, the extract was filtered through Whatman filter paper No. 40, and the filtrate was concentrated on a water bath maintained at 50°C until complete drying of the extract (Kokate, 1994).

In Vivo Hepatoprotective Activity of Hydroalcoholic Extract of *Scaphium affine* against Ethanol-Induced Hepatotoxicity in Rats

Animals

Healthy Wistar rats weighing 180 ± 20 g were used for the study. The animals were approximately two months old at the initiation of the experiment. They were housed under standard laboratory conditions at a temperature of 22 ± 2°C, relative humidity of 50 ± 10%, and a 12 h light/dark cycle. The animals were provided with standard pellet diet and water ad libitum. Before the commencement of the experiment, the animals were acclimatized to laboratory conditions for seven days. All experimental procedures were approved by the Institutional Animal Ethics Committee and conducted according to CPCSEA guidelines.

Acute Oral Toxicity Study

The acute oral toxicity study of the hydroalcoholic extract of *Scaphium affine* was performed according to OECD guideline 423 (Acute Toxic Class Method). Animals were observed continuously for behavioral changes, clinical signs of toxicity, and mortality for a period of 14 days (Alamri et al., 2025).

Experimental Design

Thirty Wistar rats were randomly divided into five groups consisting of six animals each. The hydroalcoholic extract of *Scaphium affine* was administered orally at doses of 100 and 200 mg/kg body weight. Animals were monitored throughout the study for mortality, toxicity signs, and behavioral abnormalities.

Assessment of Hepatoprotective Activity

The hepatoprotective activity of the hydroalcoholic extract of *Scaphium affine* was evaluated using an ethanol-induced hepatotoxicity model in rats. Animals were randomly allocated into five groups (n = 6). Silymarin was used as the standard hepatoprotective drug for comparison.

- **Group I (Normal Control):** Received normal saline (0.9%, p.o.) for 21 days (Berkoz et al., 2025).

- **Group II (Ethanol Control):** Received ethanol (5 mL/kg, p.o.) daily for 21 days.
- **Group III (Standard):** Received ethanol (5 mL/kg, p.o.) along with silymarin (10 mg/kg, p.o.) for 21 days (Namachivayam and Gopalakrishnan, 2023).
- **Group IV:** Received ethanol (5 mL/kg, p.o.) along with hydroalcoholic extract of *Scaphium affine* (100 mg/kg, p.o.).
- **Group V:** Received ethanol (5 mL/kg, p.o.) along with hydroalcoholic extract of *Scaphium affine* (200 mg/kg, p.o.).

At the end of the experimental period, blood samples were collected under diethyl ether anesthesia through the retro-orbital plexus. Serum was separated by centrifugation at 2500 rpm for 5 min and used for biochemical investigations. Liver tissues were excised and preserved for histopathological evaluation (Berkoz et al., 2025).

Biochemical Analysis

Blood samples collected from the retro-orbital plexus were analyzed for liver function parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, albumin, and total protein using commercially available diagnostic kits and an automated biochemical analyzer (Huang et al., 2006; Zheng et al., 2017).

The oxidative stress markers, including malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT), were estimated using standard biochemical methods (Hadwan and Abed, 2016). Tumor necrosis factor-alpha (TNF-α) levels were determined using a validated ELISA method (Farney et al., 2011).

Histopathological Evaluation

Liver tissues were fixed in 10% neutral buffered formalin for 24 h. The tissues were subsequently dehydrated in graded alcohol solutions (70%, 90%, and 100%), cleared with xylene, and embedded in paraffin wax. Sections of 3–5 µm thickness were prepared and stained with hematoxylin and eosin (H&E). The stained sections were examined under a light microscope for evaluation of hepatic architecture, inflammatory cell infiltration, fatty changes, and necrosis (Saxena et al., 2021).

