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Original Research Article

Hepatoprotective and Antioxidant Activities of Selected *Helicteres* Species in a CCl_4-Induced Acute Liver Injury Mouse Model

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ABSTRACT

Hepatoprotection refers to the capacity of a substance to prevent or mitigate liver damage caused by toxins. Medicinal plants have gained attention for their potential therapeutic effects, including antioxidant and hepatoprotective properties. This study evaluated the in vitro antioxidant activity and in vivo hepatoprotective potential of aqueous leaf and stem extracts from three *Helicteres* species: *H. lanceolata* DC., *H. hirsuta* Lour., and *H. isora* L. Antioxidant activity was assessed using the DPPH assay, and hepatoprotective effects were examined in a carbon tetrachloride (CCl_4)-induced liver injury model in male mice. Serum biochemical markers (AST, ALT, GGT, creatinine), hematological parameters, and histopathological features of liver and other organs were analyzed. The DPPH assay revealed strong antioxidant activity in all extracts (0 – 200 µg/mL), with EC_{50} values of 10.56 ± 0.31 µg/mL for *H. isora*, 10.57 ± 0.19 µg/mL for *H. hirsuta*, and 13.16 ± 0.38 µg/mL for *H. lanceolata*. In vivo experiments showed that treatment with the extracts significantly reduced liver enzyme levels ($P < 0.05$) compared to the CCl_4-only group. Hematological parameters remained largely unaffected, except for a reduction in blood glucose levels in treated groups (dose of 200 mg/kg). Among the species tested, *H. isora* extract demonstrated the most prominent protective effects, increased body weight and improved liver histology. These findings suggest aqueous extracts of *H. lanceolata*, *H. hirsuta*, and *H. isora* possess significant antioxidant and hepatoprotective activities, with *H. isora* extract showing the most significant hepatoprotective effect in mice, supporting their potential as candidates for liver disease management.

Keywords: Antioxidant activity, carbon tetrachloride-induced toxicity, Helicteres genus, Hepatoprotection, Medicinal plants.

Introduction

The liver is a vital organ involved in nearly all biochemical pathways essential for growth, immune function, nutrient metabolism, energy production, and reproduction.[1] Liver diseases are commonly caused by exposure to harmful chemicals, xenobiotics, alcohol, malnutrition, anemia, medications, autoimmune disorders, and viral infections, all of which can induce oxidative stress.[2] Reactive oxygen species (ROS) have long been recognized as key contributors to the pathophysiology of liver damage.[3] Although several drugs have been investigated for liver disease treatment, effective therapies for liver toxicity are still limited.[4] As a result, there is growing interest in identifying bioactive compounds from medicinal plants that offer safer, more effective, and affordable therapeutic options.[5–7]

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Many recent studies have focused on the pharma-pharmacological evaluation of plant-derived compounds with the potential for drug development.[8] The genus *Helicteres* Linnaeus (1753), "An xoa" (Vietnamese name), belonging to the Malvaceae family, includes approximately 60 species distributed across tropical regions of Asia and the Americas.[9,10] These are a shrub to a small tree, growing to 4 m tall (sometimes up to 8 m) with screw-shaped fruits.[11] Species in this genus are known for their diverse biological and pharmacological properties, with traditional uses supported by the isolation and structural characterization of their bioactive secondary metabolites.[11] According to [13] nine species of *Helicteres* are found in Vietnam, where they are widely used in traditional medicine for their anti-inflammatory, hepatoprotective, anticancer, antibacterial, and antioxidant properties. These plants are also employed to treat conditions such as boils, colds, measles, dysentery, and detoxification-related ailments.[12,13]

Although carbon tetrachloride (CCl_4) itself is not directly hepatotoxic, its metabolites—especially trichloromethyl free radicals—are highly reactive and lead to lipid peroxidation and oxidative liver injury.[14] [12] reported the isolation of 149 compounds from *Helicteres* species, including terpenoids, sterols, phenols, and flavonoids.[12] These compounds possess antioxidant properties that reduce lipid peroxidation and enhance endogenous antioxidant enzyme levels, potentially contributing to liver protection.[14] This study aims to evaluate the antioxidant and hepatoprotective effects of aqueous extracts from *Helicteres* species found in Vietnam against CCl_4-induced liver damage in mice.

Materials and Methods

Experimental Materials

This study investigated three species from the genus Helicteres: *Helicteres lanceolata* DC., Helicteres hirsuta Lour., and Helicteres isora L., which were collected from Cam mountain, An Giang province (10°30'8.24"N; 104°59'56.53"E), in August 2023. Plant materials were collected in accordance with the Pharmacopoeia Vietnamica, 5th Edition[15] and were identified by plant taxonomists based on morphological characteristics as described by Nguyen.[16] After taxonomic identification, voucher specimens (*Helicteres lanceolata*.2023.WE.001-012; Helicteres hirsuta.2023.WE.002-006; Helicteres isora.2023.WE.003-002) were preserved in the Plant Laboratory, Department of Basic Sciences, Can Tho Medical College. The plant parts used for extract preparation included both stems and leaves (Figure 1).

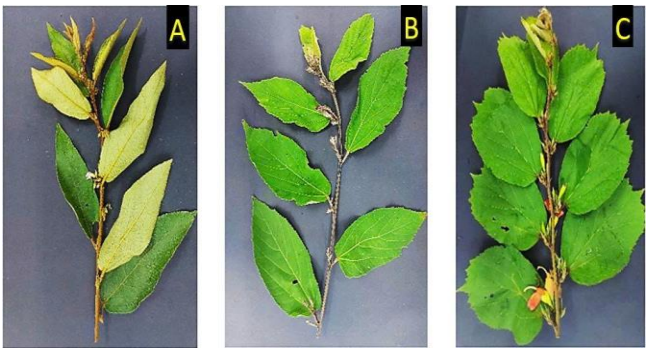

Figure 1: The morphology of plant species in the genus *Helicteres*. *A: H. lanceolata; B: H. hirsuta; C: H. isora.*

Preparation of Plant Extracts

The collected plant parts were dried at 60°C in an incubator until a constant weight was achieved, then ground into a fine powder using a laboratory grinder. The powdered material (50 g) was extracted following the method of Nguyen[17] by boiling it in 500 mL of distilled water at 100°C for 1 hour. The resulting extract was filtered through filter paper. The residue was re-extracted twice more under the same conditions, for a total of three extractions. The combined filtrates were concentrated using a rotary evaporator to obtain the crude extract, which was stored in glass containers at 4°C until use.

