International Journal of Agricultural Technology 2025 Vol. 21(6):2429-2442
DOI: https://doi.org/10.63369/ijat.2025.21.6.2429-2442
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Therapeutic effects of ethanol extract from *Erythrina fusca* Lour. fruit against carbon tetrachloride-induced liver injury in mice

Nhung, T. T. P.[1], Quoc, L. P. T.[1*] and Hoang, N. V. H.[2]

[1]Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Ho Chi Minh City, Vietnam; [2]Faculty of Medicine, Can Tho University of Medicine and Pharmacy, Can Tho City, Vietnam.

Nhung, T. T. P., Quoc, L. P. T. and Hoang, N. V. H. (2025). Therapeutic effects of ethanol extract from *Erythrina fusca* Lour. fruit against carbon tetrachloride-induced liver injury in mice. International Journal of Agricultural Technology 21(6):2429-2442.

Abstract This study investigated the hepatoprotective effects of ethanol extract from *Erythrina fusca* Lour. fruit (EFEL) against carbon tetrachloride (CCl_4)-induced liver injury in mice. EFEL was administered orally at doses of 300, 400, and 500 mg/kg for 28 days. EFEL significantly restored body weight, reduced liver weight, and normalized serum AST, ALT, ALP, and bilirubin levels compared to the CCl_4 group ($p < 0.05$). Total protein and albumin were also improved. EFEL attenuated oxidative stress by reducing malondialdehyde (MDA) and enhancing antioxidant enzymes (SOD, GSH, TAC), while decreasing catalase activity. The levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) showed a marked decrease, particularly at the 500 mg/kg dose. Histopathological findings supported the biochemical results, showing preserved hepatic architecture and reduced inflammation. These results demonstrate that EFEL exerts dose-dependent hepatoprotective effects through antioxidant and anti-inflammatory mechanisms, demonstrating its promise as a liver damage treatment.

Keywords: *Erythrina fusca* Lour., Liver injury, Hepatoprotective effects, Anti-inflammatory activity, Mice

Introduction

The liver is central to maintaining metabolic homeostasis, detoxification processes, and the production of vital biomolecules. Due to its central function in xenobiotic metabolism, it is highly susceptible to damage by toxic agents. One of the most frequently used agents to induce hepatotoxicity in research models is carbon tetrachloride (CCl_4), an industrial solvent known for its capacity to induce oxidative stress and hepatic injury. Upon activation by cytochrome P450 enzymes such as CYP2E1, CCl_4 metabolism leads to the formation of reactive intermediates, notably the trichloromethyl radical ($CCl_3^{\bullet}$) and trichloromethylperoxy radical ($CCl_3OO^{\bullet}$), which initiate lipid peroxidation,

*Corresponding Author: Quoc, L. P. T.; **Email:** lephamtanquoc@iuh.edu.vn

disrupt membrane integrity, and impair calcium homeostasis (Weber *et al*., 2003; Shokrzadeh *et al*., 2022). The consequent hepatocellular damage is further exacerbated by the activation of Kupffer cells and recruitment of neutrophils, resulting in inflammatory cascades and tissue necrosis (Johra *et al*., 2023).

Despite advances in hepatology, few effective therapies exist for chemically induced liver injury, and current synthetic drugs often present adverse side effects during prolonged use. This has driven interest in plant-derived agents with antioxidant and anti-inflammatory properties as potential hepatoprotective therapeutics (Princea *et al*., 2011).

The Fabaceae genus *Erythrina* encompasses over 130 species distributed across tropical and subtropical regions. Several species within this genus have been traditionally employed to manage fever, inflammation, liver disorders, and microbial infections (Fahmy *et al*., 2018). *Erythrina fusca* Lour., in particular, has gained attention due to its rich phytochemical profile, including flavonoids, isoflavonoids, alkaloids, pterocarpans, and tannins, demonstrated to exert recognized for their roles in combating microbial growth, modulating inflammatory responses, and preventing oxidative stress (Azmi *et al*., 2022; Anjum *et al*., 2021). Ethnomedically, various parts of the plant have been used to treat hepatic disorders, fevers, headaches, and wounds in Southeast Asian traditional medicine.

Preliminary phytochemical screenings have confirmed the presence of bioactive constituents in *E. fusca*, capable of modulating oxidative and inflammatory pathways such as MAPK, AP-1, and NF-κB (Jiménez-Cabrera *et al*., 2020). Nevertheless, the hepatoprotective potential of its fruit extract remains scientifically underexplored.

Consequently, the research focused on assessing the hepatoprotective and anti-inflammatory roles of ethanol extract obtained from *Erythrina fusca* fruit (EFEL) in a murine model of CCl_4-induced liver injury. Using biochemical assays, oxidative stress markers, proinflammatory cytokine quantification, and histopathological evaluation, the present study is provided the experimental evidence supporting the therapeutic efficacy of EFEL and its relevance for further development as a plant-based hepatoprotective agent.

