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Original Research Article

Antioxidant, Hepatoprotective and Toxicological Assessment of *Dillenia ovata* in Mice

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ABSTRACT

Dillenia ovata Wall. (DOW), collected from An Giang province, Vietnam, has been used traditionally in folk medicine. This study investigated the antioxidant and hepatoprotective potential of the crude ethanolic extract of *Dillenia ovata*. The antioxidant potential of the extract was evaluated in vitro using DPPH radical scavenging, reducing power (RP), and total antioxidant capacity (TAC) tests. Hepatoprotective effects were assessed in a Carbon tetrachloride (CCl_4)–induced liver injury model, where mice received *Dillenia ovata* extract at doses of 100, 200, or 400 mg/kg for 28 days. Biochemical analyses revealed that *Dillenia ovata*, especially at 400 mg/kg, significantly attenuated CCl_4–induced elevations in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and malondialdehyde (MDA), while restoring glutathione (GSH) levels. Histopathological examination further confirmed the protective effect by showing improved hepatic architecture. *In vivo* safety evaluation showed no signs of toxicity in both acute and sub-chronic studies in mice. These findings suggest that *Dillenia ovata* extract exhibits potent antioxidant and hepatoprotective activities, highlighting its potential as a promising source of bioactive natural products.

Keywords: *Dillenia ovata*, Antioxidant Activity, Hepatoprotection, CCl_4–Induced Liver Injury, Medicinal Herbs.

Introduction

The liver is a vital organ that regulates metabolic homeostasis by overseeing the metabolism, synthesis, and distribution of carbohydrates, lipids, and vitamins throughout the body. Therefore, the liver is a major site of free radical generation due to its high metabolic activity [1]. Overproduction of free radicals can cause oxidative stress, which damages cellular structures. The liver, being constantly exposed to agents such as alcohol, toxins, excess lipids, and metabolic byproducts, is particularly susceptible to oxidative stress. This condition can lead to hepatic inflammation and degeneration, and if sustained over time, may progress to chronic liver disease [2]. Oxidative stress in hepatic tissue disrupts the activity of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), while promoting lipid peroxidation (LPO) in the liver [3]. Currently, liver disease is a major global health problem, responsible for about 2 million deaths each year, or nearly 4% of all deaths worldwide. Cirrhosis and other chronic liver disorders are among the foremost causes of mortality worldwide, largely driven by viral hepatitis, alcohol abuse, and non-alcoholic fatty liver disease [4]. The use of natural compounds from herbs with antioxidant activity is potentially beneficial. Therefore, identifying herbs with antioxidants and hepatoprotective properties is an essential first step toward developing safe and effective liver-protective medicines. *Dillenia ovata* (DOW), a species within the Dilleniaceae family, is commonly distributed in mountainous forest regions and occurs in several Southeast Asian countries.

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Dillenia ovata has long been widely used to treat ailments such as diarrhea, arthritis, and infections. *Dillenia ovata* contains various phytochemicals, including alkaloids, flavonoids, and saponins, which contribute to its antimicrobial properties. Its extracts exhibit strong antibacterial activity against *Vibrio parahaemolyticus*, indicating potential for disease control and treatment [5]. The n-hexane extract effectively inhibits bacterial growth, outperforming methanol and ethyl acetate extracts [6]. *Dillenia ovata* exhibited moderate inhibitory activity against α-amylase and α-glucosidase, together with strong antioxidant and anti-acetylcholinesterase effects. Notably, its inhibitory activity against major enzymes implicated in diabetes and Alzheimer's disease has been investigated [4]. However, to date, there have been no reports on the antioxidant and hepatoprotective activities of *Dillenia ovata* grown in An Giang province, located in the Mekong Delta of Vietnam. Therefore, evaluating its liver-protective potential *in vivo* is essential. Such findings would provide a scientific basis for developing natural products as alternative therapies aimed at reducing the unwanted effects of current drugs. This study investigated the toxicity, as well as the antioxidant and hepatoprotective potential of *Dillenia ovata* in Carbon tetrachloride (CCl_4)–induced liver injury in mice.