Statistical analysis

GraphPad Prism version 8.0 (GraphPad Software, San Diego, USA) was used for statistical analyses. The mean ± SEM is used to express the results. For multiple comparisons, a Dunnett post hoc test was employed, with $p < 0.05$ serving as the significance level.

Results and Discussion

The present study investigated the hepatoprotective potential of the hydroalcoholic extract of *Scaphium affine* against ethanol-induced hepatotoxicity in rats. Chronic ethanol administration is known to induce oxidative stress, inflammation, lipid peroxidation, and hepatocellular damage, resulting in altered liver function and histopathological abnormalities. The findings demonstrated that the hydroalcoholic extract of *Scaphium affine* effectively protected the liver against ethanol-induced injury in a dose-dependent manner.

Body weight is an important indicator of the general health status of experimental animals. As shown in Table 1, the ethanol-treated group exhibited a reduction in body weight at the end of the study compared with the normal control group, indicating impaired metabolic activity and deteriorated health status resulting from liver injury. Treatment with the hydroalcoholic extract of *Scaphium affine* at doses of 100 and 200 mg/kg improved body weight gain, with the 200 mg/kg dose showing a greater effect. The standard drug silymarin also significantly improved body weight, indicating restoration of normal physiological functions.

The liver function parameters presented in Table 2 revealed a marked increase in serum ALT, AST, ALP, and bilirubin levels in the ethanol control group compared with the normal control group. Elevated levels of these enzymes are well-recognized indicators of hepatocellular membrane damage and increased permeability of liver cells. Ethanol administration also significantly decreased albumin and total protein concentrations, suggesting impairment of the liver's synthetic function. Pretreatment with the hydroalcoholic extract of *Scaphium affine* significantly reduced ALT, AST, ALP, and bilirubin levels while increasing albumin and total protein levels toward normal values. The hepatoprotective effect was more pronounced at the dose of 200 mg/kg and was comparable to that observed with silymarin treatment.

Oxidative stress is a key mechanism involved in ethanol-induced hepatotoxicity. The oxidative stress parameters summarized in Table 3 showed that ethanol administration significantly increased MDA levels, indicating enhanced lipid peroxidation and oxidative damage to hepatocyte membranes. Administration of the hydroalcoholic extract significantly reduced MDA levels compared with the ethanol control group, suggesting inhibition of free radical-mediated lipid peroxidation. Furthermore, treatment with the extract improved endogenous antioxidant defense systems by restoring SOD, GSH, and CAT levels. The antioxidant effect was dose-dependent, with the 200 mg/kg dose producing greater protection than the 100 mg/kg dose.

Inflammatory responses play a crucial role in the progression of alcoholic liver injury. The results

presented in Table 4 showed a significant elevation of TNF-α levels in ethanol-treated rats, indicating activation of inflammatory pathways. Treatment with the hydroalcoholic extract of *Scaphium affine* significantly reduced TNF-α concentrations compared with the ethanol control group. The reduction was more pronounced at the higher dose, suggesting that the extract possesses potent anti-inflammatory activity capable of attenuating ethanol-induced hepatic inflammation.

The biochemical findings were further corroborated by histopathological observations (Figure 1A–E). The liver section of the normal control group (Figure 1A) displayed normal hepatic architecture with well-arranged hepatocytes and intact cellular morphology. In contrast, the ethanol-treated group (Figure 1B) exhibited severe histopathological alterations, including inflammatory cell infiltration, fatty degeneration, and hepatocellular necrosis. Pretreatment with silymarin (Figure 1C) markedly reduced these pathological changes and preserved hepatic architecture. Similarly, liver sections from rats treated with the hydroalcoholic extract at doses of 100 and 200 mg/kg (Figure 1D and Figure 1E, respectively) showed considerable improvement, characterized by reduced inflammation, decreased necrosis, and restoration of normal hepatic structure. The 200 mg/kg dose demonstrated better histological protection than the 100 mg/kg dose, which was consistent with the biochemical findings.