Materials and methods

Plant collection and extract preparation

Fresh fruits of *Erythrina fusca* Lour. were collected in December 2023 from Buon Ma Thuot City, Vietnam. After selection and cleaning, the fruits were sliced, air-dried, and ground into fine powder.

For extraction, 800 g of powdered material was macerated in 2.25 L of 96% ethanol in a sealed flat-bottom container for 72 hours at room temperature under continuous agitation. The mixture was filtered through cotton and Whatman No. 1 filter paper. The ethanol extract was obtained by concentrating the filtrate at 65 °C under reduced pressure with a rotary evaporator designated as EFEL. The final product was a dark brown liquid with a characteristic aroma and slightly sweet taste.

Phytochemical screening and content of bioactive compounds

Qualitative phytochemical analysis of EFEL was conducted using standard methods to detect the presence of phenolics, flavonoids, alkaloids, saponins, proteins, terpenoids, carbohydrates, tannins, steroids, and cardiac glycosides, following the method of Bargah and Kushwaha (2017).

The Folin–Ciocalteu (FC) method was used to determine the total phenolic content (TPC). A range of sample volumes (1–5 µL, 100 mg/mL) was reacted with diluted FC reagent (1:4), sodium carbonate (75 g/L), and distilled water. After incubation for 2 hours at room temperature and centrifugation (2,000 g, 5 minutes), absorbance was recorded at 760 nm using gallic acid as the reference standard. (Bargah and Kushwaha, 2017).

Total flavonoid content (TFC) was estimated by mixing aliquots of extract (1–5 µL, 100 mg/mL) with ethanol-$AlCl_3$, sodium acetate (50 g/L), and distilled water. Absorbance was recorded at the appropriate wavelength, and results were expressed as rutin equivalents (Bargah and Kushwaha, 2017).

Experimental animals

Thirty male Swiss albino mice (30 ± 5 g; 7-8 weeks old) were obtained for the study. Animals were selected based on body weight and health status. They were housed in glass cages with sterilized wood shavings as bedding and maintained under standard laboratory conditions (22-25 °C, 55-60% humidity, 12 h light/dark cycle). Mice were provided with commercial rodent chow and filtered water ad libitum. A 7-day acclimatization period was observed before experimentation. All procedures complied with ethical standards outlined in the Basel Declaration on Animal Research (Abbott, 2010).

Experimental design

Mice were randomly assigned to six groups (n = 5 per group) and treated for 28 consecutive days via intragastric administration. Group assignments and treatments are detailed in Table 1 (Johra *et al*., 2023). Blood samples were obtained from the orbital sinus on day 29, 24 h post-final dose, and allowed to clot at room temperature for 60 min. Serum was isolated by centrifugation at 1,000 rpm for 5 min.

Following blood collection, mice were anesthetized with a combination of Xylazine and Ketamine (16 mg + 60 mg, i.m./i.p.) and euthanized via carbon dioxide inhalation. The livers were harvested, rinsed with phosphate-buffered saline, and fixed in 10% formalin prior to histopathological analysis.

Table 1. Intervention measures of ethanol extract from *E. fusca* fruit on CCl_4-induced hepatic inflammation in mice

Treatment name	Treatment type	Treatment characteristics
Normal treatment	Negative control treatment	1 mL B.W of saline (0.9% NaCl solution) daily
CCl_4 treatment	Disease treatment	CCl_4 at a dose of 1 mL/kg B.W dissolved in olive oil (ratio 1:3) twice/week
CCl_4+Silymarin treatment	Positive control treatment (Standard treatment)	CCl_4 at a dose of 1 mL/kg B.W dissolved in olive oil (ratio 1:3) twice/week + Silymarin 50 mg/kg B.W daily
CCl_4+$EFEL_{300}$ treatment	Disease treatment treatment (low dose EFEL)	CCl_4 at a dose of 1 mL/kg B.W dissolved in olive oil (ratio 1:3) twice/week + EFEL 300 mg/kg B.W daily
CCl_4+$EFEL_{400}$ treatment	Disease treatment treatment (medium dose EFEL)	CCl_4 at a dose of 1 mL/kg B.W dissolved in olive oil (ratio 1:3) twice/week + EFEL 400 mg/kg B.W daily
CCl_4+$EFEL_{500}$ treatment	Disease treatment treatment (high dose EFEL)	CCl_4 at a dose of 1 mL/kg B.W dissolved in olive oil (ratio 1:3) twice/week + EFEL 500 mg/kg B.W daily

Determination of body weight and organ weight

The Sartorius Entris 3202i-1S electronic balance (Germany) was used to measure the body weight and organ weights of the mice. The body weight of each mouse was recorded once every 7 days. To calculate the percentage of body weight gain (WG), we applied the following formula:

$$\text{WG (\%)} = \frac{\text{Body weight at the end of each week (g)}}{\text{Initial body weight (g)}} \times 100$$ (Al-Afifi *et al.*, 2018)