Materials and Methods

Preparation of Dillenia ovata extracts

Dillenia ovata were collected from An Giang province, Vietnam. Samples were dried at ambient conditions and finely ground using a DFY-600 universal high-speed grinder (Linda Machinery Co., Ltd, China) to obtain powdered material. Dried powder (470g) was extracted with 96% ethanol and centrifuged to collect the supernatant, with the procedure repeated three times. The solvent was removed under reduced pressure at 50 °C using a rotary evaporator (R-3001, GWSI, Japan) and kept at −20 °C until later analysis.

In vitro antioxidant activity

Scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals

The antioxidant capacity of *Dillenia ovata* was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

according to the modified method of Baliyan S et al., (2022) [7]. The reaction mixture consisted of 40 µL of DPPH solution (1000 µg/mL) and 960 µL of the plant extract. Incubation was carried out at 30 °C for 30 minutes in the absence of light. After incubation, the absorbance of the reaction mixture was recorded at 517 nm. Trolox served as the reference antioxidant.

Reduce power assay
The reducing power (RP) of *Dillenia ovata* extract was determined according to the modified method described by Oyaizu et al., (1986) [8]. To evaluate reducing power, 0.5 mL of ethanol extract at varying concentrations was mixed with equal volumes of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The reaction mixture was maintained at 50°C for 20 min, followed by the addition of 0.5 mL of 10% trichloroacetic acid (Sigma, Germany). The samples were then centrifuged at 3000 rpm for 10 min to separate the supernatant. An aliquot of 0.5 mL from the upper layer was mixed with 0.5 mL of distilled water and 0.1 mL of 0.1% $FeCl_3$ solution. The absorbance of the reaction mixture was recorded at 700 nm.

Total antioxidant capacity (TAC)
To assess the total antioxidant activity, the procedure was carried out based on the modified method of Prieto et al. (1999) [9]. To perform the assay, extracts of different concentrations (300 µL) were reacted with 900 µL of the reagent mixture consisting of sulfuric acid (0.6 M), sodium phosphate (28 mM), and ammonium molybdate (4 mM). The reaction mixture was maintained at 95°C for 90 min, and the absorbance was subsequently recorded at 695 nm.

Experimental animals and toxicity test
Healthy male mice (*Mus musculus*), weighing between 30–35 g, were used. The animals were provided by the Pasteur Institute, Ho Chi Minh City, Vietnam. During the experimental period, the mice were maintained under standard laboratory conditions at room temperature in the laboratory of the Department of Biology, College of Natural Sciences, Can Tho University. The animals were housed in clean plastic cages with free access to standard pellet feed and water, and a 12:12 h light–dark cycle was maintained throughout the study. Experiments were conducted with approval from the Animal Ethics Committee of Can Tho University, under Code CTU-AEC25010. The toxicity of *Dillenia ovata* was checked by acute and sub-chronic toxicity.

Acute toxicity study
The acute toxicity assay followed the guidelines of the Organization for Economic Cooperation and Development (OECD) [10]. Experimental groups received the *Dillenia ovata* ethanol extract by oral gavage at single doses of 0 or 5000 mg/kg. Both treated and control animals were monitored continuously for the first 3 h and subsequently once every 24 h for a total of 14 days, with observations focused on signs of toxicity and mortality. Following the 14-day experimental period, weight gain, blood glucose levels, liver enzymes, kidney function markers, and hematological indices were analyzed.

Sub-chronic toxicity study
This experiment was investigated following OECD 407 guidelines [11]. The mice received oral doses of *Dillenia ovata* extract (400 mg/kg/day) for 150 consecutive days. At the end of the experimental period, weight gain, blood glucose levels, liver enzyme activities, kidney function markers, and hematological indices were evaluated.