DPPH Free Radical Scavenging Activity

Extracts from leaves, stems, and a combination of stems and leaves were dissolved in methanol at varying concentrations (0 – 200 µg/mL). A DPPH solution was prepared in methanol at a concentration of 500 µg/mL. For the assay, 950 µL of each extract (or control) was added to a test tube, followed by 50 µL of the DPPH solution. The mixture was shaken vigorously and incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm using a spectrophotometer (UVS-2800, Labomed, USA). Vitamin C was used as the positive control. Each experiment was performed in triplicate. The DPPH radical scavenging activity was calculated using the following formula (Equation 1):

$$\text{Scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

where A_control is the absorbance of the control and A_sample is the absorbance of the extract. Based on the scavenging activity at different concentrations, a first-order linear regression equation was used to determine the EC_{50} value.[17]

Animal Test

Healthy Swiss albino mice (Mus musculus var. albino), weighing 28–32 g and of both sexes, were obtained from the Stem Cell Institute (Ho Chi Minh City, Vietnam). The animals were acclimatized for one week under standard laboratory conditions (12-hour light/12-hour dark cycle, 25-28ºC, 80-90% humidity) with free access to food and water. Prior to toxicity testing, the mice were fasted for 24 hours but allowed free access to water. All animal experiments were conducted in accordance with the guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Ethics Committee of Can Tho University of Medicine and Pharmacy (25.008.GV.CTUMP/PCT-HDDD).

Investigation of the hepatoprotective effect of the extract on Swiss mice with liver damage induced by CCl_4

Experimental model

The hepatoprotective activity of the plant extracts was evaluated based on the methods described by [18,] and [19] with slight modifications.[18,19] Liver injury was induced by administering carbon tetrachloride (CCl_4) at a dose of 0.5 mL/kg body weight, diluted in olive oil at a ratio of 1:4. Silymarin (40 mg/kg body weight) was used as the positive control. The Helicteres leaf extracts were tested at a dose of 400 mg/kg body weight. The experiment included six groups, each consisting of five mice (n = 5):

Group 1 (Normal control): Received distilled water only;
Group 2 (Negative control): Received CCl_4 (0.5 mL/kg) once daily;
Group 3 (Positive control): Received CCl_4 (0.5 mL/kg), followed 1 hour later by silymarin (40 mg/kg) once daily;
Group 4: Received CCl_4 (0.5 mL/kg), followed 1 hour later by *H. lanceolata* extract (400 mg/kg) once daily;
Group 5: Received CCl_4 (0.5 mL/kg), followed 1 hour later by *H. hirsuta* extract (400 mg/kg) once daily;
Group 6: Received CCl_4 (0.5 mL/kg), followed 1 hour later by *H. isora* extract (400 mg/kg) once daily.

The treatment was continued for three weeks. At the end of the experiment, the mice were weighed, anesthetized, euthanized by spinal traction, and blood was collected via cardiac puncture for hematological and biochemical analysis. Internal organs were examined macroscopically, and liver tissues were processed for histological examination to assess structural changes.

Body weight gain and Organ index

The body weight gain ratio (BWG%) was calculated according to the method described by [20] using the following formula (Equation 2):

$$BWG\% = \frac{\text{Weight of body (end)} - \text{Weight of body (start)}}{\text{Weight of body (start)}} \times 100 \quad (2)$$

After dissection, internal organs—including the heart, liver, lungs, kidneys, spleen, and testes—were collected, rinsed with physiological saline, blotted dry, and weighed. The organ index (%) was calculated as follows (Equation 3)[18]:

$$\text{Organ index \%} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100 \quad (3)$$

Hematological and Biochemical Parameters

Blood samples were collected via cardiac puncture after the mice were humanely euthanized under general anesthesia. Hematological parameters were analyzed using a CELL-DYN Ruby Automated Hematology Analyzer (Abbott, USA), and biochemical parameters were measured using a Cobas Clinical Chemistry Automatic Analyzer (Roche Ltd., Japan). Hematological parameters included blood glucose, red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and corpuscular volume (CV).[21] Biochemical parameters included creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT).[18,22]

Histological Assessment
Tissue samples of internal organs were fixed in Bouin's solution for histological analysis. The samples were sectioned using a microtome (Accu-Cut® SRM™ 200, Sakura, Japan) into 5 µm-thick slices, and stained with hematoxylin and eosin (H&E). Histological observations were carried out using an light microscope (CX23, Olympus, Japan) and images were captured using a digital camera connected to ToupView software.[23,24]

Statistical Analysis
Data were analyzed using SPSS version 26.0 software (SPSS Inc., USA). Differences among treatment groups were assessed using one-way ANOVA followed by Duncan's post-hoc test at a 95% confidence level. Results are expressed as mean ± standard deviation (SD), and $p < 0.05$ was considered statistically significant.

Results and Discussion

Extractions
The plant parts were dried, ground into a fine powder, and extracted. The results of the extraction yield are presented in Table 1. Analysis of extraction efficiency showed that all three Helicteres species yielded higher extraction output from the leaves, while the stems consistently showed lower yield. Among the solvents tested, distilled water proved to be more efficient than ethanol (data not shown). For aqueous leaf extracts, the extraction efficiency ranked as follows: *H. hirsuta* (20.11 ± 0.63%) > *H. isora* (19.17 ± 0.44%) > *H. lanceolata* (18.08 ± 0.20%), with statistically significant differences ($P < 0.05$).

