

Ameliorative role of aqueous leaf extract of *Euphorbia hirta* against carbon tetrachloride-Induced hepatotoxicity in Wistar albino rats

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Abstract

Liver diseases constitute a major global health challenge, accounting for approximately two million deaths annually, with etiologies ranging from viral hepatitis and alcohol abuse to drug-induced hepatotoxicity. The limitations of conventional hepatoprotective therapies, including high costs and adverse effects, have necessitated the exploration of medicinal plants as alternative therapeutic agents. *Euphorbia hirta* has a long history of traditional use in the management of hepatic disorders; however, its therapeutic potential against chemically-induced liver damage requires scientific validation. This study was therefore designed to investigate the ameliorative effect of the aqueous leaf extract of *E. hirta* on carbon tetrachloride (CCl₄)-induced hepatotoxicity in albino Rats, alongside its phytochemical composition and acute toxicity profile. Thirty-six (36) Wistar albino rats were randomly divided into five groups. Hepatotoxicity was induced by intraperitoneal administration of CCl₄ (1:1 in olive oil, 1 mL/kg). Group I served as the normal control (received normal saline), while Group II served as the disease control (received CCl₄ only). Groups III, IV, and V received daily oral treatment with the standard drug (Silymarin, 100 mg/kg), low-dose *E. hirta* extract (100 mg/kg), and high-dose *E. hirta* extract (300 mg/kg), respectively. Serum levels of liver function biomarkers: Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), and Total Bilirubin—were assessed, and liver tissues were examined histopathologically. Acute toxicity (LD₅₀) was determined following OECD guidelines, and preliminary phytochemical screening was conducted using standard protocols. The acute toxicity test revealed that the aqueous leaf extract of *E. hirta* was safe, with no mortality observed at a dose of 5000 mg/kg, indicating an LD₅₀ greater than 5000 mg/kg. Induction of liver damage significantly ($P < 0.05$) increased serum AST, ALT, ALP and serum total bilirubin while administration of the standard drug and *E. hirta* to rats significantly ($p < 0.05$) reduced it. Dose and time dependent responses were found in the normalization of the liver function indices. Histopathological examination further corroborated the biochemical findings; livers of CCl₄-treated rats exhibited severe hepatocellular necrosis, fatty infiltration, and inflammatory cell aggregation, whereas treatment with *E. hirta* extract markedly attenuated these lesions, with the high dose demonstrating near-normal hepatic architecture. In conclusion, the overall results showed that aqueous leaf extract of *Euphorbia hirta* has phytochemical components that can be used in treating liver damages.

Keywords: *Euphorbia hirta*, hepatoprotective, carbon tetrachloride, liver function tests, histopathology, phytochemicals

Introduction

Liver diseases represent a significant and escalating global health burden, contributing substantially to morbidity and mortality worldwide (Alolayan *et al.*, 2023) [2]. The liver, being the principal site for biotransformation, detoxification, and metabolic regulation, is continuously exposed to endogenous and exogenous insults, making it highly vulnerable to damage (Fareed *et al.*, 2024) [7]. The etiologies of hepatic disorders are diverse, encompassing viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), drug-induced liver injury, and metabolic syndromes (Bean *et al.*, 2024) [4]. NAFLD prevalence has risen significantly across every region of the world between 1990 and 2019 (Younossi *et al.*, 2020) [19], with these conditions collectively accounting for approximately two million deaths annually (Paik *et al.*, 2023). The study of hepatotoxicity has been greatly facilitated by carbon tetrachloride (CCl₄), a potent hepatotoxin that induces liver damage closely resembling human hepatic injury (Chalasaini *et al.*, 2018), characterized by marked elevations in serum AST, ALT, and ALP, alongside increased

