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In Vitro and In Vivo Hepatoprotective Evaluation of A Bioactive Fraction of Euphorbia Fusiformis Against Carbon Tetrachloride-Induced Hepatic Injury

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ABSTRACT

Background: Euphorbia fusiformis has ethnomedicinal relevance in liver disorders, and published work has demonstrated hepatoprotective activity of its tuber extract. The present study evaluates a bioactive fraction using complementary HepG2 and Wistar-rat models of carbon tetrachloride (CCl4)-induced injury. Methods: HepG2 cells were exposed to CCl4 and treated with n-butanol, aqueous or chloroform fractions (50-200 µg/mL); silymarin served as reference. Cell viability was assessed by MTT assay. Acute oral toxicity was examined over 5-2000 mg/kg. In vivo hepatoprotection was evaluated in male Wistar rats by serum ALT, AST and total bilirubin after CCl4 challenge, with test extract at 50 and 100 mg/kg and silymarin at 100 mg/kg. Results: CCl4 reduced HepG2 viability to 53.06%. The n-butanol fraction showed the strongest source-reported protection, with 94.42%, 92.00% and 97.701% viability at 50, 100 and 200 µg/mL, respectively. No overt toxicity signs were reported up to 2000 mg/kg. In rats, CCl4 increased ALT, AST and bilirubin to 110.36±1.13 U/L, 86.56±2.41 U/L and 1.51±1.36 mg/dL. Test extract at 100 mg/kg reduced these values to 51.89±1.79 U/L, 47.56±2.12 U/L and 0.59±1.74 mg/dL, approaching the silymarin group. Conclusion: The investigated fraction demonstrated substantial protection against CCl4-associated hepatic injury.

KEYWORDS: Euphorbia Fusiformis; Hepatoprotection; Hepg2; Carbon Tetrachloride; N-Butanol Fraction; Alt; Ast; Bilirubin; Mtt Assay.

INTRODUCTION

Chemical-induced liver injury is frequently accompanied by oxidative stress, membrane damage and loss of hepatocyte function. Carbon tetrachloride is a classical experimental hepatotoxicant whose reactive metabolites initiate lipid peroxidation and cellular injury. HepG2-based assays are useful for screening candidate hepatoprotective agents before animal evaluation, while serum aminotransferases and bilirubin remain practical biochemical indices of hepatic injury in vivo [1-3].

Euphorbia fusiformis Buch.-Ham. ex D.Don is an ethnomedicinal Euphorbiaceae species used traditionally for liver-related disorders. Anusuya et al. reported that ethanolic tuber extract protected Wistar rats from rifampicin-induced hepatic damage and showed a favorable toxicological profile, providing species-specific precedent for hepatoprotective investigation [4].

The present work was designed as a second, pharmacology-focused study complementary to phytochemical characterization. Its distinctive feature is the sequential comparison of solvent fractions in a CCl4-injured HepG2 model, selection of the most promising fraction, acute safety assessment and confirmation of hepatoprotection in a CCl4-challenged rat model.

MATERIALS AND METHODS

2.1 Test material and fractionation

E. fusiformis plant material was extracted and partitioned to obtain n-butanol, aqueous and chloroform fractions. authenticated the material, and a voucher specimen was deposited at MGV's Loknete Vyankatrao Hiray Science College, Nashik (authentication no. LH/PG/BOT-798; 17 February 2024).

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2.2 HepG2 cell culture and treatment design

HepG2 cells were maintained in 96-well culture plates. The supplied protocol describes treatment with serum-free DMEM, DMSO vehicle, CCl4 toxicant, silymarin reference and extract fractions. Fraction concentrations of 50, 100 and 200 µg/mL were evaluated for 24 h in the CCl4 injury model. The supplied document contains inconsistent expressions of CCl4 concentration (100 mM, 1% v/v and 1 mM in different sections).

2.3 MTT cell-viability assay

After treatment, MTT solution (5 mg/mL; 20 µL) was added and plates were re-incubated for 3 h under 5% CO2 at approximately 37 °C. Formazan crystals were dissolved and absorbance was recorded at 540 nm. Untreated cells were taken as 100% viability. Experiments were reported in triplicate. The biological principle of the assay is the conversion of tetrazolium substrate to formazan by metabolically active cells [1,2].

2.4 Acute oral toxicity

Male Wistar albino rats (150-200 g) were used. The supplied protocol states oral doses of 5, 50, 300 and 2000 mg/kg and observation for behavioral or physiological toxicity over 14 days in accordance with an OECD 423-type acute toxic class approach.

