

Hepatoprotective Effects of *N*-acetylcysteine and / or a *Tephrosia purpurea* Extract on Carbon Tetrachloride-Induced Liver Injury in Rats

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Abstract

Objective: The aim of this study is to investigate the hepatoprotective effects of *Tephrosia purpurea* extract alone and in combination with *N*-acetylcysteine against CCl₄-induced liver injury in rats.

Methods: Forty-eight male Wistar rats were randomly divided into eight experimental groups ($n = 6$ per group): normal control, olive oil negative control, CCl₄-positive control, CCl₄+NAC, three CCl₄+*Tephrosia purpurea* groups (100, 200, and 400 mg/kg), and a CCl₄+NAC+*Tephrosia purpurea* (200 mg/kg) group.

Results: Histopathological examination revealed severe hepatic necrosis, inflammatory cell infiltration, and disruption of normal hepatic architecture in the CCl₄-positive control group. In contrast, pretreatment with *Tephrosia purpurea* markedly attenuated these pathological alterations in a dose-dependent manner, with the combined administration of NAC and *Tephrosia purpurea* providing the most pronounced histological protection and preservation of hepatic tissue architecture. Similarly, biochemical analyses demonstrated significant protection against CCl₄-induced liver injury, as evidenced by improved liver function markers and attenuation of hepatic injury in the treated groups.

Conclusion: Overall, *Tephrosia purpurea* exhibited significant dose-dependent hepatoprotective activity, which was further enhanced when administered in combination with NAC.

Keywords: Hepatotoxicity, *N*-acetylcysteine, *Tephrosia purpurea* extract, liver histopathology, liver function test

Introduction

Hepatotoxicity refers to chemically induced liver injury that may present as either acute or chronic hepatic damage. As the principal organ responsible for metabolism and detoxification, the liver is particularly susceptible to injury caused by drugs, environmental pollutants, and naturally occurring toxins.¹ Understanding the mechanisms underlying hepatotoxicity is therefore essential for the development of effective preventive and therapeutic strategies. Among the experimental models used to investigate liver injury, carbon tetrachloride (CCl₄) remains one of the most widely employed hepatotoxic agents due to its ability to induce hepatic damage that resembles several pathological features of human liver disease.²

The hepatotoxic effects of CCl₄ are initiated following its metabolic activation by hepatic cytochrome P450 enzymes, leading to the generation of highly reactive trichloromethyl (CCl₃•) and trichloromethyl peroxy (CCl₃OO•) radicals.³ These reactive intermediates trigger lipid peroxidation, oxidative stress, membrane damage, inflammatory responses, and ultimately hepatocellular necrosis and liver dysfunction.⁴ Consequently, the CCl₄-induced hepatotoxicity model has been extensively utilized for evaluating the efficacy of potential hepatoprotective agents.⁵

N-acetylcysteine (NAC) is a well-established antioxidant and the standard antidote for acetaminophen-induced hepatotoxicity. Its hepatoprotective activity is primarily attributed to its ability to replenish intracellular glutathione (GSH), the major endogenous antioxidant defense system, thereby enhancing cellular protection against oxidative stress.^{6,7} In addition, NAC exhibits direct free-radical scavenging activity and modulates inflammatory pathways, further contributing to its protective effects against liver injury.^{4,6}

Interest in plant-derived hepatoprotective agents has increased considerably in recent years because many

conventional therapies may be associated with limited efficacy, adverse effects, or high treatment costs. Among these medicinal plants, *Tephrosia purpurea* (L.) Pers., commonly known in Ayurvedic medicine as “Sarapunkha,” has been traditionally used for the management of various hepatic disorders.⁸ Phytochemical investigations have revealed the presence of numerous bioactive constituents, including flavonoids, rotenoids, chalcones, alkaloids, saponins, glycosides, and isoflavones, which are believed to contribute to its diverse pharmacological properties.⁹ Previous studies have demonstrated that *Tephrosia purpurea* possesses antioxidant, anti-inflammatory, membrane-stabilizing, and hepatoprotective activities, supporting its potential as a natural therapeutic agent against liver injury.^{8,10–12}

Although the hepatoprotective effects of NAC and *Tephrosia purpurea* have been investigated individually, their combined administration has not been previously evaluated in experimental CCl₄-induced hepatotoxicity. This represents an important gap in the current literature, as the complementary mechanisms of both agents may provide enhanced hepatoprotection. While NAC primarily restores intracellular glutathione levels and neutralizes reactive oxygen species, the diverse phytoconstituents of *Tephrosia purpurea* may provide additional antioxidant, anti-inflammatory, and membrane-protective effects. Therefore, concurrent administration of both agents may offer broader protection against the multiple pathological processes involved in hepatic injury.

Accordingly, the present study aimed to evaluate the hepatoprotective effects of *Tephrosia purpurea* extract administered alone at different dose levels and in combination with *N*-acetylcysteine in rats exposed to CCl₄-induced hepatotoxicity. We hypothesized that combined treatment would provide greater protection against liver injury than either intervention administered alone.

Methodology

Experimental Animals

Forty-eight adult male Wistar albino rats weighing 180–220 g were used in this experimental study. The animals were housed at the College of Pharmacy, University of Babylon, under standard laboratory conditions with free access to food and water, maintained on a 12 h light/dark cycle, and kept at a controlled temperature of $25 \pm 2^\circ\text{C}$.

Ethical Considerations

All experimental procedures were conducted in accordance with the protocol approved by the Ethical Committee of the College of Pharmacy, University of Babylon (Approval No. A-0050, 12 October 2025).

Plant Material Characterization

Tephrosia purpurea extract (20:1 extract ratio) was obtained from KAN Phytochemicals Pvt. Ltd. (Sonipat, Haryana, India), a GMP-certified manufacturer holding ISO and organic certifications. The extract was prepared from authenticated whole plant material using Soxhlet extraction with 96% ethanol as the solvent, yielding approximately 5% w/w extract. The product was standardized at a 20:1 extract ratio according to the manufacturer's technical specifications. Major phytochemical constituents of *Tephrosia purpurea* include rutin, quercetin, rotenone, deguelin, and lupeol, along with other flavonoids, isoflavones, and rotenoids contributing to its overall bioactive profile. The extract was stored under controlled conditions ($15\text{--}25^\circ\text{C}$, relative humidity $<10\%$) in a dry, cool, and light-protected environment throughout the study period. *Tephrosia purpurea* extract powder was prepared as a fresh stock solution daily by dissolving 400 mg in 10 mL of distilled water to obtain a final concentration of 40 mg/mL. The solution was vortex-mixed until complete dispersion and formation of a uniform suspension. The required administration volume for each experimental animal was calculated individually based on the target dose and body weight.