bilirubin, decreased total protein, and histopathological changes including necrosis, steatosis, and inflammation (Fareed *et al.*, 2024) [7]. Currently available synthetic hepatoprotective drugs are limited by high costs, suboptimal efficacy, and adverse effects, necessitating exploration of natural alternatives (Qadri *et al.*, 2025). Medicinal plants have historically served as cornerstones in managing hepatic disorders (Majee *et al.*, 2023) [12], with approximately 80% of the global population relying on traditional medicine for primary health needs. Numerous plants demonstrate hepatoprotective properties through bioactive compounds including alkaloids, glycosides, flavonoids, tannins, and phenolics, which exert antioxidant, anti-inflammatory, and membrane-stabilizing effects (Kumar *et al.*, 2022) [9]. *Euphorbia hirta* (Euphorbiaceae), commonly known as asthma weed, is widely distributed across tropical regions and has been traditionally used for gastrointestinal, respiratory, and inflammatory disorders (Dharman *et al.*, 2023). Recent studies have demonstrated that *E. hirta* contains significant quantities of phenolic compounds (107.3 mg GAE/g) and flavonoids (22.9 mg QE/g) (Kumar

et al., 2022). Balasubramanian *et al.* (2022) ^[9] reported that ethanolic extracts of *E. hirta* (300 mg/kg) exhibited hepatoprotective effects in CCl₄-induced Wistar rats.

Materials and Methods

1. Chemicals, Reagents and Apparatus

All chemicals, reagents and materials used in this research were of analytical grade and were collected and used without any further purification:

2. Plant sample collection and extraction

Fresh leaf of *Euphorbia hirta* was collected from different parts of Bauchi State, Nigeria. The plant sample was authenticated at the herbarium section, Department of Science Laboratory Technology, Abubakar Tatari Ali Polytechnic, Bauchi, Nigeria. The collected plant sample was sorted to remove extraneous materials and washed to remove dirt. The leaf was allowed to dry under shade for two weeks and grinded in to a fine powder using electric blender and extracted using Soxhlet apparatus. The powder was weighed and soaked in distilled water for 24 hours, the mixture was filtered using whatman No. 1 filter paper and the residue dried and re-weighed. And then stored in air - tight containers until required.

3. Experimental Animals

The experimental animals (36 rats) weighing between 150-200 g were purchased from the animal house of Biological Sciences Department, University of Jos, Plateau State and transported to Abubakar Tatari Ali Polytechnic Bauchi. The rats were kept in a well-ventilated laboratory cages under hygienic conditions and natural light/dark cycle to acclimatize to the animal house for two weeks with free access to standard feed and portable drinking water before the commencement of the experiment.

4. Preliminary Phytochemical screening

The aqueous leaf extract of *E. hirta* was subjected to preliminary phytochemical screening using standard qualitative and quantitative protocol as described by Lakache (2016) ^[10]. The phytochemicals analyzed were alkaloids, flavonoids, phenols, tannins, saponins, cardiac glycosides, cyanogenic glycosides and terpenes/steroids.

5. Acute Toxicity (LD50) Determination

The LD₅₀ was determined as per OECD guidelines-420 (2001) ^[15], where 12 rats were divided into four groups of three rats each (Three animals were used for each step). The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to the start of dosing to allow for acclimatization to the laboratory conditions. The groups of rats were administered with stepwise graded doses of 50, 300, 2000 and 5000 mg/kg body weight. Starting with the lowest dose of 50 mg/kg, the rats were administered with increasing dose and observed for mortality and general behavior after each dose, until they were finally administered with 5000 mg/kg of the extract.

6. Experimental Design

A total of 36 rats were divided into five groups (1 to 5), with 9 rats each in 1 and 2, and 6 rats each in groups 3, 4 and 5. Liver toxicity was induced in groups 2 – 5.

Group 1: Normal rats (Not administered CCl₄ and not treated).

Group 2: Test control (administered CCl₄, and not treated)

Groups 3 and 4: (Administered CCl₄ and treated orally with aqueous leaf extract of *E. hirta* at 100mg and 300 mg/kg, respectively).

Group 5: Administered CCl₄ and treated with the standard drug livolin at dose of 10 mg/kg.