The following formula is used to determine the relative organ weight (ROW):

$$\text{ROW (\%)} = \frac{\text{The absolute organ weight (g)}}{\text{Body weight on the day of surgery (g)}}$$ (Aniagu *et al.*, 2005)

Biochemical analysis

On day 29, blood samples were collected via orbital sinus puncture into serum clot-activator tubes, centrifuged at 12,600 g for 10 min at 4 °C, and the obtained serum was stored at –150 °C in an ultra-deep freezer (MDF-1156, Japan) until analysis. Serum concentrations of AST, ALT, ALP, total protein, albumin, and total bilirubin were determined using an automated blood biochemistry analyzer (Dri-Chem NX500i, Japan).

Assessment of hepatic lipid peroxidation

The TBARS assay was employed to determine lipid peroxidation in liver tissue. Homogenates were prepared and subsequently mixed with PBS, BHA, and 0.67% TBA in a 4:5:5 ratio and heated at 100 °C for 30 minutes. After cooling, absorbance was measured at 490 nm. Malondialdehyde (MDA) equivalents were used to express lipid peroxidation.

Evaluation of antioxidant enzyme activities

The evaluation of antioxidant parameters was conducted with a UV-Visible spectrophotometer (Thermo Fisher Scientific, USA):

Reduced Glutathione (GSH): Liver homogenates were treated with phosphate buffer and hydrogen peroxide. Absorbance was measured at 450 nm at 3-minute intervals (3 replicates).

Catalase (CAT): Samples were incubated with phosphate buffer and hydrogen peroxide. Absorbance was recorded at 450 nm every 3 minutes.

Superoxide Dismutase (SOD): To determine activity, 10 µL of liver homogenate was mixed with 90 µL of phosphate buffer and 100 µL of adrenaline solution. The absorbance was recorded at 490 nm, with a reagent mixture lacking tissue serving as the blank.

Total Antioxidant Capacity (TAC): The reaction mixture (200 µL of 0.4 M acetate buffer, was mixed with 20 µL reagent II, consisting of 10 mM ABTS and 2 mM H_2O_2 in 30 mM acetate buffer at pH 3.6. The mixture was incubated for 5 min, after which absorbance was determined at 660 nm.

Measurement of proinflammatory cytokines

Commercial ELISA kits (RD Systems, Minneapolis, USA) were employed to measure serum IL-6, IL-1β, and TNF-α, as per the manufacturer's protocol.

Histopathological examination

Liver samples from the left lobes were preserved in 10% neutral-buffered formalin, dehydrated, and embedded in paraffin blocks. Thin sections (3–4 μm) were prepared, stained with H&E, and examined under brightfield microscopy (Eclipse 80i, Nikon, Japan). The analysis was performed in a blinded manner to minimize bias.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical differences between groups were analyzed using one-way ANOVA followed by Tukey's post hoc test (SPSS version 7.5). A p-value < 0.05 was considered statistically significant.

Results

Phytochemical profile and antioxidant contents

Qualitative screening of *E. fusca* fruit ethanol extract (EFEL) analysis showed that the extract contained flavonoids, alkaloids, terpenoids, carbohydrates, tannins, saponins, steroids, phenolic compounds, and proteins, but no cardiac glycosides (Table 2). The total phenolic content was 78.23 ± 4.68 mg GAE/g, and total flavonoid content was 65.42 ± 5.25 mg RE/g (Table 3), indicating a rich composition of bioactive constituents with potential antioxidant and therapeutic benefits.

Table 2. Phytochemical analysis of ethanol extracts of *E. fusca* fruit

Phytochemical constituents	The extract of *E. fusca* fruit	Phytochemical constituents	The extract of *E. fusca* fruit
Phenolic compounds	+	Terpenoids	+
Flavonoids	+	Carbohydrates	+
Alkaloids	+	Tannins	+
Saponins	+	Steroids	+
Proteins	+	Cardiac glycosides	-

Note: (+) present and (-) absent

Table 3. TPC and TFC in the ethanol extracts of *E. fusca* fruit

Sample	Total phenolic content (mg GAE/g)	Total flavonoid content (mg RE/g)
The extract of *E. fusca* fruit	78.23 ± 4.67	65.42 ± 5.25

Note: GAE: Gallic acid equivalent, RE: Rutin equivalents.

Effect of EFEL on body weight and liver index

Compared with the normal group, mice exposed to CCl_4 exhibited a significant reduction in both body weight and weight gain percentage ($p < 0.05$) (Table 4). EFEL treatment at all doses markedly reversed these changes, with the 500 mg/kg group showing a 7.25% increase in body weight over 28 days, comparable to Silymarin (7.19%) (Figure 1). Relative liver weight increased significantly in the CCl_4 group (5.9%) but was restored by EFEL to levels comparable with the control group (Table 5), indicating mitigation of CCl_4-induced hepatomegaly.