Table 1: The extraction yield of the plant parts from species of the genus *Helicteres*

Species	Plant Part	Extraction Efficiency (%)	N
H. lanceolata	Stem	8.18 ± 0.30^{b}	3
	Leaf	18.08 ± 0.20^{c}	3
H. hirsuta	Stem	7.23 ± 1.10^{a}	3
	Leaf	20.11 ± 0.63^{e}	3
H. isora	Stem	8.32 ± 0.18^{b}	3
	Leaf	19.17 ± 0.44^{d}	3

Note: Values are presented as mean ± standard deviation (n = 3 extraction process). Different superscript letters within the same column indicate statistically significant differences ($P < 0.05$).

DPPH Free Radical Scavenging Activity
Natural antioxidants have garnered growing interest from both researchers and consumers due to their potential to mitigate oxidative stress and related health disorders. Medicinal plants, in particular, are recognized as a readily available and effective source of natural antioxidants.[25] In this study, the antioxidant potential of different plant parts from species of the genus Helicteres was evaluated using the DPPH free radical scavenging assay. The antioxidant activity was quantified based on EC_{50} values, with results (Table 1) showing that aqueous leaf extracts from Helicteres species exhibited notable antioxidant capacity.
The antioxidant activity of the extracts was assessed based on their DPPH radical scavenging capacity, expressed as EC_{50} values. Lower EC_{50} values indicate higher antioxidant potency. As shown in Table 2, the aqueous leaf extracts of *H. hirsuta* and *H. isora* exhibited the strongest scavenging activities, with EC_{50} values of 10.57 ± 0.19 µg/mL and 10.56 ± 0.31 µg/mL, respectively. The leaf extract of *H. lanceolata* showed moderate activity (EC_{50} = 13.16 ± 0.38 µg/mL). All three stem extracts demonstrated weaker antioxidant effects, particularly *H. hirsuta* stem extract (EC_{50} = 39.94 ± 1.04 µg/mL). In comparison, ascorbic acid—the positive control—exhibited the highest antioxidant activity with an EC_{50} value of 4.62 ± 0.04 µg/mL. The leaf extracts of

Table 2: EC_{50} values of extracts from species of the genus *Helicteres*

Species	Plant Part	EC_{50} (µg/mL)	Linear Equation	R^2	n
H. lanceolata	Stem	12.00 ± 0.25^{bc}	y = 3.9656x + 2.3551	0.9931	9
	Leaf	13.16 ± 0.38^{c}	y = 3.6591x + 1.8768	0.9897	9
H. hirsuta	Stem	39.94 ± 1.04^{e}	y = 1.2287x + 0.9286	0.9909	9
	Leaf	10.57 ± 0.19^{b}	y = 4.8203x − 0.9550	0.9785	9
H. isora	Stem	22.83 ± 0.56^{d}	y = 2.0292x + 3.6861	0.9827	9
	Leaf	10.56 ± 0.31^{b}	y = 4.7925x − 0.5947	0.9866	9
Ascorbic acid	—	4.62 ± 0.04^{a}	y = 10.386x + 1.9998	0.9915	9

Note: Values are expressed as mean ± standard deviation (n = 9). Different superscript letters within the EC_{50} column indicate statistically significant differences ($P < 0.05$).

H. hirsuta and *H. isora* were only about 2–3 times less potent than ascorbic acid, indicating strong natural antioxidant potential. Based on these findings, the study focused on the aqueous leaf extracts of *H. lanceolata*, *H. hirsuta*, and *H. isora* to investigate their hepatoprotective potential in a mouse model of carbon tetrachloride (CCl_4)-induced liver injury.

Hepatoprotective Effects of Helicteres Extracts
CCl_4 is a well-established hepatotoxin commonly used to induce experimental liver damage. In the liver, the cytochrome P450 (CYP) enzyme system metabolizes CCl_4 into highly reactive intermediates, particularly the trichloromethyl radical ($\bullet CCl_3$), which further reacts with oxygen to form trichloromethylperoxy radicals ($\bullet CCl_3OO$). These radicals initiate lipid peroxidation and disrupt membrane integrity through covalent interactions with lipids and proteins, ultimately leading to hepatocellular damage.[26,27] The CCl_4-induced hepatotoxicity model remains widely employed for assessing the protective effects of bioactive compounds. In this study, mice were administered CCl_4 to induce liver injury and subsequently treated with the leaf extracts of *H. lanceolata*, *H. hirsuta*, and *H. isora*. After 21 days, hepatoprotective effects were assessed through hematological parameters, liver and kidney biochemical markers, body weight changes, organ weight indices, and histopathological evaluation.
As shown in Table 3, administration of carbon tetrachloride (CCl_4) significantly increased plasma levels of AST (81.33 ± 4.93 U/L), ALT (46.33 ± 5.86 U/L), and GGT (16.33 ± 6.03 U/L) in the untreated group, compared to the control group ($P < 0.05$). In contrast, treatment with Silymarin (40 mg/kg) or aqueous leaf extracts of *H. lanceolata*, *H. hirsuta*, and *H. isora* (400 mg/kg) significantly reduced these enzyme levels, bringing them closer to normal ($P < 0.05$). Plasma creatinine levels did not differ significantly between groups ($P > 0.05$), suggesting no renal impairment. Liver enzyme analysis revealed that CCl_4-treated mice without any therapeutic intervention exhibited significantly elevated levels of AST, ALT, and GGT compared to the control group ($P < 0.05$). These findings are consistent with previous reports on CCl_4-induced hepatotoxicity, where lipid peroxidation damages hepatocyte membranes, leading to the leakage of intracellular enzymes into the bloodstream.[28,29] .