2.5 CCl4-induced hepatotoxicity in rats

Animals were allocated to normal control, CCl4 control, silymarin (100 mg/kg, oral) and test-extract groups (50 and 100 mg/kg, oral). Serum ALT, AST and total bilirubin were measured as biochemical indicators of liver injury. The exact CCl4 vehicle.

2.6 Statistical analysis

The source reports values as mean±SEM and indicates n=3 for the cell-line experiment, with significance thresholds of $p<0.05$, $p<0.01$ and $p<0.001$ versus toxicant control.

RESULTS

3.1 Protective effect in CCl4-injured HepG2 cells

CCl4 exposure reduced source-reported cell viability from 100% in untreated controls to 53.06%. Among the tested fractions, the n-butanol fraction produced the strongest protective profile: 94.42%, 92.00% and 97.701% viability at 50, 100 and 200 µg/mL, respectively. The aqueous fraction yielded reported viabilities of 81.00%, 88.35% and 87.721%, whereas the chloroform fraction showed 68.34%, 32.41% and 43.82%. Silymarin produced 58.20%, 59.80% and 65.59% viability across the same concentrations.

Table 1. Source-reported HepG2 viability data after CCl4 injury.

Treatment	Concentration (µg/mL)	Mean OD	Reported viability (%)
Control	-	0.2435	100
CCl4	-	0.1292	53.06
n-Butanol + CCl4	50	0.2299	94.42
n-Butanol + CCl4	100	0.2225	92.00
n-Butanol + CCl4	200	0.2379	97.701
Aqueous + CCl4	50	0.1636	81.00
Aqueous + CCl4	100	0.2571	88.35
Aqueous + CCl4	200	0.2136	87.721
Chloroform + CCl4	50	0.1664	68.34
Chloroform + CCl4	100	0.1378	32.41
Chloroform + CCl4	200	0.1811	43.82
Silymarin	50	0.1417	58.20
Silymarin	100	0.1456	59.80
Silymarin	200	0.1597	65.59

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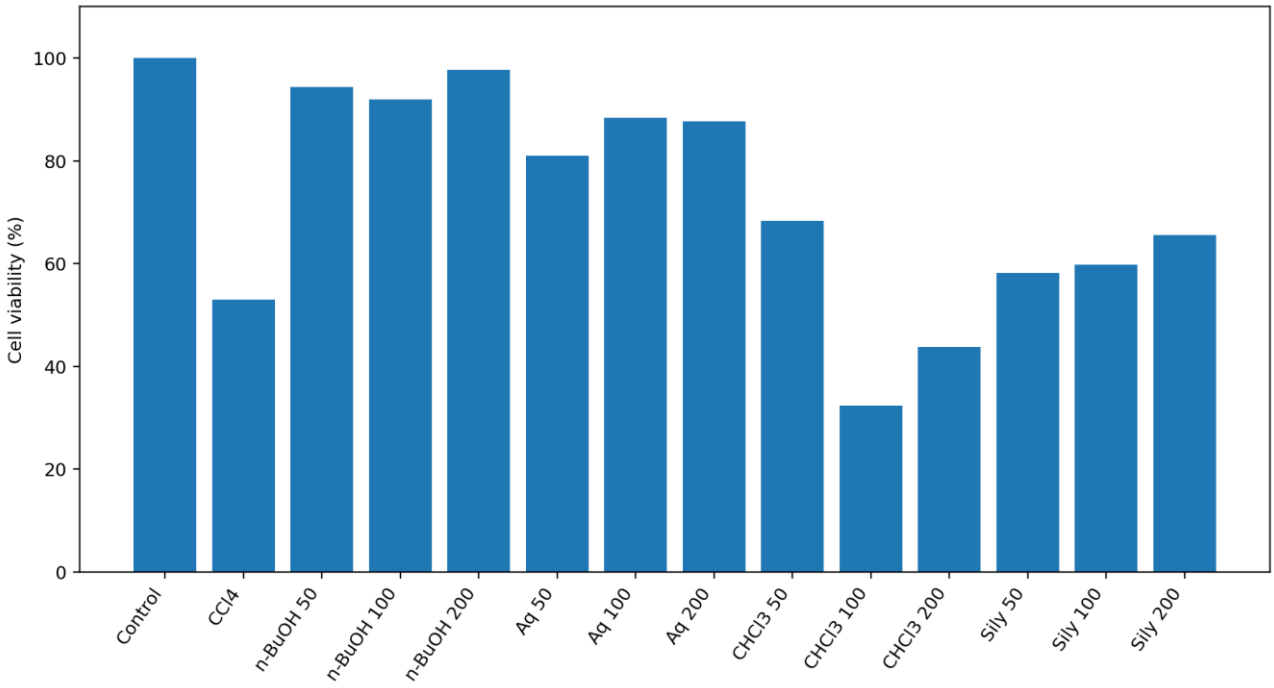

Figure 1. Viability of HepG2 cells across fractions and concentrations after CCl4 challenge.