In vivo hepatoprotective activity of Dillenia ovata extract
The hepatoprotective effect was assessed according to the procedure of Kang and Koppula (2014) [12] with modifications. Mice were divided into 7 treatment groups, with 5 mice per group:
Group 1: Normal control
Group 2: Mice administered olive oil and 1% DMSO
Group 3: Mice administered CCl_4 and 1% DMSO
Group 4: Mice administered CCl_4 and silymarin at a dose of 16 mg/kg
Groups 5 to 7: Mice administered CCl_4 and treated with *Dillenia ovata* extract at doses of 100, 200, and 400 mg/kg, respectively.
After 28 days of treatment, the following parameters were recorded: body weight, relative liver weight (liver-to-body weight ratio) and macroscopic liver appearance. The blood sample was centrifuged (3000 rpm,10 min at room temperature) for the isolation of plasma. Plasma ALT and AST activities were assessed using a semi-automated biochemical analyzer (Erba Chem-7m, Germany). Hepatic malondialdehyde (MDA) and glutathione (GSH) levels were analyzed to evaluate the effect of *Dillenia ovata* extract on endogenous oxidant and antioxidant balance in mice. The quantification of MDA and GSH was conducted following the methodologies established by Ohkawa et al., (1979) and Moron et al., (1979) respectively [13,14].

Histopathological observation
For histopathological examination, a portion of the liver was collected and fixed with 4% formaldehyde for a minimum of 24 hours. Subsequently, these tissues were gradually dehydrated with ethanol, cleared in xylene, and embedded in paraffin [15]. For histological analysis, liver specimens were trimmed into 5 µm sections using a microtome (Leica, Germany) before being stained with hematoxylin and eosin. These sections were observed under the microscope to evaluate histopathological changes.

Statistical Analysis
All data were processed using Microsoft Excel (Microsoft Corp., Washington, USA). Statistical evaluation and graph plotting were conducted with GraphPad Prism 10 software (GraphPad Software, LLC, USA). One-way ANOVA followed by Dunnett's multiple comparison test was employed to assess statistical significance among groups. Differences were considered statistically significant when the P-value was below 0.05.

Results and Discussion

In vitro antioxidant activity of Dillenia ovata extract
Plant extract antioxidants have potential health benefits in mitigating oxidative stress-related diseases. The antioxidant activity of medicinal plants is multifaceted and can be affected by various factors. Therefore, employing multiple analytical methods is recommended when assessing their antioxidant potential [16]. In this study, the antioxidant activities of *Dillenia ovata* extract were investigated using DPPH, RP and TAC assays. The antioxidant potential of *Dillenia ovata* extract was assessed *in vitro* using DPPH, reducing power (RP), and total antioxidant capacity (TAC) assays, with Trolox serving as the reference antioxidant. The concentrations required to scavenge 50% of the radicals (Half maximal inhibitory concentration, IC_{50} values) are presented in Table 1. In the DPPH assay, the *Dillenia ovata* extract showed an IC_{50} of 5.00 ± 0.07 µg/mL, which was significantly higher than that of Trolox (0.65 ± 0.01 µg/mL). The reducing power of *Dillenia ovata* (1.86 ± 0.11) was comparable to that of Trolox (1.92 ± 0.11). In contrast, the total antioxidant capacity (TAC) of *Dillenia ovata* (0.81 ± 0.04 µg/mL) was significantly lower than that of Trolox (35.02 ± 0.40 µg/mL), reflecting the extract's antioxidant potential.

Table 1: *In vitro* antioxidant effect of *Dillenia ovate extract* and Trolox

	Antioxidant	***Dillenia ovate* extract**	**Trolox**
IC_{50} (µg/mL)	DPPH	5.00 ± 0.07	0.65 ± 0.01
	RP	1.86 ± 0.11	1.92 ± 0.11
	TAC	0.81 ± 0.04	35.02 ± 0.40

Oral acute and sub – chronic toxicity study
As shown in Table 2 and Figure 1, mice treated with *Dillenia ovata* extract exhibited neither acute nor sub-chronic toxicity. After 14 days, mice receiving a 5000 mg/kg dose of *Dillenia ovata* extract showed no signs of acute toxicity or mortality. Weight gain, a sensitive indicator of general health, remained stable in the treatment group compared with controls, suggesting no toxic effects (Table 2). Similarly, blood glucose levels were unchanged, indicating that *Dillenia ovata* extract did not impair metabolic or organ function.

Biochemical analyses further confirmed the absence of toxicity: liver function markers (ALT and AST), commonly used to detect hepatocellular injury [17], showed no significant differences between treated and control groups.