Table 3: Biochemical Parameters in Experimental Mice (Mean ± SD, n = 3-5)

Parameter	Control	Silymarin	CCl_4	*H. lanceolata*	*H. hirsuta*	*H. isora*
AST (U/L)	7.00±5.20[a]	16.67±13.32[a]	81.33±4.93[b]	5.67±4.73[a]	16.33±8.50[a]	11.33±7.37[a]
ALT (U/L)	9.67±3.79[a]	19.33±12.50[ab]	46.33±5.86[c]	8.00±5.29[a]	25.67±11.02[b]	7.67±2.08[a]
GGT (U/L)	5.33±0.58[a]	5.00±3.00[a]	16.33±6.03[b]	3.33±1.53[a]	4.00±1.00[a]	3.67±0.58[a]
Creatinine (µmol/L)	63.67±2.89[a]	54.00±4.00[a]	52.33±13.65[a]	52.00±1.00[a]	60.00±10.44[a]	60.67±13.43[a]

Note: Different superscript letters within a row indicate significant differences among groups ($P < 0.05$). AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, GGT: Gamma-Glutamyl Transferase.

AST, ALT, and GGT are intracellular enzymes normally confined within hepatocytes. Disruption of cellular integrity, particularly due to oxidative damage, results in their release into circulation, making them reliable biomarkers of liver injury.[30–34] Thus, a reduction in these enzyme levels following treatment is a key indicator of hepatoprotective activity.[35] In this study, treatment with Silymarin and aqueous leaf extracts of *H. lanceolata*, *H. hirsuta*, and *H. isora* effectively restored AST, ALT, and GGT levels toward normal. Notably, *H. lanceolata* and *H. isora* extracts yielded enzyme levels comparable to those in the control group, suggesting significant hepatoprotective effects. The observed recovery may be attributed to the antioxidant properties of the extracts, which likely contributed to the regeneration of hepatocytes and restoration of liver parenchyma.[36]

Antioxidants play a critical role in neutralizing free radicals and mitigating oxidative stress, thereby preventing membrane damage and supporting hepatic recovery. These protective effects are mediated either through direct radical scavenging or by enhancing endogenous antioxidant defense systems.[37] In addition to liver function markers, kidney function was assessed by measuring serum creatinine, a key biochemical indicator for renal impairment.[33] Previous studies have shown that significant elevations in serum creatinine may reflect a loss of approximately 50% of renal function.[20] However, in the present study, no statistically significant differences in serum creatinine levels were observed among the experimental groups. These findings suggest that the administered dose of CCl_4 (0.5 mL/kg) did not induce detectable nephrotoxicity in 25 g male mice.

Hematological parameters were also evaluated. Most hematological parameters, including RBC, Hb, HCT, MCV, MCH, MCHC, MPV, PDW, and WBC, showed no significant differences across groups ($P > 0.05$) (Table 4). However, the blood glucose concentration in the CCl_4-only group (88.67 ± 49.66 mg/dL) was significantly lower than that of the control group (385.67 ± 35.08 mg/dL) ($P < 0.05$).

Table 4: Hematological Parameters in Experimental Mice (Mean ± SD, n = 3-5)

Parameter	Control	Silymarin	CCl_4	*H. lanceolata*	*H. hirsuta*	*H. isora*
Glucose (mg/dL)	385.67±35.08[c]	124.67±17.90[ab]	88.67±49.66[a]	170.67±20.50[b]	145.33±58.01[ab]	153.00±17.58[ab]
RBC (10^6/mL)	8.47±1.08[a]	9.02±0.66[a]	8.76±0.67[a]	7.65±0.20[a]	8.64±1.00[a]	9.01±0.90[a]
Hb (g/dL)	12.53±1.42[ab]	13.10±0.56[b]	12.63±1.18[ab]	10.23±0.60[a]	11.37±2.19[ab]	12.43±1.01[ab]
HCT (%)	48.40±6.33[a]	47.80±5.47[a]	48.03±4.14[a]	40.23±1.89[a]	43.93±7.11[a]	48.37±4.17[a]
MCV (µm³)	57.13±0.21[b]	52.00±4.36[a]	54.80±1.04[ab]	52.63±1.16[a]	51.50±2.70[a]	53.70±0.70[ab]
MCH (pg)	14.87±0.31[a]	14.43±0.31[ab]	14.40±0.26[ab]	13.40±0.50[a]	13.27±1.31[a]	13.80±0.36[ab]
MCHC (g/dL)	25.97±0.67[a]	25.87±0.71[a]	26.23±0.38[a]	25.43±0.49[a]	25.80±1.40[a]	25.70±0.46[a]
PCT (%)	0.66±0.05[b]	0.42±0.03[a]	0.69±0.25[b]	0.71±0.03[b]	0.72±0.02[b]	0.60±0.16[ab]
MPV (µm³)	7.50±0.00[a]	7.53±0.35[a]	7.90±0.44[a]	7.77±0.06[a]	7.57±0.21[a]	7.70±0.62[a]
PDW (µm³)	9.27±0.06[a]	10.53±1.21[a]	10.37±1.25[a]	10.23±0.06[a]	9.73±0.61[a]	10.13±1.50[a]
WBC (10^3/mL)	7.93±1.13[a]	9.62±1.87[a]	8.22±2.33[a]	6.49±2.31[a]	8.85±3.15[a]	10.09±4.45[a]

Note: Different superscript letters within a row indicate significant differences among groups ($P < 0.05$). RBC: Red Blood Cell count, Hb: Hemoglobin concentration, HCT: hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, PCT: Plateletcrit, MPV: Mean Platelet Volume; PDW: Platelet Distribution Width; WBC: White Blood Cell count.