3.2 Acute oral toxicity

No overt signs of toxicity were reported at 5, 50, 300 or 2000 mg/kg during the observation period.

3.3 Effect on serum hepatic biomarkers

CCl4 challenge markedly increased ALT, AST and total bilirubin relative to normal control. At 100 mg/kg, the test extract lowered ALT to 51.89±1.79 U/L, AST to 47.56±2.12 U/L and total bilirubin to 0.59±1.74 mg/dL. The 50 mg/kg dose produced intermediate values. Silymarin (100 mg/kg) yielded ALT 45.41±1.45 U/L, AST 39.64±1.57 U/L and bilirubin 0.63±0.97 mg/dL.

Table 2. Serum hepatic biomarkers in the CCl4-induced rat model(source-reported mean±SEM).

Group	ALT (U/L)	AST (U/L)	Total bilirubin (mg/dL)
Control	35.45±2.64	25.36±2.45	0.41±0.02
CCl4 (1 mL/kg)	110.36±1.13	86.56±2.41	1.51±1.36
Silymarin 100 mg/kg	45.41±1.45	39.64±1.57	0.63±0.97
Test extract 50 mg/kg	65.17±1.23	67.36±1.91	0.89±0.61
Test extract 100 mg/kg	51.89±1.79	47.56±2.12	0.59±1.74

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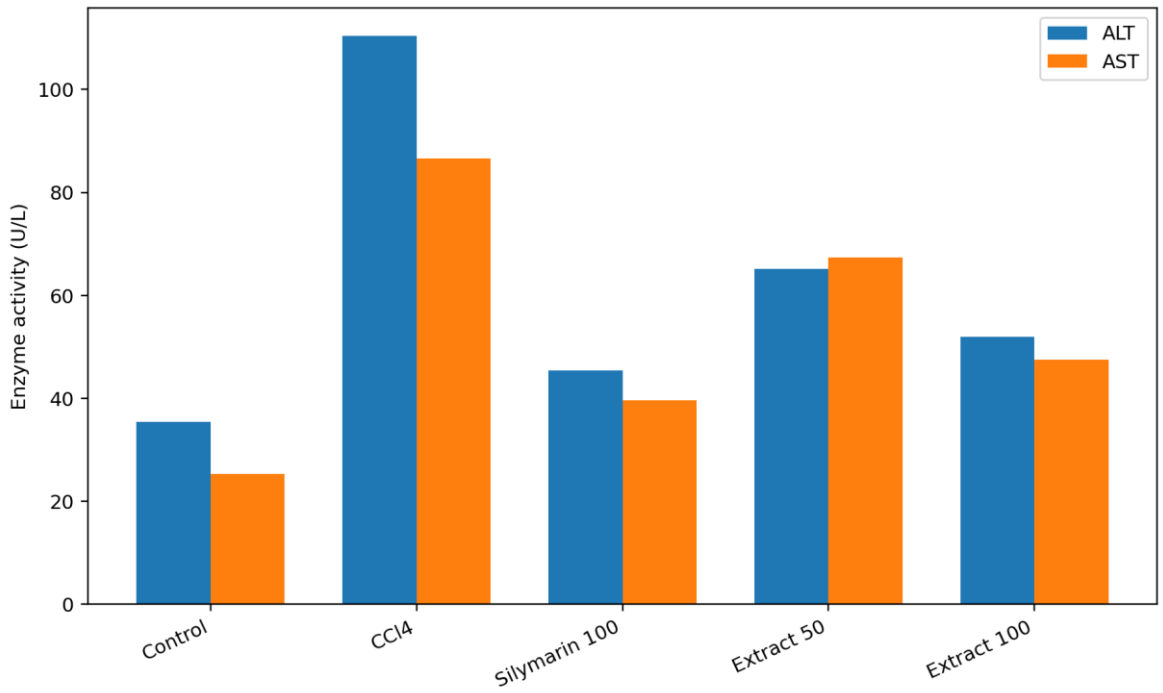

Figure 2. ALT and AST values across experimental groups.