Table 4. Effect of *E. fusca* fruit ethanol extract on the body weight of CCl_4-administered mice

Time	Body weight (g)					
	Normal treatment	CCl_4 treatment	CCl_4+ Silymarin treatment	CCl_4 + $EFEL_{300}$ treatment	CCl_4 + $EFEL_{400}$ treatment	CCl_4 + $EFEL_{500}$ treatment
"0"	30.55 ± 0.12^{a}	30.61 ± 0.12^{a}	30.85 ± 0.08^{b}	31.04 ± 0.07^{cd}	31.08 ± 0.04^{d}	30.95 ± 0.08^{bc}
1 week	31.23 ± 0.11^{b}	29.99 ± 00.11^{a}	31.46 ± 0.08^{c}	31.33 ± 0.06^{b}	31.45 ± 0.04^{c}	31. 34 ± 0.06^{b}
2 weeks	32.16 ± 0.11^{d}	29.83 ± 0.11^{a}	31.93 ± 0.06^{c}	31.68 ± 0.06^{b}	31.74 ± 0.08^{bc}	31.85 ± 0.07^{cd}
3 weeks	33.59 ± 0.11^{d}	28.04 ± 0.11^{a}	32.69 ± 0.06^{c}	32.26 ± 0.05^{b}	32.31 ± 0.06^{b}	32.58 ± 0.06^{c}
4 weeks	34.09 ± 0.11^{d}	26.92 ± 0.12^{a}	33.26 ± 0.06^{c}	32.85 ± 0.05^{b}	32.94 ± 0.09^{b}	33.19 ± 0.06^{e}

Letters (a-e) stand for treatment differences ($p < 0.05$).

Biochemical markers of liver function

CCl_4 exposure significantly elevated serum AST, ALT, ALP, and total bilirubin levels while reducing total protein and albumin ($p < 0.05$). EFEL treatment reversed these alterations in a dose-dependent method, with the greatest recovery occurring at 500 mg/kg. It is supported the hepatoprotective capacity of EFEL via normalization of liver function parameters (Table 6).

Table 5. Effect of *E. fusca* fruit ethanol extract on liver weight of CCl_4-administered mice

Treatments	Semi-field experiment	
	Plant length (cm)	Canopy diameter (cm)
Water treatment	18.23 ± 1.11^{a}	17.59 ± 1.29^{a}
Fipronil treatment	27.35 ± 1.13^{e}	26.39 ± 1.25^{e}
EEMP2 treatment	21.88 ± 1.11^{b}	21.11 ± 1.24^{b}
EEMP4 treatment	22.79 ± 1.16^{b}	21.91 ± 1.26^{bc}
EEMP6 treatment	23.78 ± 1.17^{bc}	22.95 ± 1.34^{bc}
EEMP8 treatment	24.86 ± 1.12^{cd}	23.99 ± 1.18^{cd}
EEMP10 treatment	26.81 ± 1.12^{de}	25.87 ± 1.18^{de}

Letters (a-e) stand for treatment differences ($p < 0.05$).

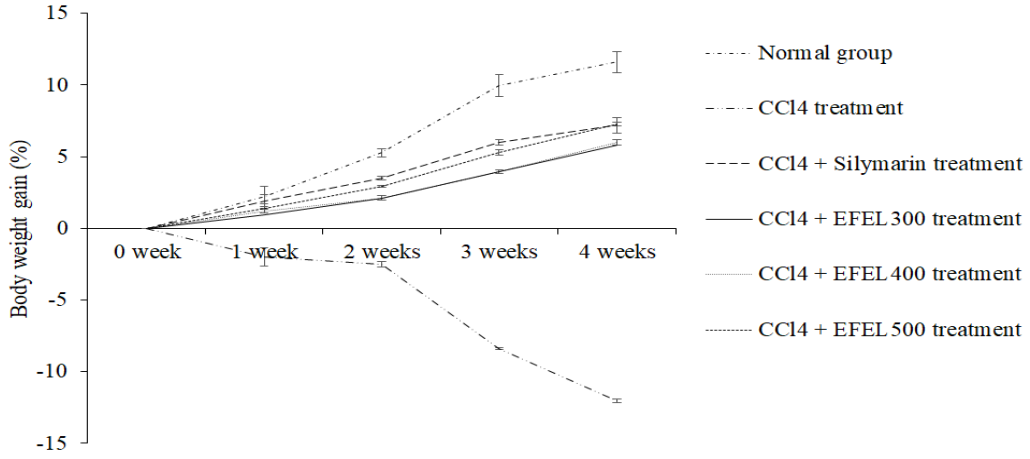

Figure 1. Body weight gain levels alterations in mice treated with CCl_4 and *E. fusca* fruit extract, and Silymarin

Lipid peroxidation and oxidative stress biomarkers

CCl_4 significantly increased hepatic MDA levels (3.07 ± 0.13 nmol/mg protein), indicating elevated lipid peroxidation. EFEL markedly reduced MDA concentrations, particularly at 500 mg/kg (1.93 ± 0.11 nmol/mg), similar to Silymarin (1.59 ± 0.13 nmol/mg) (Figure 2). These findings confirmed the antioxidant potential of EFEL in attenuating oxidative liver damage.