Three rats were removed from groups 1 and 2, 48 hours after CCl₄ administration and humanely sacrificed for blood sample to confirm the inducement of liver damage. The Rats in the treatment groups were treated for a total of four weeks. However, three rats were removed from each group after the first two weeks of treatment, sacrificed and blood and liver samples collected for analysis. The remaining rats were sacrificed after 4 weeks blood sample and liver samples were collected.

7. Biochemical analysis of Liver function markers

Liver function indices were determined enzymatically using Randox kits According to the manufacturer's instruction. Aspartate Amino transferase and Alanine Amino transferase were determined as described by Reitman and Frankel (1957), Total protein by Tietz (1995) ^[16, 18], conjugated and direct bilirubin by Jendrassik and Grof (1938) and albumin by Doumas *et al.*, (1971) ^[6].

8. Histopathological Analysis of Liver Tissues

Histopathological studies of liver tissues was carried out using a standard laboratory procedure according to the method described by Kiernan (2008) ^[8].

Results and discussions

1. Phytochemical analysis

Results of the qualitative and quantitative phytochemicals analysis of the aqueous leaf extract of *Euphorbia hirta* revealed the presence of five (5) out of the seven phytochemicals tested. Tannins, phenols, cardiac glycosides, alkaloids and steroids were present while flavonoids and saponins were absent (Table 1). The extract was found to have high concentrations of glycosides (8.95g) followed by phenols (7.25g) and then alkaloids (4.27g). The concentration of tannins (0.56g) and steroids (0.15g) were very low. The predominance of glycosides and phenols in the extract is consistent with recent GC-MS studies on *E. hirta* which identified glycosides as major bioactive compounds, and aligns with findings reporting significant phenolic content in aqueous extracts of the plant (Kumar *et al.*, 2022; Rodrigues *et al.*, 2024) ^[9, 17]. These findings are consistent with recent ethnopharmacological studies validating the traditional use of water-based preparations of *E. hirta* for treating hepatic disorders and support the development of standardized phytotherapeutic formulations from this medicinal plant (Kumar *et al.*, 2022) ^[9].

Table 1: Phytochemical screening of the aqueous leaf extract of *Euphorbia hirta*

Constituent	Concentration (g/100g)
Flavonoids	ND
Saponins	ND
Alkaloids	4.27 ± 0.57
Glycosides	8.95 ± 0.03
Phenols	7.25 ± 0.05
Tannins	0.56 ± 0.08
Steroids	0.15 ± 0.03

Key: ND = not detected

2. Acute Toxicity

The extract up to a dose of 5000 mg/kg weight did not cause any death after 72 h treatment in all rats. Also behavioral changes in skin and fur, eyes, mucus convulsion, salivation,

diarrhea and lethargy were not observed in treated groups. The absence of death at all doses up to 5000 mg/kg shows that the LD₅₀ of the aqueous leaf extract of *Euphorbia hirta* is above 5000 mg/kg body weight. (Table 2).

Table 2: Acute toxicity of *Euphorbia hirta* aqueous leaf extract in rats

Groups	Doses (mg/Kg)	Number of rats	Mortality
1	50	3	0/3
2	300	3	0/3
3	2000	3	0/3
4	5000	3	0/3

3. Effect of aqueous leaf extract of *Euphorbia hirta* on CCl₄ induced liver damage.

Administration of carbon tetrachloride significantly ($p < 0.05$) elevated serum liver function indices in test control rats compared.

Normal controls, consistent with the established hepatotoxic mechanism of CCl₄, which disrupts hepatocellular membrane integrity, leading to enzyme leakage and impaired hepatic synthetic and excretory functions (Fareed *et al.*, 2024) [7].

Table 3: Effect of aqueous leaf extract of *E. hirta* on liver function indices of CCl₄-induced liver damaged rats.