Experimental Design

After the acclimatization period, 48 rats were randomly assigned to eight experimental groups ($n = 6$ per group). This sample size was selected to accommodate biological variation among animals and to provide a robust and reliable assessment of treatment effects on biochemical and histopathological parameters while minimizing unnecessary animal use.

The normal control group received no treatment. The negative control group received olive oil (0.5 mL/kg, intraperitoneally, twice weekly). The CCl_4 -positive control group received CCl_4 (1 mL/kg, intraperitoneally, prepared as a 1:1 mixture with olive oil) twice weekly for 28 consecutive days to induce hepatotoxicity. The CCl_4 +NAC group received NAC (150 mg/kg/day, orally) prior to CCl_4 administration. Three treatment groups received *Tephrosia purpurea* extract at doses of 100, 200, or 400 mg/kg/day orally before CCl_4 administration and were designated as the CCl_4 +*Tephrosia purpurea* (100 mg/kg), CCl_4 +*Tephrosia purpurea* (200 mg/kg), and CCl_4 +*Tephrosia purpurea* (400 mg/kg) groups, respectively.

The combination group received NAC (150 mg/kg/day) and *Tephrosia purpurea* extract (200 mg/kg/day, orally) prior to CCl_4 administration and was designated as the CCl_4 +NAC+*Tephrosia purpurea* group. CCl_4 was administered to all pretreated groups approximately 30 minutes after pretreatments using the same regimen described above. All oral treatments were administered once daily by gastric gavage. The treatment regimen was continued for 28 days. No mortality was reported during the experimental period.

The selected dose of CCl_4 was based on previous studies.¹³ The NAC dose was selected according to established experimental hepatoprotection protocols.¹⁴ Dose selection for *Tephrosia purpurea* was guided by previously published studies utilizing comparable dose ranges in experimental animals. Low, moderate, and high doses were adopted to allow the assessment of potential dose-dependent responses in the present study.^{8,12}

At the end of the experimental period, the animals were fasted overnight and anesthetized with ketamine (100 mg/kg, intramuscularly) and xylazine (10 mg/kg, intramuscularly). Following confirmation of deep anaesthesia, blood samples (approximately 3.5–5 mL) were collected by cardiac puncture in accordance with accepted ethical procedures. After euthanasia, liver tissues were excised and processed for histopathological examination.

Blood samples were allowed to clot in gel tubes at 37°C for 30 min and were subsequently centrifuged at 5000 rpm for 5 min. The separated serum was used for the determination of ALT, AST, ALP, total bilirubin, albumin, and total protein using commercial biochemical reagent kits (BioLABO, France) according to the manufacturer's instructions.

Liver specimens were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for microscopic examination. Histopathological alterations, including hepatocellular degeneration, necrosis, inflammatory cell infiltration, and architectural distortion, were evaluated semi-quantitatively by a blinded histopathologist according to the morphological criteria described by Rosai and Ackerman's Surgical Pathology.¹⁵

Each histopathological lesion was scored individually according to the predefined morphological criteria. The total histopathological damage score for each liver specimen was calculated by summing the scores of all evaluated lesions.

Based on the composite histopathological damage score, hepatic injury was classified as mild (0–3), moderate (4–5), or severe (6–7). Microscopic evaluation and scoring were performed blinded to the treatment allocation to minimize observer bias (Table 1).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 9.0.0 (La Jolla, USA) for Microsoft Windows. Data were expressed as mean $\pm$ standard deviation (SD). The normality of data distribution was assessed before analysis via Shapiro-Wilk test. Comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Tukey HSD post hoc test for multiple comparisons for normally distributed data. While for not normally distributed data, Kruskal wallis test was performed (for histopathological

Table 1. **Semi-quantitative batts-ludwig grading system for hepatic activity and injury (adapted from rosai and ackerman's surgical pathology¹⁵**

Grade	Activity level	Histopathological criteria (Morphological description)
0	No activity	No tissue injury or inflammation; portal inflammation only; no interface activity; no lobular activity
1	Minimal activity	Portal inflammation; minimal interface activity; occasional lobular apoptotic hepatocytes, mild cytoplasmic/perinuclear vacuolation, or slight hepatocyte swelling.
2	Mild activity	Interface activity involving some or all portal tracts; mild lobular activity with little hepatocyte damage, degenerative changes, or localized necrosis.
3	Moderate activity	Interface activity involving all portal tracts; moderate lobular activity with obvious hepatocyte damage, stromal changes, and prominent cellular vacuolation
4	Severe activity	Severe activity accompanied by bridging necrosis; prominent diffuse hepatocyte damage, extensive centrilobular/panzonal necrosis, and severe architectural distortion.

results). A *p*-value of less than 0.05 was considered statistically significant.

Results

Histopathological Findings

Histopathological examination of liver sections stained with H&E revealed normal hepatic architecture in both the normal control and negative control groups. Hepatic cords were regularly arranged around the central vein, with intact hepatocytes and open hepatic sinusoids (Figure 1A&B).

In contrast, liver sections from the CCl₄-positive control group demonstrated severe hepatic injury (score 3), characterized by extensive centrilobular and panzonal necrosis, pyknotic nuclei, loss of normal lobular architecture, marked disorganization of hepatic cords, dense inflammatory cell infiltration, and prominent hepatocellular vacuolation (Figure 1C).

The CCl₄+NAC group exhibited only mild histological injury (score 1), with partial preservation of normal hepatic architecture, mild inflammatory cell infiltration, and slight sinusoidal dilatation with reduced vascular congestion (Figure 1D).

The CCl₄+*Tephrosia purpurea* (100 and 200 mg/kg) groups exhibited moderate hepatic injury (score 2), characterized by hepatocellular swelling, moderate inflammatory cell infiltration, focal necrotic areas, and congestion of the central vein associated with sinusoidal dilatation (Figure 1E&F). The CCl₄+*Tephrosia purpurea* 400 mg/kg group demonstrated a greater degree of protection, showing only mild hepatocellular swelling, minimal inflammatory cell infiltration, near-normal hepatic cord organization, and mild or absent vascular congestion (Figure 1G).