Treatment with Silymarin and Helicteres extracts partially restored blood glucose levels toward normal. As shown in Table 4, most hematological indices remained unchanged across all groups ($P > 0.05$), indicating no systemic hematotoxicity under the experimental conditions. Interestingly, CCl_4-treated mice exhibited significantly lower blood glucose levels (88.67 ± 49.66 mg/dL) compared to the control group (385.67 ± 35.08 mg/dL) ($P < 0.05$), consistent with previous reports indicating hypoglycemia as a secondary effect of hepatocellular damage and impaired gluconeogenesis.[38] However, treatment with Silymarin or aqueous leaf extracts of Helicteres species partially restored blood glucose levels to normal, suggesting a limited effect of these interventions on glucose metabolism.

Body weight gain and Organ index

As shown in Figure 2, after 21 days of experimentation, the control group exhibited the highest body weight gain ratio (16.52 ± 2.29%). In contrast, the CCl_4-only group showed a significantly lower weight gain (3.19 ± 2.81%) ($P < 0.05$). Mice treated with Silymarin (2.71 ± 1.15%), *H. lanceolata* extract (4.56 ± 2.76%), and *H. hirsuta* extract (7.43 ± 3.20%) also showed relatively low weight gain, not significantly different from the CCl_4 group ($P > 0.05$). However, mice treated with *H. isora* extract showed a significantly higher body weight gain (9.27 ± 2.53%) compared to the untreated CCl_4 group ($P < 0.05$), indicating a partial protective effect.

CCl_4-treated animals typically exhibit clinical signs such as reduced activity, anorexia, poor nutrient absorption, dull fur, fatigue, and diminished weight gain.[39] As a result, body weight loss is a hallmark of CCl_4-induced hepatotoxicity.[20] In this study, rats in the untreated CCl_4 group, as well as those treated with Silymarin, *H. lanceolata*, or *H. hirsuta* extracts, showed significantly lower weight gain compared to the control group (Figure 2). Interestingly, rats treated with *H. isora* extract demonstrated improved weight gain, though still lower than control values, suggesting a potential role in supporting metabolic recovery. The results of organ weight analysis are summarized in Table 5. No .significant differences were observed in the weights of the heart, lungs, kidneys, spleen, pancreas, or testes among the groups ($P > 0.05$), except for the liver. The CCl_4-only group exhibited a significantly higher liver index (5.27 ± 0.14%) compared to the control group (4.42 ± 0.33%) ($P < 0.05$).

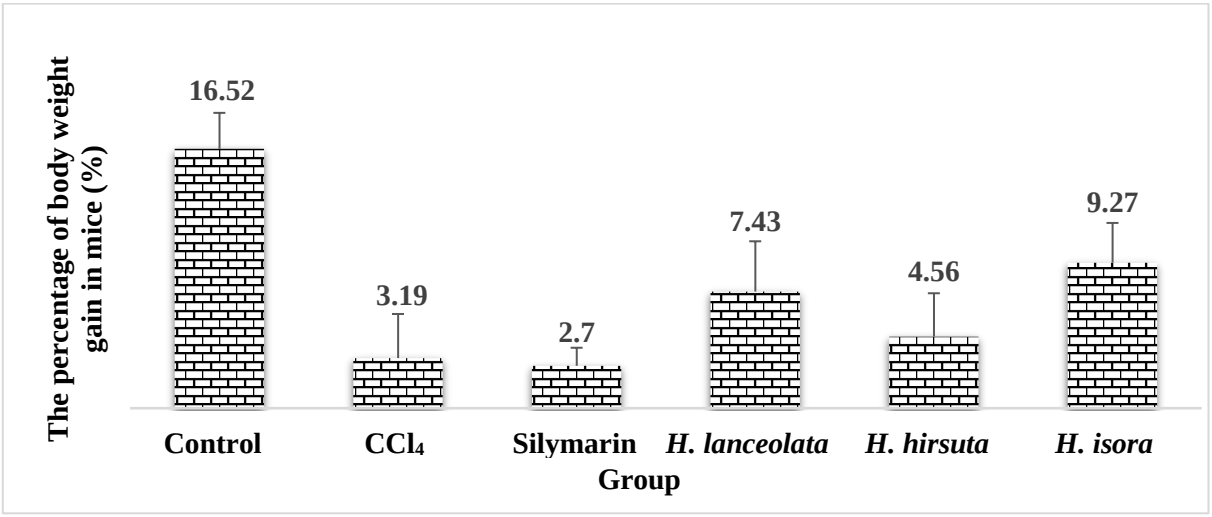

Figure 2: Body weight gain ratio (BWG%) of mice after 21 days of treatment. Data are expressed as mean ± SD ($n = 5$). Different letters indicate significant differences among groups ($P < 0.05$).

Table 5: Organ Weight Indices of Experimental Mice (Mean ± SD, n = 3-5)

Organ	Control	CCl_4	Silymarin	*H. lanceolata*	*H. hirsuta*	*H. isora*
Heart (%)	0.52±0.16^{a}	0.58±0.19^{a}	0.44±0.05^{a}	0.48±0.09^{a}	0.48±0.06^{a}	0.45±0.02^{a}
Liver (%)	4.42±0.33^{a}	5.27±0.14^{b}	4.78±0.83^{ab}	5.13±0.26^{ab}	5.38±0.13^{b}	5.46±0.49^{b}
Lungs (%)	0.63±0.07^{ab}	0.53±0.05^{a}	0.76±0.11^{bc}	0.69±0.14^{abc}	0.82±0.08^{c}	0.80±0.10^{bc}
Kidneys (%)	1.21±0.09^{ab}	1.37±0.07^{abc}	1.31±0.27^{abc}	1.12±0.16^{a}	1.49±0.08^{bc}	1.54±0.20^{c}
Spleen (%)	0.66±0.23^{a}	1.08±0.40^{a}	0.86±0.03^{a}	0.82±0.12^{a}	0.91±0.21^{a}	0.83±0.12^{a}
Pancreas (%)	0.40±0.02^{a}	0.37±0.04^{a}	0.38±0.06^{a}	0.46±0.12^{a}	0.41±0.08^{a}	0.42±0.05^{a}
Testes (%)	0.59±0.14^{a}	0.72±0.06^{ab}	0.68±0.10^{ab}	0.71±0.08^{ab}	0.79±0.12^{b}	0.66±0.03^{ab}

Note: Different superscript letters within a row indicate statistically significant differences among groups ($P < 0.05$).