Group/ Treatment		ALT (u/l)	AST (u/l)	ALP (u/l)	T.Bilirubin (mg/dl)	D.Bilirun (mg/dl)	T.Protein (g/dl)	Albumin (g/dl)
Group I Normal Control	2 wks	18.30±0.25	20.12±0.42 ^{ab}	173.53±0.41 ^a	2.08±0.08 ^a	1.73±0.02 ^a	6.76±0.20 ^a	3.89±0.30
	4 wks	18.36±0.19 ^{ab}	19.92±0.59 ^{ab}	173.0±0.82 ^{ab}	2.13±0.52 ^{ab}	1.74±0.01 ^a	6.71±0.23 ^{ab}	3.74±0.08 ^{ab}
Group II Test Control	2 wks	102.80±0.67 ^{*b}	96.15±1.27 ^{*b}	178.50±1.91 ^{*b}	7.04±0.16 ^{*b}	1.07±0.01 ^{*b}	2.12±0.02 ^{*b}	1.03±0.43 ^{*b}
	4 wks	92.0±2.45 ^{*b}	88.5±2.52 ^{*b}	161.5±1.91 ^{*b}	6.80±0.01 ^{*b}	1.11±0.08 ^{*b}	3.84±0.02 ^{*b}	1.95±0.05 ^{*b}
Group III 100 mg/kg Extract	2 wks	154.30±1.88 ^{*ab}	98.20±1.56 ^{*b}	164.75±3.30 ^{*ab}	7.02±0.86 ^{*b}	1.09±0.014 ^{*b}	1.35±0.06 ^{*b}	0.9±0.2 ^{*ab}
	4 wks	81.63±2.81 ^{*ab}	87.0±0.25 ^{*b}	155.50±2.38 [*]	6.15±0.19 ^{*ab}	1.19±0.01 ^{*b}	3.93±0.03 ^{*b}	2.02±0.03 ^{*b}
Group IV 300mg/Kg Extract	2 wks	84.18±1.75 ^{*ab}	77.50±0.38 ^{*ab}	171.0±0.10 ^a	6.54±0.47 ^{*ab}	1.16±0.03 ^{*ab}	4.52±0.02 ^{*ab}	2.1±0.2 ^{*ab}
	4 wks	70.89±0.87 ^{*ab}	68.0±0.14 ^{*ab}	159±0.96 ^{*ab}	5.02±0.07 ^{*ab}	1.62±0.02 ^{*ab}	4.72±0.02 ^{*ab}	2.59±0.02 ^{*ab}
Group V Standard Control (Livolin 10mg/Kg)	2 wks	42.40±0.87 ^{*a}	33.37±0.31 ^{*a}	172.95±0.10 ^a	2.24±0.05 ^a	1.76±0.06 ^a	5.70±0.35 ^{*a}	2.6±0.1 ^{*a}
	4 wks	48.50±1.22 ^{*a}	40.13±0.25 ^{*a}	155.0±0.82 ^{*a}	2.90±0.01 ^{*a}	1.82±0.01 ^a	4.95±0.01 ^{*a}	2.88±0.01 ^{*a}

Values are presented as mean ± SD, n= 3. Values with *, a, b Statistically different down the group at (P<0.05)

- Statistically different compared to normal control
- a. Statistically different compared to test control
- b. Statistically different compared to standard control

2 wks: after two weeks treatment, 4 wks: after four weeks treatment, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase and ALP: Alkaline Phosphatase

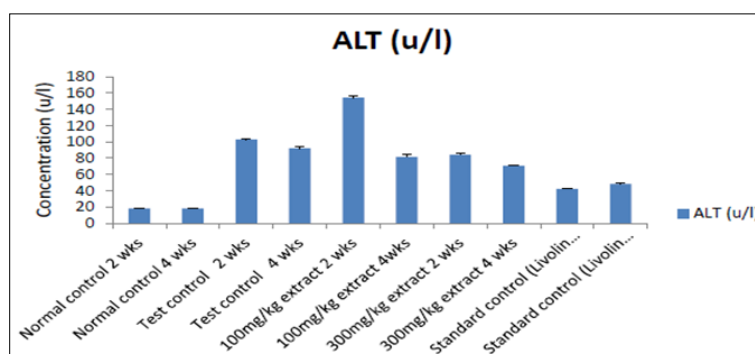


Fig 2: Effect of aqueous leaf extract of *E. hirta* on CCl₄ induced liver damage on serum ALT levels