The hepatoprotective effect of *Scaphium affine* may be attributed to the presence of phytoconstituents such as flavonoids, phenolic compounds, tannins, and other antioxidant constituents. These bioactive compounds are known to scavenge reactive oxygen species, inhibit lipid peroxidation, stabilize hepatocyte membranes, enhance endogenous antioxidant defenses, and suppress inflammatory mediators. The improvement observed in liver function markers (Table 2), oxidative stress parameters (Table 3), inflammatory markers (Table 4), and histopathological features (Figure 1A–E) collectively supports the hepatoprotective efficacy of the extract.

The results obtained from body weight analysis (Table 1), liver function studies (Table 2), oxidative stress assessment (Table 3), inflammatory marker evaluation (Table 4), and histopathological examination (Figure 1A–E) indicate that the hydroalcoholic extract of *Scaphium affine* possesses significant hepatoprotective activity against ethanol-induced liver damage. The higher dose (200 mg/kg) produced greater protection and showed effects comparable to those of the standard drug silymarin, suggesting its potential as a promising natural therapeutic agent for the management of hepatic disorders.

Table 1: Mean Body Weight Change

The values are given as mean ± S.E.M. (n = 6). When compared to the control group, values are statistically significant at $p<0.05$ (one-way ANOVA followed by Dunnett's test).

Groups	Drug	Dose	Body weight (g)	
			Onset of study	End of study
I	Normal Control	0.5% CMC 1 ml/kg, p.o.	210.80 ± 2.10	229.60 ± 2.45
II	Control (Ethanol)	5 mL/kg b.wt.	223.50 ± 1.90	217.40 ± 2.00
III	Ethanol+Silymarin	10 mg/kg p.o.	236.10 ± 2.30	242.90 ± 2.70
IV	Ethanol+ Hydroalcoholic extract of *Scaphium affine*	100 mg/kg p.o.	232.40 ± 2.15	239.80 ± 2.25
V	Ethanol+ Hydroalcoholic extract of *Scaphium affine*	200 mg/kg p.o.	243.20 ± 2.40	249.90 ± 2.85

Table 2: Effect of Hydroalcoholic Extract of *Scaphium affine* on Liver Function Parameters in Ethanol-Induced Hepatotoxicity in Rats

Group	Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)	Albumin (mg/dL)	Total Protein (g/dL)
I	Normal Control	42.60 ± 5.20	95.80 ± 9.90	102.40 ± 9.85	0.92 ± 0.08	4.35 ± 0.32	7.25 ± 0.55
II	Ethanol Control	138.40 ± 9.15	268.40 ± 11.75	245.30 ± 11.20	2.45 ± 0.15	2.10 ± 0.25	4.10 ± 0.48
III	Ethanol + Silym	58.90 ±	142.60 ±	148.60 ±	1.28 ± 0.10	3.95 ± 0.28	6.85 ± 0.5

	arin (10 mg/kg)	6.40	8.55	7.10			0
IV	Ethanol + *S. affine* (100 mg/kg)	82.30 ± 7.85	182.30 ± 9.40	182.90 ± 8.45	1.72 ± 0.12	3.10 ± 0.30	5.80 ± 0.52
V	Ethanol + *S. affine* (200 mg/kg)	68.70 ± 6.95	160.90 ± 8.75	165.40 ± 7.90	1.55 ± 0.11	3.55 ± 0.27	6.20 ± 0.47

Table 3: Effect of Hydroalcoholic Extract of *Scaphium affine* on Oxidative Stress Markers in Ethanol-Induced Hepatotoxicity in Rats