No treatment group showed significant improvement in liver index compared to the CCl_4-only group. Organ weight indices were also evaluated as indicators of physiological changes. No significant differences were observed in the weights of the heart, lungs, kidneys, spleen, pancreas, or testes among the groups (Table 5). However, liver weight was significantly elevated in the untreated CCl_4 group relative to the control group, which may be attributed to triglyceride accumulation, impaired lipoprotein secretion, or CCl_4-induced inflammation, infiltration, and fibrosis.[40,41] Although liver weights were lower in the groups treated with Silymarin and Helicteres extracts, the differences were not statistically significant ($P > 0.05$).

Macroscopic Morphology of Internal Organs

Macroscopic examination of internal organs revealed distinct differences between the control and treated groups. In the control group, the liver exhibited a smooth, glossy surface with a soft texture and a characteristic dark red coloration. In contrast, the liver of CCl_4-treated mice (untreated group) appeared swollen and pale, with a rough and firm surface—indicative of hepatic damage (Figure 3).

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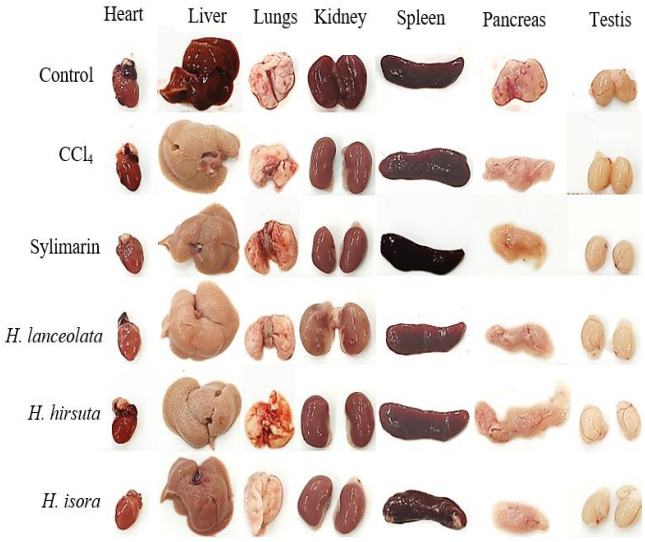

Figure 3: Macroscopic morphology of selected internal organs from mice in different treatment groups.

Mice treated with Silymarin or Helicteres extracts showed varying degrees of morphological improvement. These livers exhibited reduced swelling, smoother surfaces, and more natural coloration compared to the CCl_4 group. Among these, the silymarin-treated and *H. isora*-treated groups showed the most pronounced recovery in gross liver morphology, whereas the *H. lanceolata* and *H. hirsuta* groups showed milder improvements.

No obvious morphological abnormalities were observed in other internal organs (heart, lungs, kidneys, spleen, pancreas, and testes) across all groups. These organs maintained a normal appearance, with smooth surfaces and no visible signs of congestion, necrosis, or fibrosis (Figure 3).

Livers from CCl_4-treated mice showed swelling and pallor, while treatment with Silymarin or *H. isora* extract alleviated visible liver damage. The liver in the control group shows a smooth, shiny surface with dark red coloration, indicating normal hepatic condition. In contrast, the CCl_4-treated group exhibits marked pathological changes, including pale color, swelling, surface roughness, and firm texture—characteristics consistent with hepatotoxicity. Treatment with Silymarin and *H. isora* extract resulted in noticeable improvements in liver morphology, with reduced swelling and a more natural coloration.

Milder improvements were observed in the H. lanceolata and H. hirsuta groups.

No macroscopic abnormalities were observed in the heart, lungs, kidneys, spleen, pancreas, or testis across all groups, as these organs appeared smooth and free from visible lesions, congestion, necrosis, or fibrosis.

Histological Observation of Liver Tissues

To further support the biochemical findings, histopathological examination of liver tissues was conducted. In the control group (Figure 4A), hepatocytes were uniform in size, arranged in regular hepatic cords radiating toward the central vein. Sinusoidal capillaries were clearly visible between hepatocyte rows. No lipid droplets or signs of necrosis were observed. In contrast, the CCl_4-only group (Figure 4B) showed disorganized hepatic architecture, hepatocellular swelling, and indistinct sinusoidal spaces. Numerous phagocytic cells were found infiltrating the tissue. Hepatocytes displayed cytoplasmic vacuolation due to lipid accumulation, and several nuclei showed signs of degeneration or condensation. Groups treated with Silymarin (Figure 4C) and Helicteres extracts (Figure 4D–F) showed varying degrees of structural improvement. Hepatocyte arrangement became more organized, cytoplasmic swelling was reduced, and sinusoidal spaces became more distinguishable. The *H. isora*-treated group exhibited the most notable restoration of normal hepatic architecture among the extract-treated groups.

As shown in Table 6, mice in the CCl_4-only group exhibited the largest hepatocyte diameter (30.63 ± 2.57 µm), significantly higher than that of all other groups ($P < 0.05$). Conversely, this group had the smallest nuclear diameter (7.23 ± 1.68 µm), suggesting hepatocellular swelling and nuclear condensation; hallmarks of cellular damage. The nuclear-to-cell diameter ratio is a useful histological indicator of hepatic integrity. A higher ratio generally indicates healthier hepatocytes, with balanced cell and nuclear size.