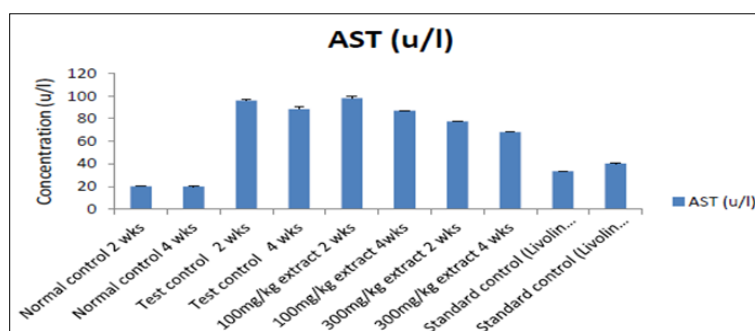


Fig 3: Effect of aqueous leaf extract of *E. hirta* on CCl₄ induced liver damage on serum AST levels

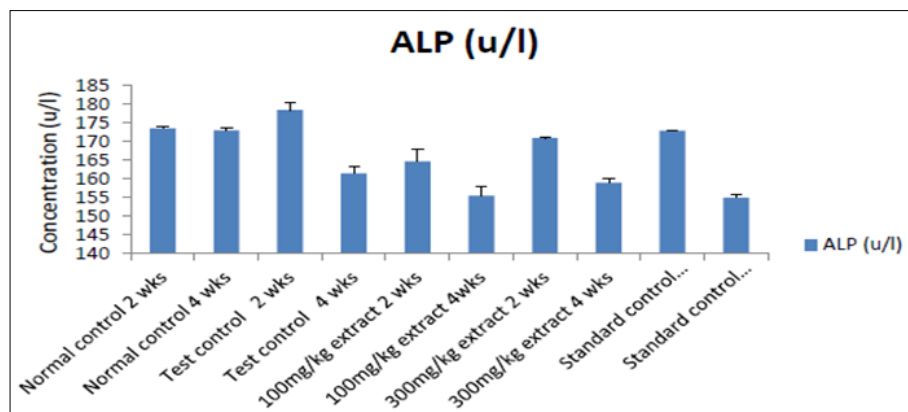


Fig 4: Effect of aqueous leaf extract of *E. hirta* on CCl₄ induced liver damage on serum concentration of direct bilirubin levels

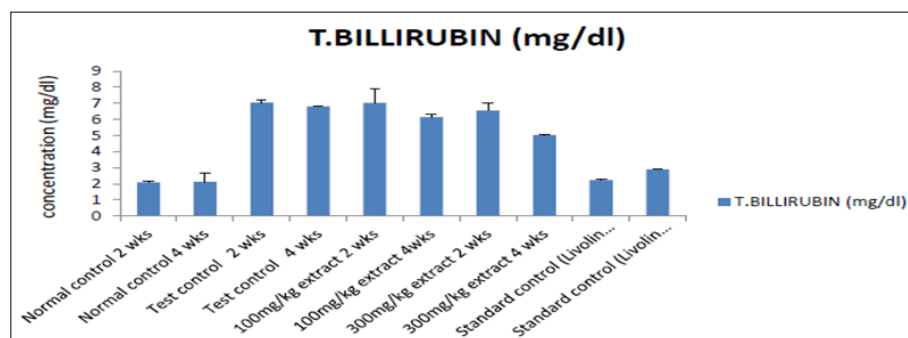


Fig 5: Effect of aqueous leaf extract of *E. hirta* on CCl₄ induced liver damage on serum concentration of total bilirubin levels