Table 2: Observed parameters in acute and sub-chronic toxicity studies

Parameters	Unit	Acute toxicity		Sub-chronic toxicity	
		Control	DOW	Control	DOW
Weight gain	g	0.54±0.21	1.90±0.24	31.30±1.60	35.02±0.87
Blood glucose	mg/dL	163.60±12.72	165.20±10.18	129.60±8.44	145.80±5.09
AST	U/L	123.20±30.90	221.40±87.91	111.60±20.01	59.40±14.48
ALT	U/L	35.40±16.01	112.60±147.85	48.00±12.23	42.20±10.69
Urea	mmol/L	2.38±0.91	3.60±1.14	4.64±2.22	6.96±0.71
Creatinine	mmol/L	54.00±11.40	66.20±11.05	84.00±27.02	80.20±7.09
WBC	$10^3/mm^3$	4.02±0.63	4.66±0.93	7.28±1.38	7.50±0.90
NEU	%	19.24±2.89	10.88±2.04	10.54±1.97	17.90±2.21
LYM	%	69.22±5.08	80.62±2.70	81.22±2.19	70.00±1.56
MONO	%	10.04±2.59	7.04±1.91	6.96±0.97	11.06±1.59
RBC	%	9.29±0.39	8.29±2.13	9.54±0.57	9.75±0.52
HGB	g/dL	15.34±0.90	13.58±3.60	15.20±0.86	15.08±0.54
PLT	$10^3/mm^3$	1009.80±124.40	607.80±222.50	809.40±123.50	773.40±93.40

Note: AST - aspartate aminotransferase, ALT - alanine aminotransferase; WBC - White blood cell; NEU – Neutrophils; LYM – Lymphocyte; MONO – Monocyte; BASO – Basophils; RBC - Red blood cell; HGB – Haemoglobin; PLT - Platelet Count.

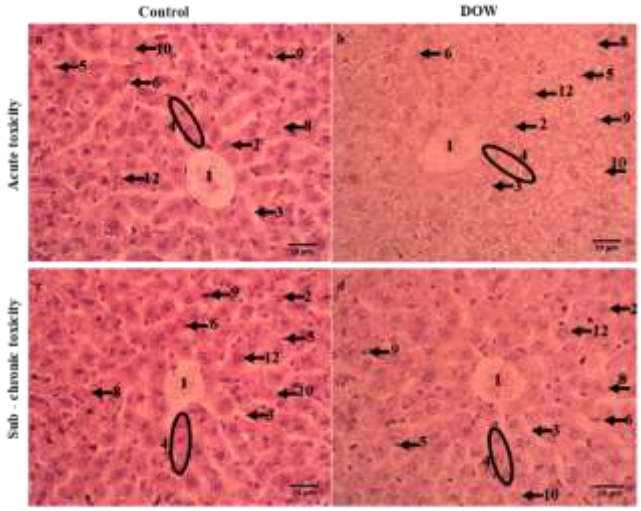

Figure 1: Hematoxylin and eosin-stained sections of liver tissue in acute and sub – chronic toxicity. (a-c) Normal control (distilled water); (b) DOW at 5000 mg/kg; (d) DOW at 400 mg/kg. Note: *1: Vein; 2: Hepatocyte; 3: Hepatic sinusoid; 4: Hepatocyte cord; 5: Mononuclear cell; 6: Kupffer cell; 7: Bile duct; 8: Karyolytic cell; 9: Pyknotic cell; 10: Karyoless cell; 11: Lipid droplet; 12: Swollen hepatocyte.*