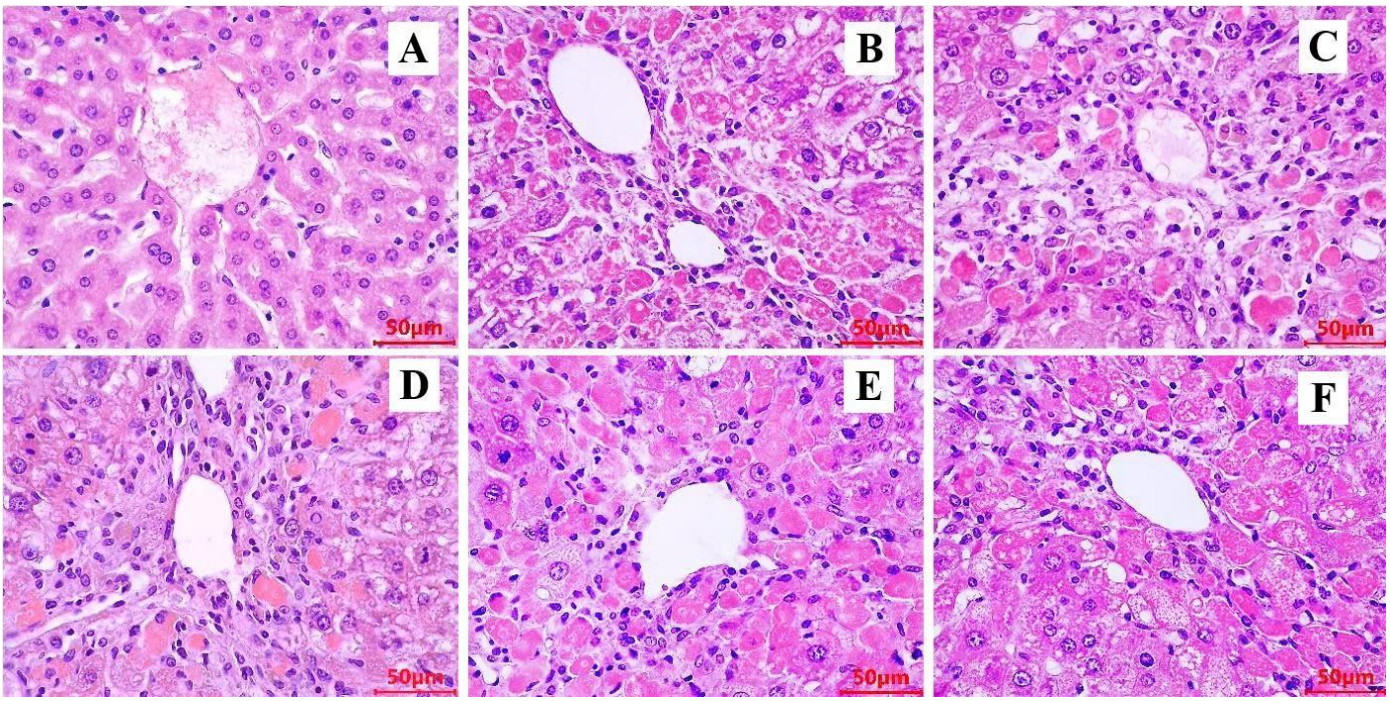

Figure 4: Histological sections of mouse liver stained with hematoxylin and eosin (H&E), observed at 400× magnification. *A: Control; B: CCl_4; C: Silymarin; D: H. lanceolata; E: H. hirsuta; F: H. isora.*

Table 6: The cell diameter, nucleus diameter, and the nucleus-to-cell diameter ratio of mouse hepatocytes

Group	Hepatocyte Diameter (µm)	Nuclear Diameter (µm)	Nucleus/Cell Diameter Ratio
Control	18.82 ± 1.24[a]	8.69 ± 1.46[b]	0.46 ± 0.08[b]
CCl_4	30.63 ± 2.57[d]	7.23 ± 1.68[a]	0.24 ± 0.05[a]
Silymarin	21.79 ± 2.23[bc]	11.08 ± 1.70[c]	0.51 ± 0.09[c]
H. lanceolata	21.71 ± 2.03[bc]	11.74 ± 1.71[d]	0.54 ± 0.08[d]
H. hirsuta	21.47 ± 1.69[b]	12.30 ± 1.50[de]	0.57 ± 0.08[e]
H. isora	22.27 ± 2.02[c]	11.91 ± 1.62[e]	0.54 ± 0.08[d]

Note: Data are presented as mean ± standard deviation (n = 30). Different superscript letters in the same column indicate significant differences among groups ($P < 0.05$).

The ratio was lowest in the CCl_4 group (0.24 ± 0.05), confirming severe hepatocellular damage. In contrast, the highest ratios were observed in the groups treated with *H. hirsuta* (0.57 ± 0.08), *H. isora* (0.54 ± 0.08), and *H. lanceolata* (0.54 ± 0.08), followed by Silymarin (0.51 ± 0.09). The control group had a moderate ratio (0.46 ± 0.08). These results suggest that Helicteres extracts, particularly *H. hirsuta* and *H. isora*, may offer substantial protection against CCl_4-induced hepatic injury, as indicated by their ability to maintain a healthy nucleus-to-cell size balance.

The liver sections from untreated CCl_4-exposed mice exhibited severe architectural disruption, including hepatocyte disarray, cytoplasmic swelling, indistinct sinusoidal capillaries, and infiltration of phagocytic cells—hallmarks of hepatocellular damage. Lipid droplets were abundant in the cytoplasm, leading to cell enlargement and signs of lipid degeneration. The hepatocyte nuclei were irregular in shape (Figure 3-B), with many displaying condensation or degeneration, consistent with previous observations.[42,43]

Mice treated with Silymarin following CCl_4-induced liver injury demonstrated marked histological improvements (Figure 3C). Although some hepatocytes still contained lipid droplets, these were fewer and smaller in size, resulting in reduced cytoplasmic swelling. Hepatocytes were more regularly arranged around the central vein, and sinusoidal capillaries became distinguishable. Nuclear morphology normalized, and infiltration of phagocytic cells was significantly reduced. Furthermore, signs of liver regeneration, including binucleated hepatocytes, were observed—supporting the hepatoprotective role of Silymarin.[25,44] Similarly, liver sections from CCl_4-treated mice administered Helicteres leaf extracts also showed histological improvements (Figure 3D–F). Hepatocyte swelling was reduced, and the nuclei appeared round and regular. While lipid droplets were still present, they were fewer and smaller than those in the untreated group. Some areas of necrosis and degenerative changes remained, indicating partial recovery.