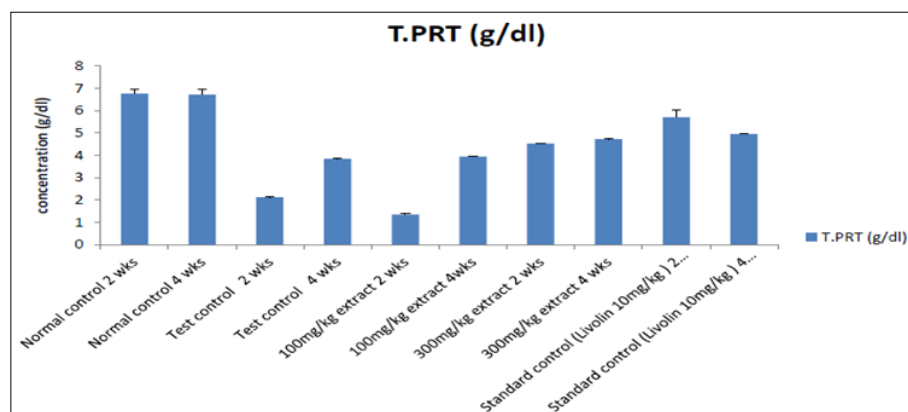


Fig 6: Effect of aqueous leaf extract of *E. hirta* on CCl₄ induced liver damage on serum concentration of total protein levels

4. Histopathology of the liver

The histopathological examination of liver tissues provided compelling morphological evidence supporting the biochemical findings. The normal control rats exhibited unremarkable hepatic architecture with well-preserved hepatocytes, central veins, and sinusoidal spaces (Plate 1). In contrast, the livers of CCl₄-treated (test control) rats displayed pathological alterations, including areas of fibrosis, vascular congestion, and extensive hepatocellular damage (Plate 2), confirming the potent hepatotoxic effect of CCl₄ (Fareed *et al.*, 2024) [7]. Following two weeks of treatment, rats receiving the (100 mg/kg) of *E. hirta* still exhibited areas of inflammation, fibrosis, and necrosis (Plate 3), whereas those treated with (300 mg/kg) and Livolin (10 mg/kg) showed unremarkable liver tissue comparable to the normal control (Plates 4 and 5). After four weeks of treatment, rats given 100 mg/kg of the extract continued to show areas of cytoplasmic vacuolation (Plate 6), while those receiving 300 mg/kg of the extract and Livolin

demonstrated near-normal hepatic architecture (Plates 7 and 8).

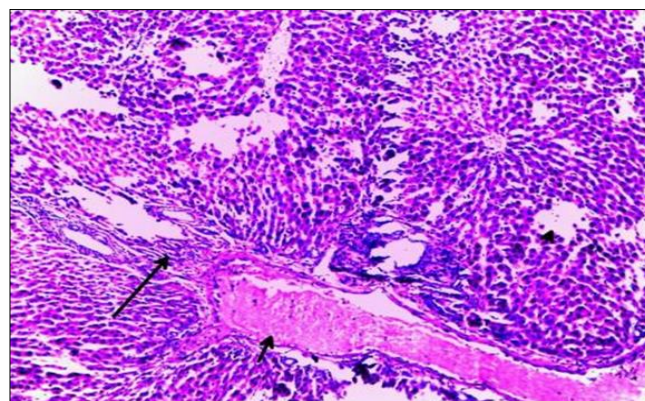


Plate I: histopathological examination of liver of normal control rats. Show remarkable liver tissues (X 100)