Table 6. Effects of *E. fusca* fruit ethanol extract on the biochemistry of mice with CCl_4-induced hepatitis

Parameters	Normal treatment	CCl_4 treatment	CCl_4 + Silymarin treatment	CCl_4 + $EFEL_{300}$ treatment	CCl_4 + $EFEL_{400}$ treatment	CCl_4 + $EFEL_{500}$ treatment
Total protein (g/dL)	5.78 ± 0.12^{f}	3.12 ± 0.11^{a}	5.16 ± 0.13^{e}	4.22 ± 0.12^{b}	4.62 ± 0.12^{c}	4.94 ± 0.12^{d}
Albumin (g/dL)	3.46 ± 0.06^{f}	1.62 ± 0.05^{a}	3.26 ± 0.05^{e}	2.58 ± 0.04^{b}	2.81 ± 0.04^{c}	3.12 ± 0.05^{d}
AST (U/L)	17.84 ± 0.13^{a}	52.54 ± 0.14^{f}	20.97 ± 0.12^{b}	42.68 ± 0.11^{e}	34.94 ± 0.12^{d}	21.16 ± 0.12^{c}
ALT (U/L)	18.61 ± 0.12^{a}	56.39 ± 0.24^{f}	22.15 ± 0.12^{b}	45.39 ± 0.22^{e}	37.22 ± 0.26^{d}	23.01 ± 0.12^{c}
ALP (U/L)	132.76 ± 8.85^{a}	466.76 ± 6.82^{e}	156.77 ± 10.53^{b}	234.2 ± 7.18^{d}	236.63 ± 7.65^{c}	167.22 ± 8.34^{b}
Total bilirubin (µmol/L)	0.28 ± 0.11^{a}	1.08 ± 0.14^{e}	0.42 ± 0.11^{ab}	0.88 ± 0.11^{d}	0.71 ± 0.12^{c}	0.46 ± 0.11^{b}

Letters (a-e) stand for treatment differences ($p < 0.05$).

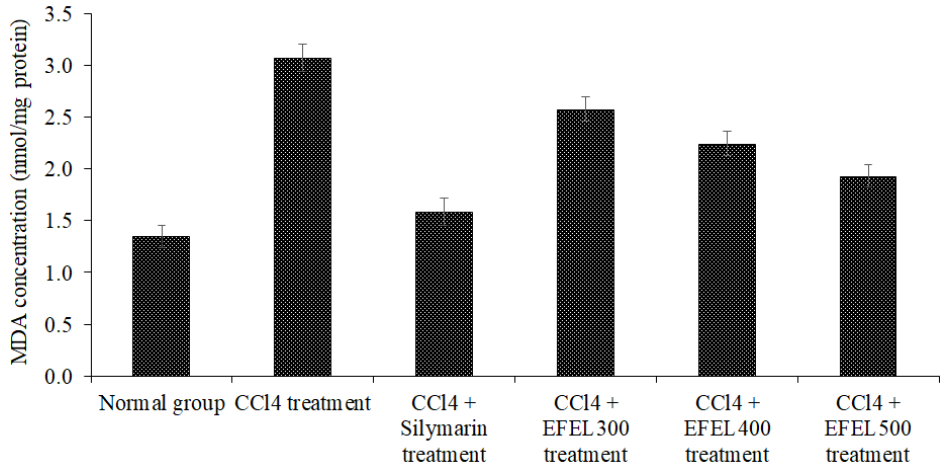

Figure 2. Malondialdehyde (MDA) level alterations in mice treated with CCl_4 and *E. fusca* fruit extract, and Silymarin

Antioxidant enzyme activities

CCl_4 reduced hepatic levels of GSH, SOD, and TAC while increasing CAT activity ($p < 0.05$). EFEL treatment significantly restored antioxidant enzyme levels and normalized CAT activity (Table 7). These results suggested that EFEL enhanced endogenous antioxidant defense mechanisms against CCl_4-induced oxidative stress.