Kidney function markers (urea and creatinine) also remained within normal ranges [18], indicating no renal impairment. Hematological parameters were unaffected as well. Red blood cell counts remained stable, and white blood cell subtypes (lymphocytes, neutrophils, monocytes) were not significantly altered, suggesting no immunological or inflammatory response [19]. Moreover, no signs of organ hypertrophy—a typical early marker of chemical toxicity—were observed. As shown in Figure 1, mice administered *Dillenia ovate extract* at a dose of 5000 mg/kg showed no evidence of tissue fibrosis or inflammation. Overall, these findings demonstrate that *Dillenia ovata* extract, even at elevated doses of 5000 mg/kg, is safe and does not induce toxicity in mice during the study period. Subchronic toxicity studies are essential for determining appropriate dosages for long-term research, as they assess the effects of repeated exposure to substances over a significant portion of an animal's lifespan [20]. Sub-chronic toxicity was assessed by administering 400 mg/kg/day of DOW extract to mice for 150 days (Table 2). The final body weight of treated mice (31.30 ± 1.60 g) exhibited no significant variation from the control group (35.02 ± 0.87 g). Blood glucose levels (129.60 ± 8.44 mg/dL) were also comparable to controls (145.80 ± 5.09 mg/dL). In addition, ALT, AST, urea, and creatinine levels showed no significant differences between groups, indicating no liver or kidney injury. Hematological profiles were likewise unaffected, with no significant alterations in red or white blood cell counts following long-term administration of *Dillenia ovata* extract. Furthermore, no evidence of tissue degeneration was observed in histopathological staining (Figure 1).

Effect of Dillenia ovata on body weight and liver-to-body weight ratio in CCl_4–exposed mice

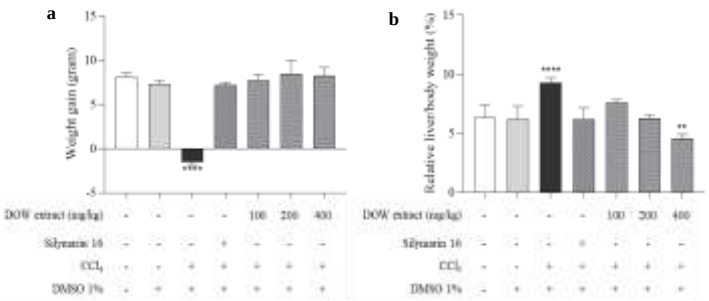

Figure 2: Effect of CCl_4, DOW extract and silymarin on body weight (a) and relative organ weights of liver (b) in mice. Data are presented as mean ± SEM. Significance levels: **P < 0.01, ****P < 0.0001.

Hepatic diseases are recognized as major metabolic disorders, contributing markedly to global illness and death rates. Consequently, medicinal plants with hepatoprotective potential have attracted significant scientific interest. In recent years, many experimental studies have evaluated the liver-protective activities of herbal extracts in animal models [21]. This work evaluated the hepatoprotective potential of *Dillenia ovata* against CCl_4–induced liver injury. Body weight is one of the key indicators reflecting the health status of an organism.

Therefore, the body weight gain and the relative ratio between body weight and liver weight were recorded in mice across different experimental groups. The increase in body weight after 28 days of treatment is presented in Figure 2.

After 28 days of treatment, an elevation in body weight was observed across all experimental groups. However, a reduction in body weight was observed in the group of mice administered CCl_4. The results indicated that mice treated with CCl_4 without any therapeutic intervention exhibited symptoms such as diarrhea, loss of appetite, and reduced activity, which may have contributed to the weight loss observed. In control group, mice receiving distilled water and those administered olive oil with 1% DMSO showed the most pronounced weight gain, reaching 8.14 ± 0.42 g and 7.32 ± 0.41 g, respectively (Figure 2a).

Mice with CCl_4–induced liver injury treated with *Dillenia ovata* extract (100 mg/kg, 7.76 ± 0.65 g) showed a comparatively greater gain in body weight. When the *Dillenia ovata* extract was increased to 200 mg/kg (8.44 ± 1.53 g) and 400 mg/kg (8.28 ± 0.89 g), the bodyweight gain was comparable to that of control group.

As shown in Figure 2b, the liver-to-body weight ratio was significantly increased in the CCl_4–treated group compared to the control groups, indicating liver enlargement due to hepatotoxicity. Mice administered CCl_4 alone showed the highest liver-to-body weight ratio (9.28 ± 0.43 %), whereas the normal control groups receiving distilled water and 1% DMSO had lower ratios (6.36 ± 1.04 % and 6.18 ± 1.13 %, respectively). Treatment with silymarin significantly reduced the liver-to-body weight ratio (6.17 ± 0.97 %), suggesting a protective effect. Similarly, *Dillenia ovata* extract at 100 mg/kg (7.61 ± 0.25 %), 200 mg/kg (6.27 ± 0.32 %), and 400 mg/kg (4.60 ± 0.33 %) showed a dose-dependent reduction in the liver-to-body weight ratio, approaching values observed in control groups. These findings suggest that *Dillenia ovata* extract may help mitigate liver hypertrophy caused by CCl_4–induced toxicity.