The CCl₄+ NAC+ *Tephrosia purpurea* combination group exhibited the most pronounced hepatoprotection. Liver architecture was largely preserved, with only slight cloudy swelling, mild cytoplasmic vacuolation, limited inflammatory cell infiltration, and minimal vascular congestion (Table 2; Figure 1H), this group demonstrated the lowest histological injury score among all treatment groups.

Histopathological examination of liver sections stained with H&E revealed normal hepatic architecture in both the normal control and negative control groups. Hepatic cords were regularly arranged around the central vein, with intact hepatocytes and open hepatic sinusoids (Figure 1A&B).

In contrast, liver sections from the CCl₄-positive control group demonstrated severe hepatic injury, with the highest median composite histopathological damage score (6.5; IQR: 6.0–7.0). Histopathological changes were characterized by extensive centrilobular and panzonal necrosis, pyknotic nuclei, loss of normal lobular architecture, marked disorganization of hepatic cords, dense inflammatory cell infiltration, and prominent hepatocellular vacuolation (Figure 1C).

The CCl₄ + NAC group exhibited only mild histological injury, with a median composite histopathological damage score of 1.5 (IQR: 0.75–2.25). Liver sections showed partial preservation of normal hepatic architecture, mild inflammatory cell infiltration, and slight sinusoidal dilatation with reduced vascular congestion (Figure 1D).

The CCl₄+ *Tephrosia purpurea* (100 mg/kg) group exhibited moderate hepatic injury, with a median composite histopathological damage score of 5.0 (IQR: 4.75–5.0). Histopathological changes included hepatocellular swelling, moderate inflammatory cell infiltration, focal necrotic areas, and congestion of the central vein associated with sinusoidal dilatation (Figure 1E).

The CCl₄ + *Tephrosia purpurea* (200 mg/kg) group also exhibited moderate hepatic injury, with a median composite histopathological damage score of 4.0 (IQR: 4.0–5.0). Histopathological findings were similar to those observed in the 100 mg/kg group but with less severe alterations (Figure 1F).

The CCl₄ + *Tephrosia purpurea* (400 mg/kg) group demonstrated a greater degree of protection, with a median composite histopathological damage score of 2.0 (IQR: 0.75–2.25), corresponding to mild hepatic injury. Liver sections showed only mild hepatocellular swelling, minimal inflammatory cell infiltration, near-normal hepatic cord organization, and mild or absent vascular congestion (Figure 1G).

The CCl₄ + NAC + *Tephrosia purpurea* combination group exhibited the most pronounced hepatoprotection, with the lowest median composite histopathological damage score among the treated groups (1.0; IQR: 0.0–1.25). Liver architecture was largely preserved, with only slight cloudy swelling, mild cytoplasmic vacuolation, limited inflammatory cell infiltration, and minimal vascular congestion (Figure 1H).

Biochemical Findings

The biochemical findings were consistent with the histopathological observations. No significant differences (*P* > 0.05) were observed between the normal control and negative control