Table 7. Antioxidant status in the liver of CCl_4-induced hepatitis mice treated with ethanol extract of *Erythrina fusca* fruit

Parameters	Normal treatment	CCl_4 treatment	CCl_4+ Silymarin treatment	CCl_4 + $EFEL_{300}$ treatment	CCl_4 + $EFEL_{400}$ treatment	CCl_4 + $EFEL_{500}$ treatment
SOD (mM/min/mg tissue)	29.75 ± 0.11^{f}	14.88 ± 0.11^{a}	24.59 ± 0.11^{e}	21.09 ± 0.11^{b}	22.37 ± 0.11^{c}	23.06 ± 0.12^{d}
CAT (mM/min/g tissue)	18.26 ± 0.11^{a}	38.52 ± 0.11^{f}	22.83 ± 0.12^{b}	27.25 ± 0.12^{e}	26.46 ± 0.12^{d}	24.54 ± 0.11^{c}
GSH (nM/mg tissue)	4.99 ± 0.11^{f}	2.41 ± 0.12^{a}	4.46 ± 0.12^{e}	3.22 ± 0.13^{b}	3.83 ± 0.11^{c}	4.12 ± 0.11^{d}
TAC (nM/mg protein)	28.74 ± 1.07^{f}	11.93 ± 0.57^{a}	25.66 ± 1.11^{e}	17.11 ± 0.99^{b}	19.29 ± 1.05^{c}	22.99 ± 1.15^{d}

Letters (a-e) stand for treatment differences ($p < 0.05$).

Anti-inflammatory effects of FEEL

Serum concentrations of TNF-α, IL-1β, and IL-6 increased markedly after CCl_4 administration ($p < 0.05$). Treatment with EFEL attenuated these cytokines in a dose-dependent fashion, with the 500 mg/kg group exhibiting the strongest suppression (Figure 3). It indicated potent anti-inflammatory activity, supporting EFEL's role in modulating hepatic inflammation.

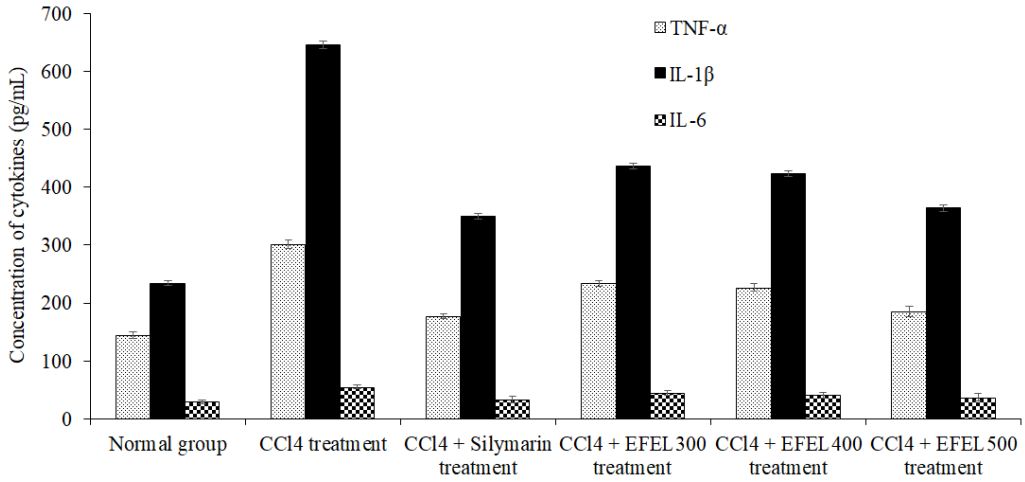

Figure 3. IL-6, IL-1β, and TNF-α levels alterations in mice treated with CCl_4 and *E. fusca* fruit extract, and Silymarin

Macroscopic and histopathological evaluation

Gross liver morphology in the CCl_4 group enlarged, pale livers with yellow nodules, while EFEL and Silymarin treatments restored normal red color, smooth texture, and reduced liver mass (Figure 4A). Histologically, CCl_4 induced severe hepatic lesions, including central vein dilation, necrosis, inflammatory infiltration, and bile duct damage. In contrast, EFEL and Silymarin significantly preserved hepatic architecture, reduced inflammatory cell presence, and prevented cellular degeneration (Figure 4B). These findings confirmed the hepatoprotective and regenerative effects of EFEL.