Effect of DOW on liver morphology in CCl_4–exposed mice

The external morphology of the mouse liver is also an important indicator for assessing the extent of hepatic injury, alongside parameters such as body weight gain and relative liver weight [22]. Macroscopic images of the liver from different experimental groups are presented in Figure 3. The livers from the normal control (Figure 3a) and olive oil + 1% DMSO groups (Figure 3b) displayed a smooth, shiny surface and dark red coloration. In contrast, the liver from the CCl_4–treated group without any therapeutic intervention (Figure 3c) appeared enlarged with a rough and hardened surface. The liver showed heterogeneous coloration, with large areas appearing pale and irregular dark spots visible on the surface. Signs of necrosis were also observed in some regions of the liver in this group. These findings suggest that oral administration of CCl_4 induced significant liver damage, including fibrosis and necrosis.

In the group treated with silymarin at a dose of 16 mg/kg (Figure 3d), the liver morphology showed marked improvement. The liver surface appeared smoother compared to the untreated CCl_4 group, although full recovery to the appearance of a normal liver was not achieved. In the *Dillenia ovata* extract-treated groups (Figure 3e-g), the liver surface appeared smooth, with progressively redder coloration as the dosage increased. At the dose of 400 mg/kg, the liver of mice showed surface texture and coloration similar to those observed in the normal control group.

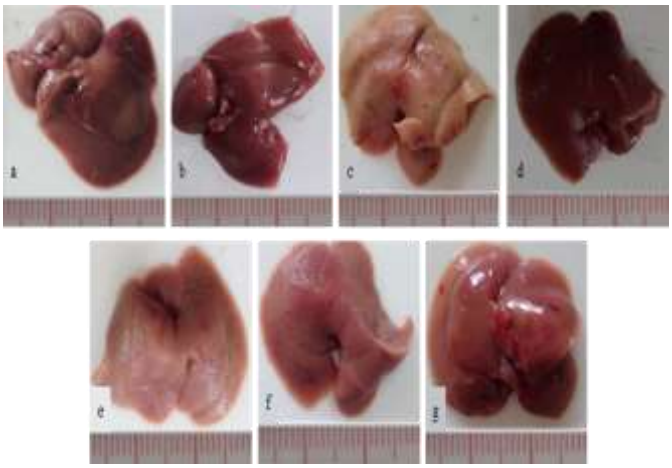

Figure 3: Gross morphology of the liver: (a) Normal control (distilled water); (b) 1% DMSO-treated group; (c) Disease control (CCl_4-treated); (d) CCl_4 + silymarin; (e) CCl_4 + DOW at 100 mg/kg; (f) CCl_4 + DOW at 200 mg/kg; (g) CCl_4 + DOW at 400 mg/kg.

Effect of Dillenia ovataextract on aminotransferases, glutathione and lipid peroxidation in the liver

CCl_4 is commonly applied as a chemical agent to establish experimental models of liver injury, allowing the evaluation of hepatoprotective activity in vivo [23]. The hepatic lesions produced by CCl_4 share several pathological features with viral hepatitis, making it a reliable model for toxicological studies. One of the hallmarks of CCl_4 intoxication is the elevation of serum aminotransferases (AST and ALT), reflecting disruption of hepatocyte membrane integrity and leakage of cytoplasmic enzymes into the bloodstream. Serum activities of aminotransferases are well-established markers of hepatocellular damage, were assessed to determine the protective role of *Dillenia ovata* extract [24].

As shown in Table 3, the CCl_4–treated group exhibited markedly elevated levels of AST, ALT, and MDA, accompanied by a pronounced reduction in GSH, confirming the successful induction of hepatic injury. Silymarin treatment (16 mg/kg) markedly improved these parameters, significantly lowering MDA levels (14.98 ± 3.01) and restoring GSH content (1081.80 ± 190.50) compared to the CCl_4 + DMSO group. AST and ALT levels were restored to near-normal values, showing no significant difference from the normal control group.