Group	Treatment	MDA (µmol/L)	SOD (U/mg)	GSH (µmol/mg)	CAT (U/mg)
I	Normal Control	1.42 ± 0.32	8.92 ± 0.28	103.20 ± 7.60	102.40 ± 7.90
II	Ethanol Control	4.80 ± 0.55	4.12 ± 0.21	225.40 ± 11.85	226.10 ± 11.60
III	Ethanol + Silymarin (10 mg/kg)	1.75 ± 0.40	8.15 ± 0.26	129.70 ± 6.95	130.20 ± 6.85
IV	Ethanol + *S. affine* (100 mg/kg)	2.85 ± 0.48	6.35 ± 0.24	169.80 ± 8.20	178.90 ± 8.10
V	Ethanol + *S. affine* (200 mg/kg)	2.30 ± 0.42	7.72 ± 0.27	158.60 ± 9.40	165.30 ± 9.75

Table 4: Effect of Hydroalcoholic Extract of *Scaphium affine* on Inflammatory Marker in Ethanol-Induced Hepatotoxicity in Rats

Group	Treatment	TNF-α (pg/mL)
I	Normal Control	98.60 ± 7.85
II	Ethanol Control	232.40 ± 13.10
III	Ethanol + Silymarin (10 mg/kg)	131.20 ± 8.05
IV	Ethanol + *Scaphium affine* (100 mg/kg)	176.90 ± 9.20
V	Ethanol + *Scaphium affine* (200 mg/kg)	162.30 ± 10.40

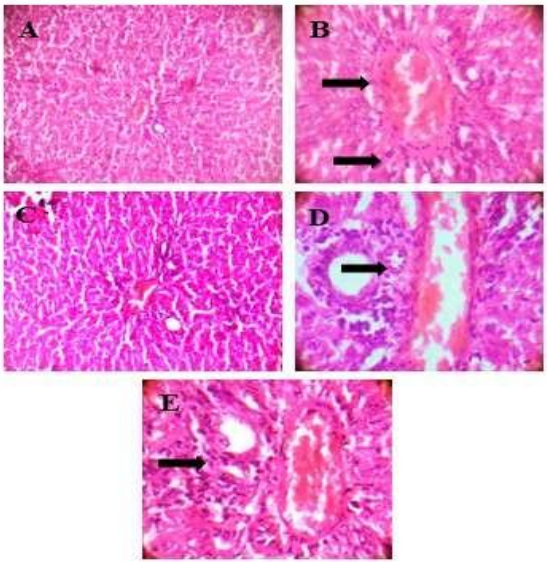

Figure: 1A-E

Histological analysis of liver sections from various 200: section of liver tissue pretreatment with 100 and 200 mg/kg hydroalcoholic extract of *Scaphium affine*, followed by ethanol; the black arrow indicates moderate to mild inflammation and tissue necrosis.

Conclusion

The present study demonstrated that the hydroalcoholic extract of *Scaphium affine* seeds possesses significant hepatoprotective activity against ethanol-induced liver injury in Wistar rats. Ethanol administration caused marked hepatic damage, as evidenced by elevated serum levels of ALT, AST, ALP, bilirubin, lipid peroxidation marker (MDA), and inflammatory cytokine TNF-α, along with reduced levels of albumin, total protein, and endogenous antioxidant enzymes such as SOD, GSH, and CAT. Treatment with the hydroalcoholic extract of *Scaphium affine* significantly ameliorated these biochemical alterations in a dose-dependent manner. The extract also improved body weight changes and restored normal liver architecture, as confirmed by histopathological examination, which showed reduced inflammation, fatty degeneration, and hepatocellular necrosis. The hepatoprotective effect of the extract was more pronounced at the dose of 200 mg/kg and was comparable to that of the standard drug silymarin. The observed hepatoprotective activity may be attributed to the

antioxidant and anti-inflammatory properties of the phytoconstituents present in *Scaphium affine*.

The findings suggest that the hydroalcoholic extract of *Scaphium affine* has considerable potential as a natural hepatoprotective agent and may be useful in the prevention and management of alcohol-induced liver disorders. Further studies are warranted to isolate the active constituents and elucidate the precise molecular mechanisms responsible for its hepatoprotective effects.

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