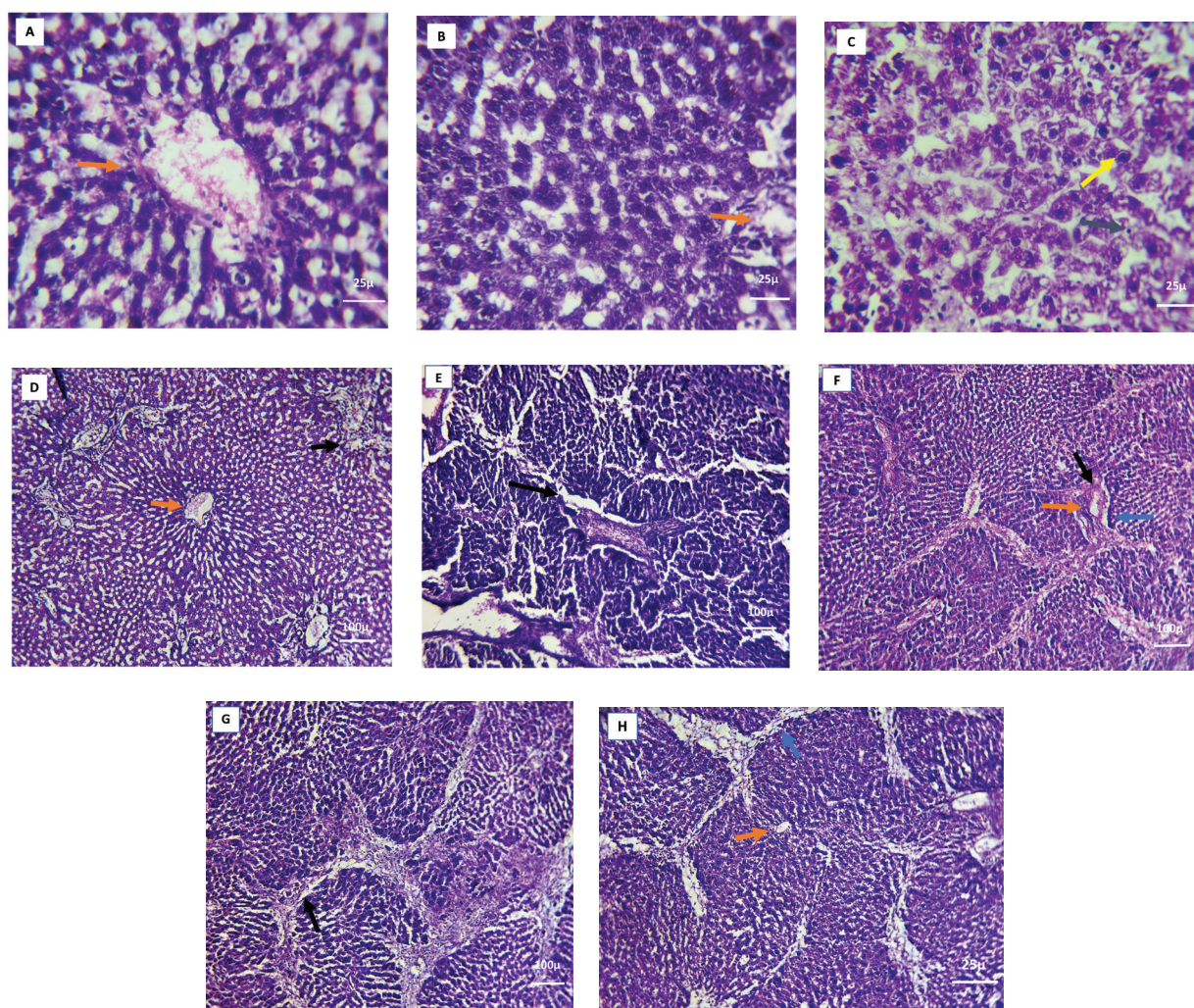


Fig. 1 A & B: Histological section of rat liver for negative control group stained with hematoxylin and eosin (H&E) showing normal hepatic architecture. The central vein (orange arrow) is surrounded by normally arranged hepatic cords with intact hepatocytes and patent hepatic sinusoids. (40 x). C: Histological section of rat liver for positive control group, exhibit score 3 damage (sever), stained with H&E. Massive centrilobular or panzonal necrosis (death of liver cells) with pyknotic nuclei(gray arrow). Loss of the typical lobular structure, with severe disorientation of hepatic cords, dense inflammatory cell infiltration (yellow arrow). High levels of neutrophil and lymphocyte aggregation in the portal and parenchymal areas, and vacuolation of hepatocytes. (40 x). D: Histological section of rat liver for reference treatment group stained with H&E exhibit score 1 damage (mild) showing the protective effect of NAC pretreatment against CCl_4 -induced hepatotoxicity. Partial restoration of normal hepatic architecture , mild inflammatory cell infiltration, and slight sinusoidal dilation (black arrow) with reduced congestion, the central vein (orange arrow). E: Histological section of rat liver for pretreatment with *Tephrosia purpurea* 100 mg/kg groups stained with H&E showing moderate(score 2) hepatocyte swelling (edema), moderate inflammatory cell infiltration with focal necrotic area, and marked congestion of the central vein with sinusoidal dilatation (black arrow). (X 10). F: Histological section of rat liver for pretreatment with *Tephrosia purpurea* 200 mg/kg groups stained with H&E showing moderate (score 2) hepatocyte swelling (edema), moderate inflammatory cell infiltration with focal necrotic area (blue arrow), and marked congestion of the central vein (orange arrow) with sinusoidal dilatation (black arrow). (X 10). G: Histological section of rat liver for pretreatment with *Tephrosia purpurea* 400 mg/kg stained with H&E showing mild swelling (edema) of hepatocyte, mild infiltration of inflammatory cells, near-normal appearance of hepatic cords with minimal sinusoidal dilation (black arrow), and central vein show mild or no congestion. H: Histological section of rat liver for pretreatment with combination (CCl_4 + NAC+ *Tephrosia purpurea* 200 mg/kg) stained with H&E, overall hepatic cord arrangement is generally preserved, slight cloudy swelling, mild vacuolation in the cytoplasm of hepatocytes, and mild infiltration of lymphocytes (blue arrow) or leukocytes within the portal areas or parenchyma, and central vein show mild or no congestion(orange arrow).

groups in any of the measured liver function parameters. In contrast, the CCl_4 -positive control group showed significant elevations in serum ALT, AST, ALP, and total bilirubin levels, accompanied by significant reductions in albumin and total protein concentrations compared with the negative control group ($p < 0.001$) (Table 3).

Pretreatment with NAC significantly attenuated these biochemical alterations ($P < 0.05$). *Tephrosia purpurea* similarly produced dose-dependent improvements in all liver function parameters, with the 400 mg/kg dose achieving the greatest biochemical normalization among the single-agent treatment groups (Tables 4–9).

Table 2. **Effects of *Tephrosia purpurea*, NAC, and their combination on serum liver biochemical parameters in CCl₄-induced hepatotoxicity**

Groups	ALT (U/L) Mean ± SD	AST (U/L) Mean ± SD	ALP (U/L) Mean ± SD	Total bilirubin (mg/dL) Mean ± SD	Albumin (g/dL) Mean ± SD	Total protein(g/dL) Mean ± SD
Normal control (n = 6)	44.78 ± 0.51	122.72 ± 0.67	121.01 ± 4.05	0.11 ± 0.01	3.90 ± 0.29	7.18 ± 0.38
Negative control (olive oil) n = 6	44.82 ± 0.31	122.92 ± 0.52	120.51 ± 4.11	0.11 ± 0.01	3.91 ± 0.21	7.01 ± 0.53
Positive control (CCl ₄)(n = 6)	134.11 ± 4.18	208.61 ± 2.51	203.33 ± 1.86	0.89 ± 0.06	2.11 ± 0.17	5.01 ± 0.18
Reference treatment (NAC+CCl ₄)(n = 6)	55.13 ± 3.24	135.54 ± 2.73	138.84 ± 0.75	0.16 ± 0.01	3.55 ± 0.24	6.65 ± 0.22
<i>Tephrosia purpurea</i> 100 mg/kg +CCl ₄ (n = 6)	85.25 ± 2.52	152.75 ± 1.02	155.83 ± 0.75	0.26 ± 0.02	3.22 ± 0.12	6.11 ± 0.13
<i>Tephrosia purpurea</i> 200 mg/kg +CCl ₄ (n = 6)	72.01 ± 1.89	146.82 ± 1.16	142.01 ± 1.26	0.16 ± 0.01	3.35 ± 0.12	6.40 ± 0.21
<i>Tephrosia purpurea</i> 400 mg/kg +CCl ₄ (n = 6)	56.58 ± 3.69	135.74 ± 2.94	138.82 ± 0.73	0.16 ± 0.04	3.46 ± 0.15	6.71 ± 0.24
Combination (CCl ₄ +NAC+ <i>Tephrosia purpurea</i> 200 mg/kg) (n = 6)	46.63 ± 1.66	128.21 ± 4.26	132.01 ± 1.41	0.11 ± 0.01	3.83 ± 0.12	7.05 ± 0.31

Data are presented as mean ± SD (n = 6 per group).