Table 3: Levels of liver enzymes (AST and ALT), malondialdehyde (MDA), and glutathione (GSH) in liver tissues

Treatment	AST (U/L)	ALT (U/L)	MDA (nM/g)	GSH (nM/g)
Normal mice	163.80 ± 46.80	32.20 ± 9.20	8.15 ± 0.77	199.10 ± 67.40
DMSO 1%	$164.60^{ns} \pm 32.50$	$28.00^{ns} \pm 6.50$	$7.05^{ns} \pm 3.55$	$194.70^{ns} \pm 16.40$
CCl_4, DMSO 1%	$1682.20^{****} \pm 304.20$	$989.20^{****} \pm 309.50$	$220.90^{****} \pm 2.32$	$29.10^{ns} \pm 14.30$
CCl_4, silymarin 16	$173.60^{ns} \pm 32.10$	$94.20^{ns} \pm 5.40$	$14.98^{**} \pm 3.01$	$1081.80^{****} \pm 190.50$
CCl_4, DOW100	$766.80^{***} \pm 95.30$	$962.80^{****} \pm 61.80$	$18.55^{***} \pm 1.13$	$287.70^{ns} \pm 5.60$
CCl_4, DOW200	$344.80^{ns} \pm 53.60$	$221.20^{ns} \pm 60.90$	$11.37^{ns} \pm 0.86$	$664.00^{****} \pm 21.40$
CCl_4, DOW400	$236.80^{ns} \pm 33.80$	$81.00^{ns} \pm 14.00$	$6.29^{ns} \pm 0.49$	$1021.10^{****} \pm 21.60$

Note: *ns = no significant, **P < 0.01, ***P < 0.001 ****P < 0.0001 indicates a statistically significant difference.*

Dillenia ovata extract administration produced a dose-dependent improvement. At 100 mg/kg, DOW significantly reduced MDA levels (18.55 ± 1.13, $p < 0.001$), but AST and ALT remained high, with no significant change in GSH. At 200 mg/kg and 400 mg/kg, AST, ALT, and MDA levels showed a significant reduction relative to the CCl_4–only group and approached the values observed in the normal control mice. Conversely, GSH content increased markedly relative to the CCl_4–induced liver injury group (664 ± 21.40 at 200 mg/kg and 1021.10 ± 21.60 at 400 mg/kg).

In hepatocytes, CCl_4 undergoes metabolic activation via cytochrome P450 enzymes, generating a highly reactive trichloromethyl radical (CCl_3•), which rapidly reacts with oxygen to form the trichloromethyl peroxyl radical (CCl_3OO•) [25]. These free radicals trigger a chain reaction of lipid peroxidation, which is commonly assessed by measuring its byproduct, malondialdehyde (MDA) [26]. In this study, an increased MDA level was observed in the livers of CCl_4–treated animals, consistent with findings from previous research. *Dillenia ovata* administration resulted in a significant decrease in liver MDA levels compared with the CCl_4–treated group, suggesting that it inhibited lipid peroxidation and the propagation of oxidative damage in hepatic cells.

Glutathione (GSH) acts as an important antioxidant, playing a key role in protecting cells from the harmful effects of free radicals, reducing endogenous oxidants, and mitigating exogenous oxidative stress[27]. Mice with CCl_4–induced liver injury showed a reduction in hepatic GSH levels. Effective hepatoprotective agents can restore GSH levels in the liver [28]. The ability to regulate hepatic GSH levels in mice is presented in Table 3. *Dillenia ovata* extract displayed dose-related hepatoprotective effects, with maximal recovery observed at 400 mg/kg, similar to the effect produced by the standard hepatoprotective agent, silymarin.