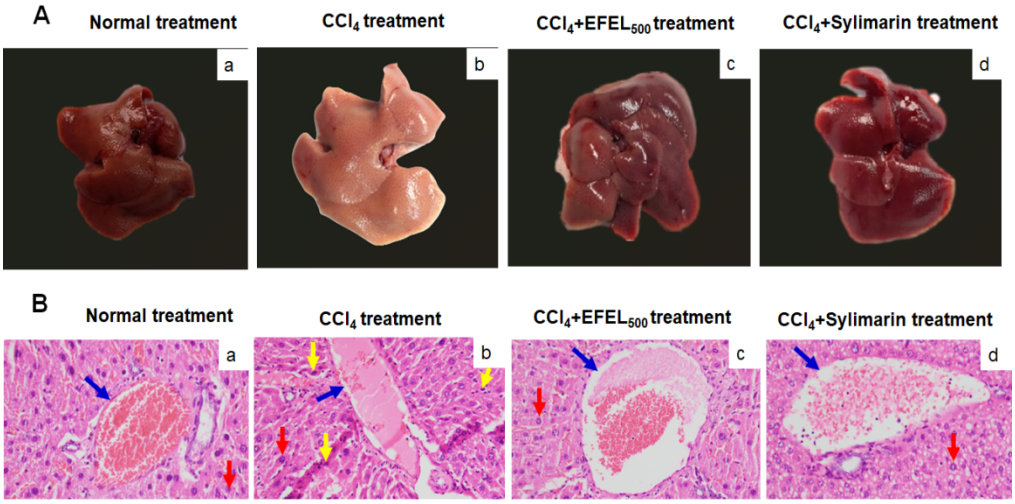

Figure 4. Macroscopic and microscopic images of mouse liver treated with *E. fusca* fruit ethanol extract: A - Macroscopic image of the mouse liver, B - Hematoxylin and eosin (H&E)-stained liver slices under a microscope magnification ×200. Note: Green arrows indicate central veins, red arrows indicate liver cells, and yellow arrows indicate inflammatory cells.

Discussion

The liver plays a central role in detoxification and metabolism and is vulnerable to xenobiotic-induced oxidative damage. This study demonstrated that the ethanol extract of *Erythrina fusca* fruit (EFEL), rich in flavonoids, phenolics, terpenoids, saponins, and tannins, exerts potent hepatoprotective effects against CCl_4-induced hepatic injury. These phytochemicals assist in protecting cells and restore metabolism because of their well-known anti-inflammatory, antioxidant, and membrane-stabilizing qualities(Tungmunnithum *et al*., 2018; Nunes *et al*., 2020).

Administration of CCl_4 led to significant weight loss and hepatomegaly, consistent with hepatic edema, inflammation, and metabolic dysfunction (Lee *et al*., 2019). EFEL treatment restored body weight and reduced relative liver weight in a dose-dependent manner, suggesting improved hepatic function and reduced tissue damage. This protective effect is attributable to the suppression of trichloromethyl radical formation and attenuation of lipid accumulation and fibrosis.

Biochemically, CCl_4 exposure elevated serum markers (AST, ALT, ALP, bilirubin) and decreased protein and albumin levels, indicating hepatocellular damage and impaired synthetic function. EFEL reversed these changes, particularly at 500 mg/kg, highlighting its potential in restoring hepatocyte integrity and liver detoxification capacity. Similar trends have been reported with other plant extracts exhibiting hepatoprotective properties (Kalantari *et al*., 2010; Dutta *et al*., 2018).

Oxidative stress, a key mediator of CCl_4 toxicity, was confirmed by increased hepatic MDA levels. EFEL significantly reduced MDA, reflecting its capacity to suppress lipid peroxidation. Moreover, EFEL improved endogenous antioxidant defenses by enhancing GSH, SOD, and TAC levels while normalizing CAT activity, aligning with previous findings on plant-based antioxidants (Pradeep *et al*., 2005; Munteanu and Apetrei, 2021).

Cytokine analysis further validated EFEL's anti-inflammatory potential. CCl_4 markedly increased TNF-α, IL-1β, and IL-6 levels, which were significantly attenuated by EFEL treatment. This immunomodulatory effect may facilitate hepatocyte regeneration by modulating Kupffer cell activation and downstream STAT3 signaling (Tsai *et al*., 2021; Huang *et al*., 2012).

Histopathological examination corroborated the biochemical findings. EFEL preserved hepatic architecture, reduced inflammatory infiltrates, and prevented vascular congestion and collagen deposition, indicating effective protection against necrosis and fibrosis (Dong *et al*., 2016).

Collectively, the findings underscore the multifaceted hepatoprotective action of EFEL through antioxidative, anti-inflammatory, and tissue-preserving mechanisms. These results support its therapeutic potential in managing hepatic disorders and warrant further molecular investigations to elucidate its specific targets and clinical applicability.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

Abbott, A. (2010). Basel declaration defends animal research. Nature, 468:742.

Al-Afifi, N. A., Alabsi, A. M., Bakri, M. M. and Ramanathan, A. (2018). Acute and sub-acute oral toxicity of *Dracaena cinnabari* resin methanol extract in rats. BMC Complementary Medicine and Therapies, 18:50.

Aniagu, S. O., Nwinyi, F. C., Akumka, D. D., Ajoku, G. A., Dzarma, S., Izebe, K. S., Ditse, M., Nwaneri, P. E. C., Wambebe, C. and Gamaniel, K. (2005). Toxicity studies in rats fed nature cure bitters. African Journal of Biotechnology, 4:72-78.

Anjum, A., Sultan, M. Z., Ferdosh, S., Islam, M. K., Rashid, M. A., Nahar, L. and Sarker, S. D. (2021). Flavonoid, pterocarpans and steroid from *Erythrina fusca* Lour. growing in Bangladesh: isolation, and antimicrobial and free-radical scavenging activity. Journal of Medicinal Plants, 20:37-46.