Table 3. **Median histopathological damage scores of liver tissues of experimental groups (n = 6 rats per group)**

Parameter	Groups (n = 6)	Median (IQR)
Histopathology damage scores	Normal	0.0 (0.0–0.0)
	Olive oil	0.0 (0.0–0.0)
	CCl ₄	6.5 (6.0–7.0)
	NAC+ CCl ₄	1.5 (0.75–2.25)
	<i>Tephrosia purpurea</i> 100 mg/kg+ CCl ₄	5.0 (4.75–5.0)
	<i>Tephrosia purpurea</i> 200 mg/kg+ CCl ₄	4.0 (4.0–5.0)
	<i>Tephrosia purpurea</i> 400 mg/kg+ CCl ₄	2.0 (0.75–2.25)
	Combination (CCl ₄ + NAC + <i>Tephrosia purpurea</i> 200)	1.0 (0.0–1.25)

Table 4. **Mean serum ALT levels in different experimental groups**

Parameter	Groups (n = 6)	Mean ± SD	P-value vs CCl ₄	CI 95%
Alanine aminotransferase (ALT) (U/L)	Normal	44.78 ± 0.51	< 0.0001	44.24–45.32
	Olive oil	44.82 ± 0.31	< 0.0001	44.46–45.18
	CCl ₄	134.11 ± 4.18	–	129.7–138.4
	NAC+ CCl ₄	55.13 ± 3.24	< 0.0001	51.73–58.53
	<i>Tephrosia purpurea</i> 100 mg/kg+ CCl ₄	85.25 ± 2.52	< 0.0001	82.60–87.90
	<i>Tephrosia purpurea</i> 200 mg/kg+ CCl ₄	72.01 ± 1.89	< 0.0001	70.01–73.99
	<i>Tephrosia purpurea</i> 400 mg/kg+ CCl ₄	56.58 ± 3.69	< 0.0001	52.71–60.46
	Combination (CCl ₄ +NAC+ <i>Tephrosia purpurea</i> 200)	46.63 ± 1.66	< 0.0001	44.89–48.38

SD: standard deviation. P > 0.05: statistically non-significant; P < 0.05: statistically significant.

Table 5. Mean serum AST levels in different experimental groups

Parameter	Groups	Mean $\pm$ SD	P-value vs CCl ₄	CI 95%
Aspartate aminotransferase AST (U/L)	Control	122.72 $\pm$ 0.67	< 0.0001	122.0–123.4
	Olive oil	122.92 $\pm$ 0.52	< 0.0001	122.3–123.4
	CCl ₄	208.61 $\pm$ 2.51	–	206.0–211.3
	NAC+ CCl ₄	135.54 $\pm$ 2.73	< 0.0001	132.6–138.4
	<i>Tephrosia purpurea</i> 100 mg/kg+ CCl ₄	152.75 $\pm$ 1.02	< 0.0001	151.6–153.7
	<i>Tephrosia purpurea</i> 200 mg/kg+ CCl ₄	146.82 $\pm$ 1.16	< 0.0001	145.6–148.1
	<i>Tephrosia purpurea</i> 400 mg/kg+ CCl ₄	135.74 $\pm$ 2.94	< 0.0001	132.6–138.8
	Combination (CCl ₄ +NAC+ <i>Tephrosia purpurea</i> 200)	128.21 $\pm$ 4.26	< 0.0001	123.7–132.6

SD: standard deviation. $P > 0.05$: statistically non-significant; $P < 0.05$: statistically significant.

Table 6. Mean serum of ALP levels in different experimental groups

Parameter	Groups	Mean $\pm$ SD	P-value vs CCl ₄	CI 95%
Alkaline phosphatase (ALP) (U/L)	Control	121.01 $\pm$ 4.05	< 0.0001	116.8–125.2
	Olive oil	120.51 $\pm$ 4.11	< 0.0001	116.1–124.8
	CCl ₄	203.33 $\pm$ 1.86	–	201.4–205.3
	NAC+ CCl ₄	138.84 $\pm$ 0.75	< 0.0001	138.0–139.6
	<i>Tephrosia purpurea</i> 100 mg/kg+ CCl ₄	155.83 $\pm$ 0.75	< 0.0001	155.0–156.6
	<i>Tephrosia purpurea</i> 200 mg/kg+ CCl ₄	142.01 $\pm$ 1.26	< 0.0001	140.7–143.3
	<i>Tephrosia purpurea</i> 400 mg/kg+ CCl ₄	138.82 $\pm$ 0.73	< 0.0001	138.0–139.6
	Combination (CCl ₄ +NAC+ <i>Tephrosia purpurea</i> 200)	132.01 $\pm$ 1.41	< 0.0001	130.5–133.5

SD: standard deviation. $P > 0.05$: statistically non-significant; $P < 0.05$: statistically significant.

Table 7. Mean serum total bilirubin levels in different experimental groups

Parameter	Groups	Mean $\pm$ SD	P-value vs CCl ₄	CI 95%
Total Bilirubin (mg/dL)	Control	0.11 $\pm$ 0.01	< 0.0001	0.09583–0.1175
	Olive oil	0.11 $\pm$ 0.01	< 0.0001	0.1005–0.1295
	CCl ₄	0.89 $\pm$ 0.06	–	0.8263–0.9537
	NAC+ CCl ₄	0.16 $\pm$ 0.01	< 0.0001	0.1485–0.1715
	<i>Tephrosia purpurea</i> 100 mg/kg+ CCl ₄	0.26 $\pm$ 0.02	< 0.0001	0.2352–0.2848
	<i>Tephrosia purpurea</i> 200 mg/kg+ CCl ₄	0.16 $\pm$ 0.01	< 0.0001	0.1562–0.1738
	<i>Tephrosia purpurea</i> 400 mg/kg+ CCl ₄	0.16 $\pm$ 0.04	< 0.0001	0.1194–0.2139
	Combination (CCl ₄ + NAC + <i>Tephrosia purpurea</i> 200)	0.11 $\pm$ 0.01	< 0.0001	0.09241–0.1309

SD: standard deviation. $P > 0.05$: statistically non-significant; $P < 0.05$: statistically significant.