Histological examination of the kidneys revealed normal structural features, including well-defined glomeruli, clear urinary spaces, and intact proximal and distal convoluted tubules. The lung tissue showed preserved architecture with visible bronchioles, arterioles, and alveolar sacs. Longitudinal sections of heart tissue demonstrated normal cardiac muscle fibers, with visible striations and intercalated discs, although the alignment of fibers appeared slightly irregular. In the spleen, the reticular cell network and supporting reticular fibers were clearly observed, with cells properly distributed within the lacunar spaces. The pancreas showed intact exocrine acini and distinct islets of Langerhans, without any observable abnormalities. Cross-sections of testicular tissue revealed normal seminiferous tubule architecture, with identifiable Leydig cells, spermatogonia, spermatocytes, and spermatozoa, indicating active spermatogenesis (Figure 5)

These histopathological findings were consistent with the biochemical results. The elevated levels of AST, ALT, and GGT strongly correlated with hepatocellular membrane disruption, including central lobular necrosis, swelling, inflammatory infiltration, and steatosis—hallmarks of CCl_4-induced oxidative injury. [29,45] To quantitatively support these observations, hepatocyte and nuclear diameters were measured around the central vein.

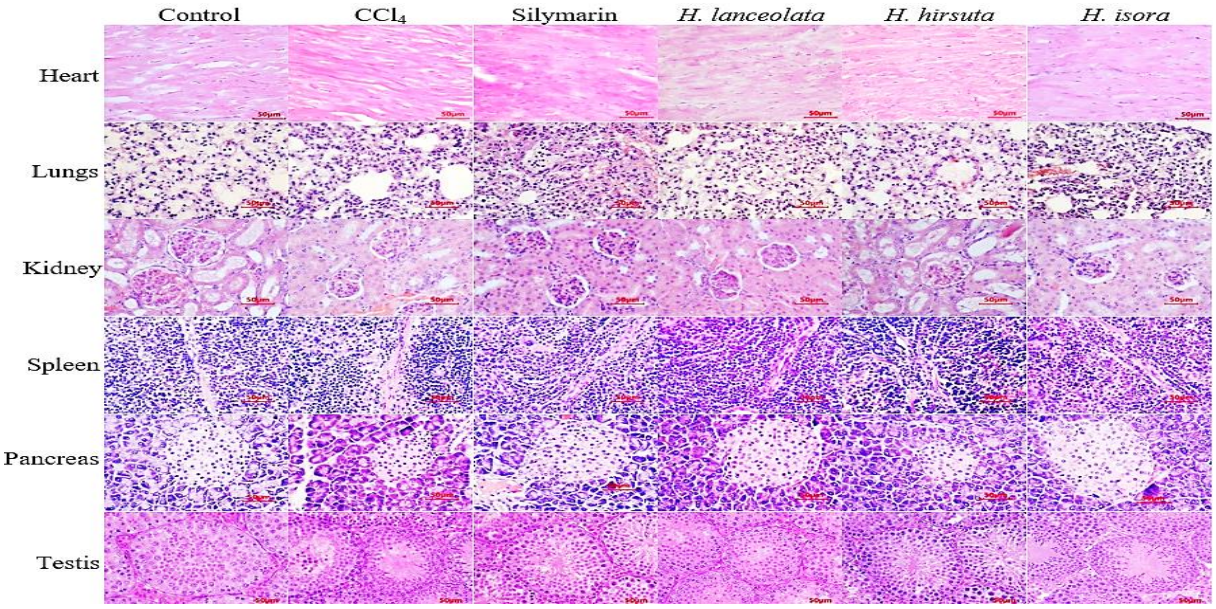

Figure 5: Histological sections of internal organs stained with hematoxylin and eosin (H&E) at 400× magnification.

Treatment with Helicteres leaf aqueous extracts resulted in significant improvement in the nucleus-to-cell diameter ratio, suggesting reduced swelling and restoration of nuclear morphology.[46] Additionally, histological evaluation of other internal organs—including the heart, lungs, kidneys, spleen, pancreas, and testes—revealed no abnormal changes across the experimental groups.[47] This supports the organ-specific hepatotoxicity of CCl_4 under the conditions of this study and indicates that the administered Helicteres spps leaf aqueous extracts did not induce pathological alterations in non-hepatic tissues.

Conclusion

In conclusion, aqueous leaf extracts of *Helicteres lanceolata*, *Helicteres hirsuta*, and *Helicteres isora* at a dose of 400 mg/kg exhibited notable hepatoprotective effects in CCl_4-induced liver injury in mice, comparable to those of the standard hepatoprotective drug Silymarin. This was evidenced by the significant reduction in serum aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transferase levels, restoration of hepatocyte morphology and nuclear integrity, and improvements in liver histoarchitecture. The hepatoprotective properties of these leaf extracts are likely attributable to their strong antioxidant activities, which mitigate lipid peroxidation by scavenging free radicals and enhancing endogenous antioxidant defense mechanisms. Among the three species, *H. isora* leaves demonstrated the most pronounced antioxidant and hepatoprotective efficacy. These findings suggest that *Helicteres* species, particularly *H. isora* leaves, hold promise as potential sources of natural hepatoprotective agents. Further investigations are warranted to isolate active compounds and elucidate the molecular mechanisms underlying their protective effects.

Conflict of interest

The author declares no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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