Azmi, A. S., Amini, M. H., Azman, M. F. S. N. and Salim, F. (2022). Antibacterial activity and phytochemical screening of *Erythrina fusca* Lour. leaf extract (Fabaceae). Science Letters, 16:60-71.

Bargah, R. K. and Kushwaha, P. K. (2017). Extractions, phytochemical screening and in-vitro antioxidant activity of *Cassia fistula* extracts. International Journal of Research in Pharmacy and Chemistry, 7:518-524.

Dong, S., Chen, Q. L., Song, Y. N., Sun, Y., Wei, B., Li, X. Y., Hu, Y. Y., Liu, P. and Su, S. B. (2016). Mechanisms of CCl_4-induced liver fibrosis with combined transcriptomic and proteomic analysis. Journal of Toxicological Sciences, 41:561-572.

Dutta, S., Chakraborty, A. K., Dey, P., Kar, P., Guha, P., Sen, S., Kumar, A., Sen, A. and Chaudhuri, T. K. (2018). Amelioration of CCl_4 induced liver injury in swiss albino mice by antioxidant rich leaf extract of *Croton bonplandianus* Baill. PLoS One, 13:0196411.

Fahmy, N. M., Al-Sayed, E., El-Shazly, M. and Singab, A. S. (2018). Comprehensive review on flavonoids biological activities of *Erythrina* plant species. Industrial Crops and Products, 123:500-538.

Huang, H. L., Wang, Y. J., Zhang, Q. Y., Liu, B., Wang, F. Y., Li, J. J. and Zhu, R. Z. (2012). Hepatoprotective effects of baicalein against CCl_4-induced acute liver injury in mice. World Journal of Gastroenterology, 18:6605-6613.

Jiménez-Cabrera, T., Bautista, M., Velázquez-González, C., Jaramillo-Morales, O. A., Guerrero-Solano, J. A., Urrutia-Hernández, T. A. and O-Arciniega, M. D. (2020). Promising antioxidant activity of *Erythrina* genus: an alternative treatment for inflammatory pain. International Journal of Molecular Sciences, 22:248.

Johra, F. T., Hossain, S., Jain, P., Bristy, A. T., Emran, T., Ahmed, R., Sharker, S. M., Bepari, A. K. and Reza, H. M. (2023). Amelioration of CCl_4-induced oxidative stress and hepatotoxicity by *Ganoderma lucidum* in Long Evans rats. Scientific Reports, 13:9909.

Kalantari, H., Jalali, M., Jalali, A., Mahdavinia, M., Salimi, A., Juhasz, B., Tosaki, A. and Gesztelyi, R. (2010). Protective effect of *Cassia fistula* fruit extract against bromobenzene-induced liver injury in mice. Human & Experimental Toxicology, 30:1039-1044.

Lee, Y. S., Cho, I. J., Kim, J. W., Lee, M. K., Ku, S. K., Choi, J. S. and Lee, H. J. (2019). Hepatoprotective effects of blue honeysuckle on CCl_4-induced acute liver damaged mice. Food Science & Nutrition, 7:322-338.

Munteanu, I. G. and Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A Review. International Journal of Molecular Sciences, 22:3380.

Nunes, C. D. R., Arantes, M. B., Pereira, S. M. D. F., Cruz, L. L. D., Passos, M. D. S., Moraes, L. P. D., Vieira, I. J. C. and Oliveira, D. B. D. (2020). Plants as sources of anti-inflammatory agents. Molecules, 25:3726-3739.

Pradeep, K., Mohan, C. V. R., Anand, K. G. and Karthikeyan, S. (2005). Effect of pretreatment of *Cassia fistula* Linn. leaf extract against subacute CCl_4 induced hepatotoxicity in rats. Indian Journal of Experimental Biology, 43:526-530.

Princea, E. S., Parameswarib, P. and Khan, R. M. (2011). Protective effect of *Ricinus communis* leaves extract on carbon tetrachloride induced hepatotoxicity in albino rats. Iranian Journal of Pharmaceutical Sciences, 7:269-278.

Shokrzadeh, M., Khalvati, R., Hosseinzadeh, M. H., Ayatifard, M. and Habibi, E. (2022). Effects of hydroalcoholic extract of *Lepidium sativum* L. on carbon tetrachloride-induced hepatotoxicity in mice. Pharmaceutical and Biomedical Research, 8:225-231.

Tsai, M. Y., Yang, W. C., Lin, C. F., Wang, C. M., Liu, H. Y., Lin, C. S., Lin, J. W., Lin, W. L., Lin, T. C., Fan, P. S., Hung, K. H., Lu, Y. W. and Chang, G. R. (2021). The ameliorative effects of fucoidan in thioacetaide-induced liver injury in mice. Molecules, 26:1937.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A. and Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines, 5:93.

Weber, L. W. D., Boll, M. and Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. Critical Reviews in Toxicology, 33:105-36.

(Received: 21 November 2024, Revised: 30 July 2025, Accepted: 26 August 2025)