Table 8. Mean serum albumin levels in different experimental groups

Parameter	Groups	Mean $\pm$ SD	P-value vs CCl ₄	CI 95%
Albumin (g/dL)	Normal	3.90 $\pm$ 0.29	< 0.0001	3.589–4.211
	Olive oil	3.91 $\pm$ 0.21	< 0.0001	3.690–4.110
	CCl ₄	2.11 $\pm$ 0.17	–	1.936–2.297
	NAC+ CCl ₄	3.55 $\pm$ 0.24	< 0.0001	3.295–3.805
	<i>Tephrosia purpurea</i> 100 mg/kg+ CCl ₄	3.22 $\pm$ 0.12	< 0.0001	3.085–3.315
	<i>Tephrosia purpurea</i> 200 mg/kg+ CCl ₄	3.35 $\pm$ 0.12	< 0.0001	3.221–3.479
	<i>Tephrosia purpurea</i> 400 mg/kg+ CCl ₄	3.46 $\pm$ 0.15	< 0.0001	3.309–3.625
	Combination (CCl ₄ + NAC + <i>Tephrosia purpurea</i> 200)	3.83 $\pm$ 0.12	< 0.0001	3.725–3.942

SD: standard deviation. $P > 0.05$: statistically non-significant; $P < 0.05$: statistically significant.

Table 9. Mean serum total protein levels in different experimental groups

Parameter	Groups	Mean $\pm$ SD	P-value vs CCl ₄	CI 95%
Total protein (g /dL)	Normal	7.18 $\pm$ 0.38	< 0.0001	6.777–7.589
	Olive oil	7.01 $\pm$ 0.53	< 0.0001	6.456–7.578
	CCl ₄	5.01 $\pm$ 0.18	–	4.824–5.209
	NAC+ CCl ₄	6.65 $\pm$ 0.22	< 0.0001	6.413–6.887
	<i>Tephrosia purpurea</i> 100 mg/kg+ CCl ₄	6.11 $\pm$ 0.13	0.0021	5.977–6.256
	<i>Tephrosia purpurea</i> 200 mg/kg+ CCl ₄	6.40 $\pm$ 0.21	0.0005	6.170–6.630
	<i>Tephrosia purpurea</i> 400 mg/kg+ CCl ₄	6.71 $\pm$ 0.24	< 0.0001	6.456–6.977
	Combination (CCl ₄ + NAC + <i>Tephrosia purpurea</i> 200)	7.05 $\pm$ 0.31	< 0.0001	6.727–7.373

SD: standard deviation. $P > 0.05$: statistically non-significant; $P < 0.05$: statistically significant.

Among all groups, the CCl₄+NAC+*Tephrosia purpurea* combination produced the most substantial improvement in liver function parameters, with values approaching those observed in the negative control group (Tables 3–9).

The effects of the different treatments on liver function biomarkers were evaluated through statistical analysis (Tables 3–9), as illustrated in Figures 2–7.

Discussion

The present study demonstrated that *Tephrosia purpurea* extract exerts significant hepatoprotective effects against CCl₄-induced liver injury in rats, as demonstrated by both histopathological and biochemical findings. In addition, co-administration of *Tephrosia purpurea* with NAC resulted in greater protection than either treatment alone, leading to improved preservation of hepatic architecture and liver function. Notably, the histopathological findings closely paralleled the biochemical results, as reductions in liver injury markers were accompanied by marked improvements in hepatic tissue integrity.

The cross-section of liver tissue from the normal and olive oil groups showed normal hepatic architecture, characterized by intact hepatocytes arranged in regular hepatic cords, normal central veins, and narrow sinusoidal spaces.

Histological analysis revealed an increase in tissue damage after CCl₄ as shown by the presence of rapid coagulation necrosis of hepatocytes adjacent to the central vein (centrilobular necrosis), characterised by shrunken, hyperosinophilic cells exhibiting nuclear loss, condensation, and/or fragmentation, the distribution of injury severity that indicating severe liver damage, exhibited marked histological alterations, including hepatocellular swelling, inflammatory cell infiltration, sinusoidal dilatation, central venous congestion, and focal areas of hepatocellular necrosis.

Furthermore, the findings of this experimental study revealed that pretreatment with NAC was the mean of severity score was mild, show an improvement in liver architecture, reduced inflammatory cell infiltration, mild sinusoidal dilatation, and decreased central vein congestion.

Pretreatment with *Tephrosia purpurea* extract significantly attenuated the severity of liver injury induced by CCl₄ across all tested doses demonstrated by biochemical and histological outcomes that can be attributed to its rich phytochemical composition, including flavonoids, glycosides, and rotenoids.⁹ These compounds possess inherent antioxidant and free radical scavenging properties. These findings are consistent with those reported by Gora et al., who demonstrated that supplementation of *Tephrosia purpurea* extract (500 mg/kg) could ameliorate the hepatotoxic action of arsenic.¹⁶

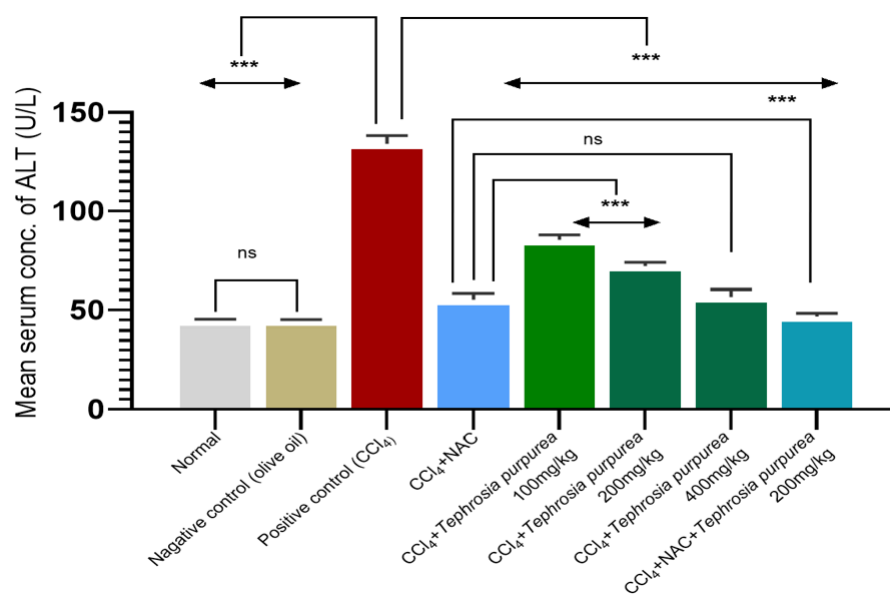


Fig. 2 Comparison of mean serum ALT levels among different experimental groups.