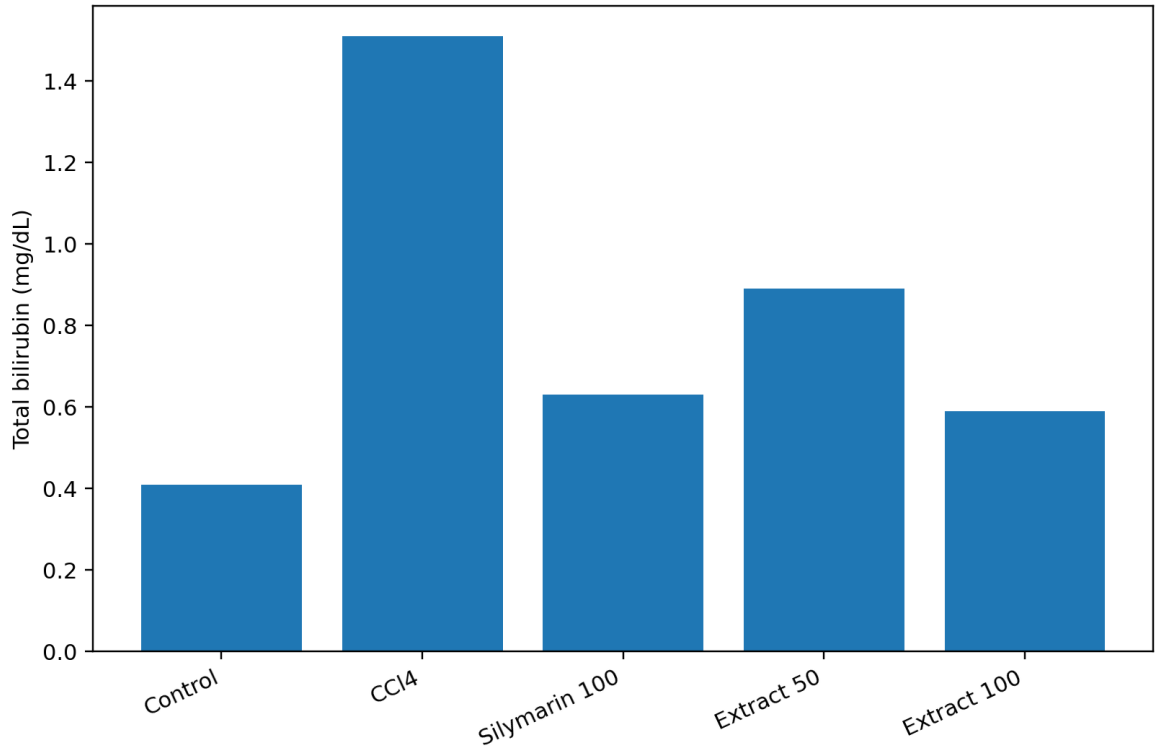

Figure 3. Total bilirubin values across experimental groups.

DISCUSSION

The combined cell-line and animal findings indicate a hepatoprotective signal in the supplied dataset. CCl4 substantially reduced HepG2 viability, while the n-butanol fraction restored source-reported viability to above 90% at all tested concentrations and reached 97.701% at 200 µg/mL. CCl4 is widely used to model oxidative hepatocellular injury, and published HepG2 studies likewise demonstrate reductions in viability and changes in oxidative and enzymatic injury markers following exposure [1,2].

The in vivo biomarker pattern supports the cell-based observation. Relative to the CCl4 group, the 100 mg/kg

extract group showed markedly lower ALT and AST and a lower bilirubin value. Because aminotransferase elevation reflects loss of hepatocyte membrane integrity and bilirubin accumulation can accompany impaired hepatic handling, the coordinated movement of these endpoints is compatible with functional protection. Nevertheless, histopathology and oxidative-stress biomarkers would materially strengthen the mechanistic interpretation.

The findings are also biologically plausible in the context of prior E. fusiformis research. A previous Journal of Ethnopharmacology study reported significant hepatoprotection by ethanolic tuber extract against rifampicin-induced hepatic injury and found no major toxicity in acute and subchronic testing [4]. The present dataset differs by using CCl4 injury and fraction-level screening, which may help narrow the activity to a chemically enriched fraction rather than a crude extract.

CONCLUSION

The bioactive fraction of E. fusiformis demonstrated substantial protection against CCl4-associated injury in HepG2 cells and improved serum ALT, AST and bilirubin in a rat hepatotoxicity model. The n-butanol fraction was the strongest in-vitro, while the 100 mg/kg test dose produced the most favorable in-vivo biochemical profile.

Declarations

Ethics approval: IAEC/CPD/415/12. with CPCSEA under Registration No. 316/PO/ReBi/S/2000/CPCSEA, dated 02/11/2022.

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