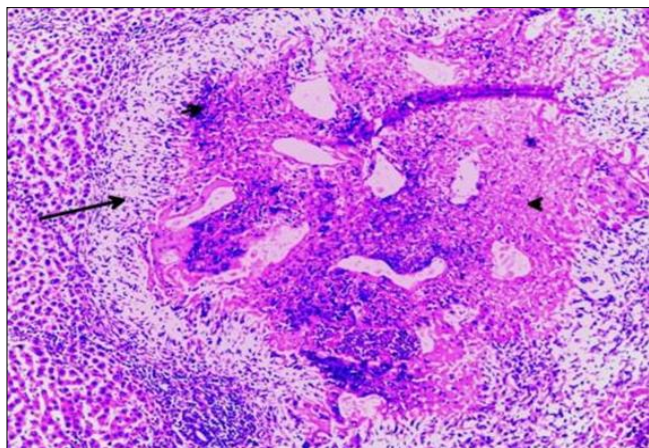


Plate II: Histopathological examination of carbon-tetrachloride – induced liver damage of rats in the untreated test group. The black arrows shows areas of fibrosis, vascular congestion and liver damaged (X 100)

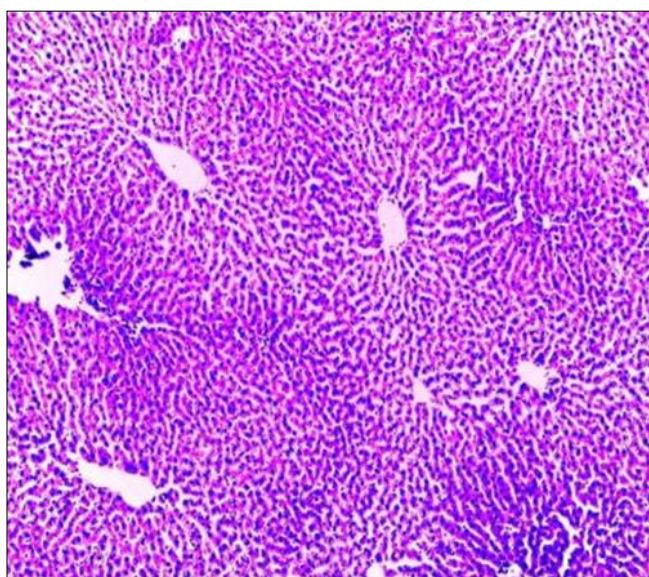


Plate III: Histopathological examination of livers of rats treated with 300 mg/kg of *Euphorbia hirta* for two weeks after CCl₄-induced liver damage. The black arrow shows unremarkable liver tissue (X 100)

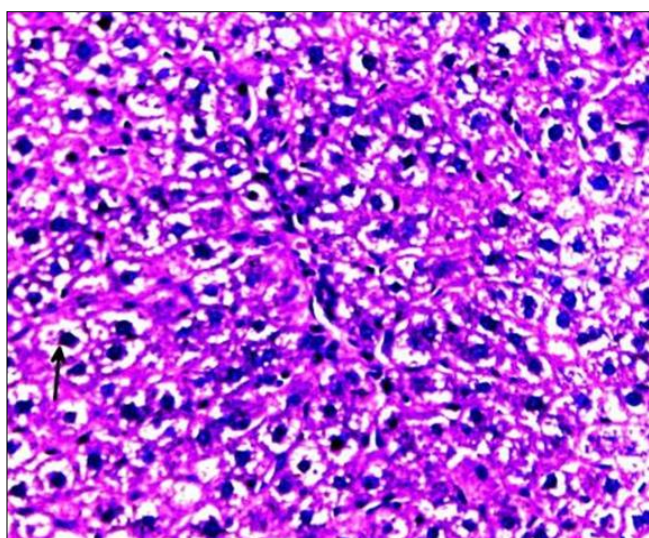


Plate IV: Histopathological examination of livers of rats treated with drug livolin for two weeks after CCl₄-induced liver damage. The black arrow shows unremarkable liver tissue (X 100)