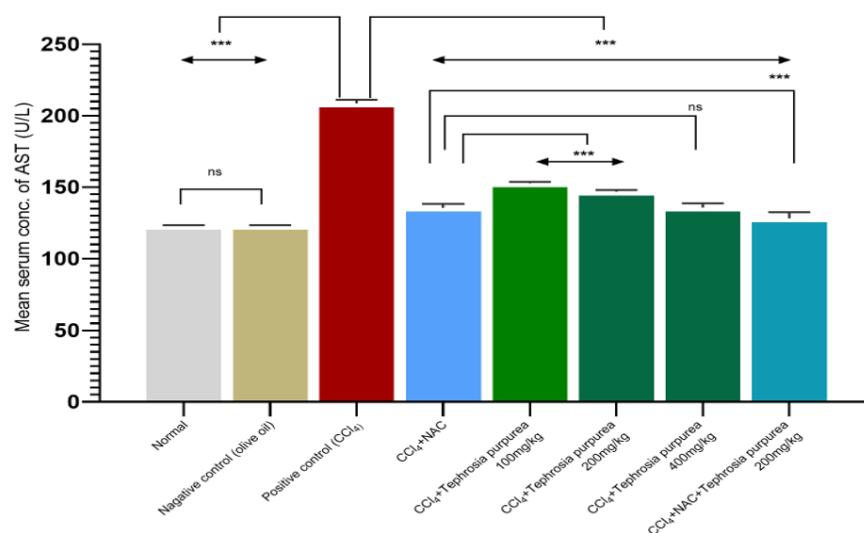


Fig. 3 Comparison of mean serum AST levels among different experimental groups.

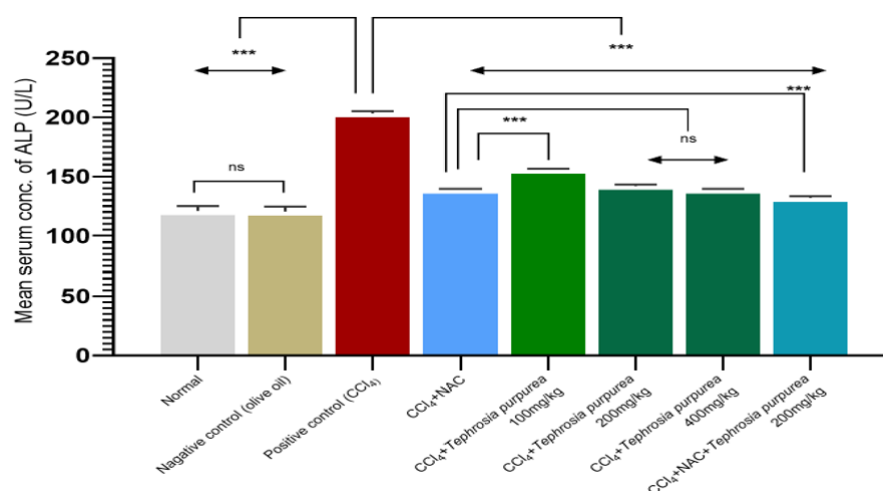


Fig. 4 Comparison of mean serum ALP levels among different experimental groups.

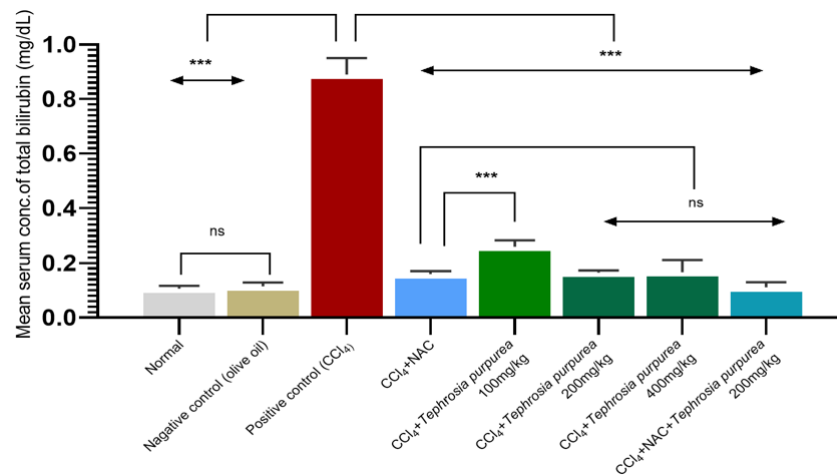


Fig. 5 Comparison of mean serum bilirubin levels across different study groups.

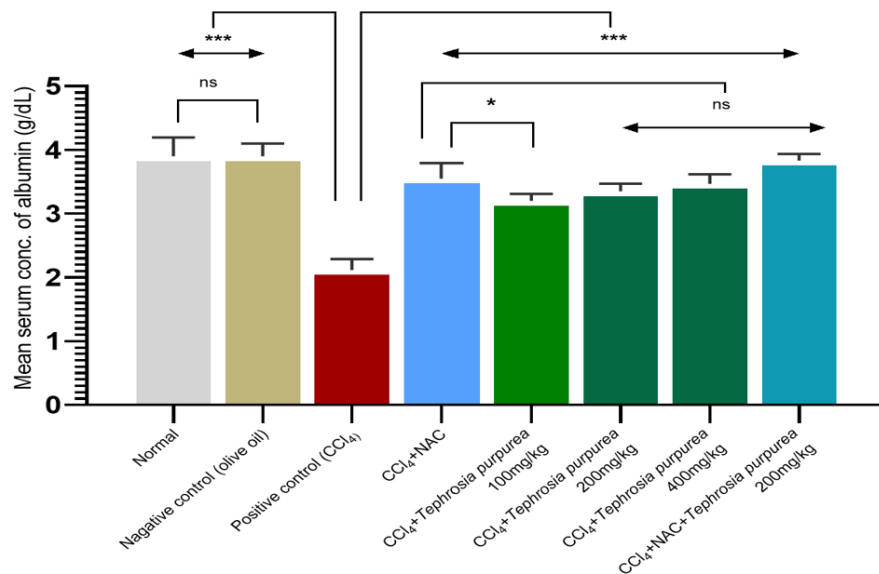


Fig. 6 Comparison of mean serum albumin levels among different study groups.