Histopathological analysis of the effects of Dillenia ovata in CCl_4*–exposed mice*

Histopathological analysis offers direct visual confirmation of the protective action of certain compounds against CCl_4–induced acute liver damage [29]. As shown in Figure 4a, the normal control group exhibited intact hepatic architecture, with well-defined portal triads, uniformly polygonal hepatocytes, and radiating hepatic sinusoids free of inflammatory or fibrotic changes. Administration of olive oil followed by 1% DMSO for 28 days (Figure 4b) did not alter liver histology compared with controls, indicating that the solvent combination was histologically safe for *Dillenia ovata* extract preparation. In the CCl_4–only group (Figure 4c), the hepatic lobular structure was severely disrupted, with widespread hepatocellular necrosis, fibrosis, inflammatory infiltration, vascular wall rupture, and bile duct proliferation, confirming that CCl_4 caused extensive liver injury.

Treatment with *Dillenia ovata* extract promoted dose-dependent recovery. At 100 mg/kg (Figure 4e), hepatocytes remained irregularly arranged with persistent lipid accumulation and vascular sclerosis. At 200 mg/kg (Figure 4f), most hepatocytes regained normal morphology and sinusoidal organization, though portal triad restoration was incomplete and steatosis persisted. At 400 mg/kg (Figure 4g), hepatic architecture was largely normalized, with abundant healthy hepatocytes, well-organized hepatic cords, and clearly identifiable portal triads. In comparison, silymarin at 16 mg/kg (Figure 4d) improved fibrosis but left numerous damaged cells and disorganized hepatic cords, demonstrating a lower hepatoprotective effect than *Dillenia ovata* extract.

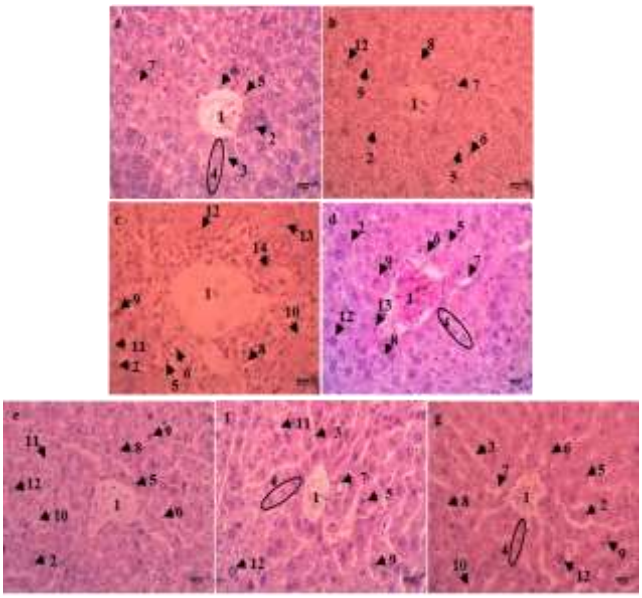

Figure 4: Hematoxylin and eosin-stained sections of liver tissue after treatment with DOW extract. (a) Normal control (distilled water); (b) 1% DMSO-treated group; (c) Disease control (CCl_4-treated); (d) CCl_4 + silymarin; (e) CCl_4 + DOW at 100 mg/kg; (f) CCl_4 + DOW at 200 mg/kg; (g) CCl_4 + DOW at 400 mg/kg. *1: Vein; 2: Hepatocyte; 3: Hepatic sinusoid; 4: Hepatocyte cord; 5: Mononuclear cell; 6: Kupffer cell; 7: Bile duct; 8: Karyolytic cell; 9: Pyknotic cell; 10: Karyoless cell; 11: Lipid droplet; 12: Swollen hepatocyte; 13: Fibrotic structure*

Conclusion

In this study, *Dillenia ovata* extract was assessed for its in vitro antioxidant capacity and its protective action against CCl_4–mediated acute hepatic toxicity in mice. *Dillenia ovata* extract exhibited no acute or sub-chronic toxicity, indicating its safety for further application. The liver-protective effect of *Dillenia ovata* extract may result from its antioxidant mechanisms, including free radical neutralization, prevention of lipid peroxidation, and enhancement of endogenous antioxidant enzymes. This study provides a valuable reference for the potential development of *Dillenia ovate extract* as a remedy to prevent oxidative stress–induced liver damage.

Conflict of Interest

The author`s declare no conflict 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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