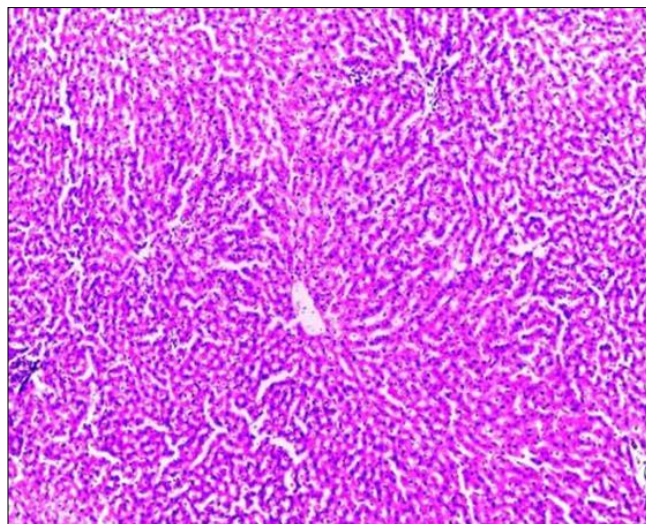


Plate V: Histopathological examination of livers of rats treated with 100 mg/kg of *Euphorbia hirta* for four weeks after CCl₄-induced liver damage. The black arrow shows areas of cytoplasmic vacuolation (X 100)

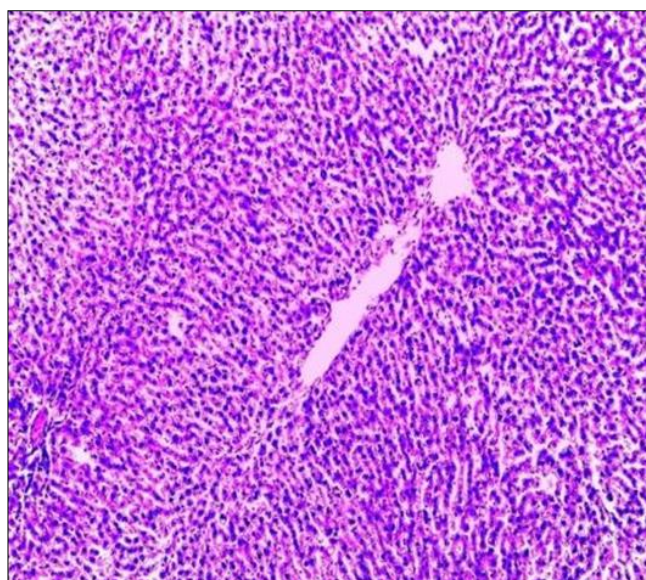


Plate VI: Histopathological examination of livers of rats treated with 300 mg/kg of *Euphorbia hirta* for four weeks after CCl₄-induced liver damage. The black arrow shows unremarkable liver tissue (X 100)

Plate VIII: Histopathological examination of livers of rats treated with drug livolin for four weeks after CCl₄-induced liver damage. The black arrow shows unremarkable liver tissue (X 100)

Conclusion

The findings of this study demonstrate that the aqueous leaf extract of *Euphorbia hirta* possesses significant therapeutic potential against CCl₄-induced hepatotoxicity in albino rats, as evidenced by the dose- and time-dependent normalization of serum liver function biomarkers (AST, ALT, ALP, bilirubin, total protein, and albumin) and the restoration of near-normal hepatic histoarchitecture. The acute toxicity study confirmed the safety of the extract (LD₅₀ > 5000 mg/kg), supporting its traditional ethnopharmacological use in the management of liver disorders and positioning *E. hirta* as a promising candidate for the development of safe, effective, and affordable phytotherapeutic agents.

Recommendations

Further studies are recommended to isolate and characterize the specific bioactive compounds responsible for the hepatoprotective activity, elucidate the underlying molecular mechanisms through investigation of inflammatory cytokines and signaling pathways, and conduct chronic toxicity studies to establish the long-term safety profile. Additionally, well-designed clinical trials are warranted to evaluate the efficacy and safety of standardized *E. hirta* formulations in human patients with liver diseases, alongside the development of quality-controlled phytotherapeutic products for clinical use.

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