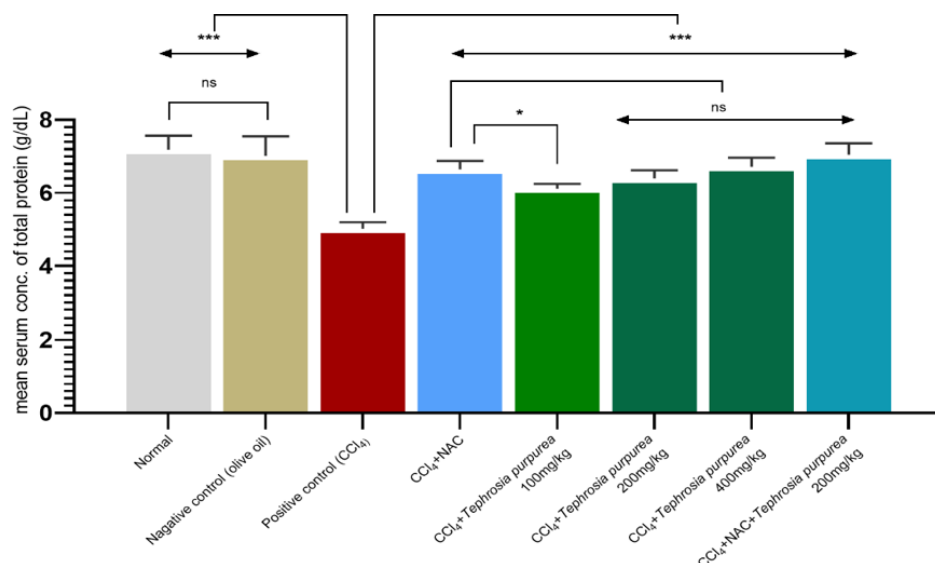


Fig. 7 Comparison of mean serum total protein levels among experimental groups.

These findings are in agreement with those reported by Palbag et al., who studied the hepatoprotective activity of ethanol extract of the roots of *Tephrosia purpurea* against CCl_4 inducing hepatotoxicity in rats. Similarly, oral administration of *Tephrosia purpurea* resulted in a significant decreased biochemical markers of liver damage, and histopathological examination exhibited almost normal architecture, as compared to the CCl_4 -treated group.¹⁰

Furthermore, the degree of histological improvement appeared to be dose-dependent, with higher doses producing more pronounced protective effects. The present results are in agreement with earlier conclusions of Khatri et al., who evaluated the hepatoprotective activity of aerial parts of *Tephrosia purpurea* (100, 300, and 500 mg/kg/day) against thioacetamide-induced hepatotoxicity and stated that histology of the liver sections of the animals treated with the extracts showed a dose-dependent reduction of necrosis.⁸ Also Gupte et al., showed similar result when investigated the hepatoprotective efficacy of ethanolic extract of *Tephrosia purpurea* (100, 200, and 300 mg/kg) against Aflatoxin B1 (AFB1) induced liver injury, *Tephrosia purpurea* concluded that *Tephrosia purpurea* possess hepatoprotective efficacy against AFB1 induced oxidative injury and maximum recovery was seen with 300 mg/kg.¹⁷

However, the pretreatment with *Tephrosia purpurea* demonstrated a dose-dependent reduction in these elevated liver enzyme levels these patterns were consistent with the findings of Desai,¹⁸ who studied the hepatoprotective effects of ethanolic extract of the root of *Tephrosia purpurea* (200 mg/kg, and 400 mg/kg) against thioacetamide-induced hepatotoxicity in experimental Wistar albino rats and concluded that the ethanolic extract of *Tephrosia purpurea* root has a significant protective effect on liver injuries, the 400 mg/kg dose showed a greater reduction in serum enzyme level compare to 200 mg/kg dose.

Notably, the combination of *Tephrosia purpurea* with NAC demonstrated a more pronounced improvement in liver histoarchitecture compared with either treatment alone. This superior efficacy observed in the combination-treated group may be explained by the complementary mechanisms of action of both agents. NAC primarily enhances endogenous antioxidant defenses through glutathione replenishment and free-radical scavenging, whereas *Tephrosia purpurea* provides additional antioxidant and anti-inflammatory effects through its diverse phytochemical constituents. The simultaneous targeting of multiple pathways involved in oxidative stress and hepatic injury may account for the enhanced protection observed in the combination group. Importantly, the combination produced greater hepatoprotection than either intervention administered alone under the conditions of the present study.

From a translational perspective, these findings suggest that combining a clinically established antioxidant such

as NAC with a bioactive medicinal plant extract may represent a promising multi-target approach for mitigating liver injury associated with oxidative stress. Such an approach may enhance therapeutic efficacy while potentially reducing the dependence on higher doses of individual agents. Nevertheless, further preclinical and clinical investigations are required before any therapeutic recommendations can be made.

Despite the encouraging findings, several limitations should be acknowledged. First, the study was conducted exclusively in male Wistar rats using a single experimental model of hepatotoxicity, which may limit the generalizability of the results to other species, sexes, or clinical settings. Second, the specific phytochemical constituents responsible for the observed hepatoprotective effects were not isolated or quantified. Third, mechanistic investigations involving oxidative stress biomarkers, inflammatory mediators, and molecular signaling pathways were not performed. Finally, long-term safety and chronic toxicity assessments of the extract were beyond the scope of the present study. Therefore, future studies should focus on elucidating the underlying molecular mechanisms, characterizing the active constituents, evaluating different dose combinations, and assessing long-term efficacy and safety in additional experimental models.

Conclusion

The present study demonstrated that *Tephrosia purpurea* extract produced significant dose-dependent hepatoprotective effects against CCl_4 -induced hepatotoxicity in rats. Furthermore, co-administration of *Tephrosia purpurea* with N-acetylcysteine resulted in greater protection than either treatment alone, as evidenced by improvements in both biochemical and histopathological parameters. These findings suggest that the combination of NAC and *Tephrosia purpurea* may represent a promising therapeutic strategy for the management of oxidative stress-related liver injury and warrants further mechanistic and clinical investigation.

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Conflict of Interest

None to declare. ■

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