

Protective Effect of Solvent Fractions of Methanolic Bark Extract of *Euphorbia tirucalli* on Carbon Tetrachloride-Induced Hepatic Damage

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ABSTRACT

Liver disorders remain a major global health concern, and the search for safe plant-based hepatoprotective agents continues to gain importance. In this study, the hepatoprotective activity of different solvent fractions of the methanolic bark extract of *Euphorbia tirucalli* was evaluated using a carbon tetrachloride (CCl₄)-induced hepatotoxicity model in rats. CCl₄ administration produced significant liver injury, as evidenced by marked elevation of serum SGPT, SGOT, ALP, and total bilirubin levels compared to normal control animals, confirming successful induction of hepatotoxicity. Treatment with various fractions of *Euphorbia tirucalli* resulted in a significant reduction in these biochemical markers, indicating restoration of liver function. The effect was found to be dose dependent in all treatment groups. Among all fractions tested, the ethyl acetate fraction showed the most pronounced hepatoprotective activity, particularly at 400 mg/kg. It significantly normalized liver enzyme levels and bilirubin concentration, with effects comparable to the standard drug silymarin. Percentage protection analysis further supported the biochemical findings, with the ethyl acetate fraction demonstrating the highest protective effect against CCl₄-induced hepatic damage. Histopathological observations also confirmed these results, showing severe necrosis, fatty changes, and inflammatory infiltration in the toxic control group, whereas treated groups exhibited varying degrees of hepatic recovery. The ethyl acetate fraction showed near-normal liver architecture with minimal cellular damage. In conclusion, the results indicate that the bark extract fractions of *Euphorbia tirucalli* possess significant hepatoprotective activity. This effect is likely due to antioxidant phytoconstituents that reduce oxidative stress and stabilize hepatocyte membranes. The ethyl acetate fraction emerged as the most active, suggesting its potential for further phytochemical isolation and development of hepatoprotective agents.

Keywords: *Euphorbia tirucalli*, hepatoprotection, carbon tetrachloride, liver toxicity, phytochemicals, biochemical markers, histopathology.

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INTRODUCTION

The liver is a key metabolic organ responsible for regulating carbohydrate, protein, and lipid metabolism, detoxifying xenobiotics, synthesizing plasma proteins, producing bile, and storing essential nutrients. Due to its continuous detoxification activity, it remains highly exposed to

toxic substances, making it prone to injury. Consequently, liver disorders represent a significant global health burden associated with high morbidity and mortality [1].

Hepatotoxicity refers to liver damage induced by drugs, chemicals, toxins, or infections. Carbon tetrachloride (CCl₄) is widely used in experimental

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studies to induce liver injury. It is metabolized by hepatic cytochrome P450 enzymes into reactive free radicals, which trigger lipid peroxidation, oxidative stress, membrane damage, hepatocellular necrosis, and inflammation. These changes are reflected by elevated serum markers such as SGPT, SGOT, ALP, and total bilirubin [2,3].

Although synthetic drugs are available for liver disorders, their clinical use is often limited due to side effects and incomplete protection. This has encouraged the exploration of plant-derived hepatoprotective agents. Medicinal plants are rich in bioactive compounds with antioxidant and anti-inflammatory properties, making them promising alternatives for liver protection [4].

Euphorbia tirucalli L. (Euphorbiaceae), commonly known as Pencil Tree or Milk Bush, is traditionally used for treating inflammation, infections, and skin disorders. Phytochemical studies have revealed the presence of flavonoids, phenolics, terpenoids, and steroids, which may contribute to its pharmacological effects [5,6].

Oxidative stress plays a major role in liver injury progression; therefore, antioxidant-rich plants may offer hepatoprotective benefits. Previous reports suggest that *Euphorbia tirucalli* possesses antioxidant and cytoprotective activity, supporting its potential role in liver protection [7].

Fractionation of plant extracts using solvents of different polarity helps isolate bioactive constituents and identify the most active fraction responsible for pharmacological effects. This approach aids in understanding the compounds contributing to hepatoprotection [8].

Based on this rationale, the present study was designed to evaluate the hepatoprotective activity of different solvent fractions of the methanolic bark extract of *Euphorbia tirucalli* against CCl₄-induced hepatotoxicity in rats using biochemical parameters, percentage protection, and histopathological analysis.

MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

The bark of *Euphorbia tirucalli* L. was obtained from Tirupati, Andhra Pradesh, India, in the month of August 2025. The plant material was taxonomically identified and confirmed by Dr. K. Madhava Chetty (Department of Botany, Sri Venkateswara University, Tirupati, India). A voucher specimen (ET-2025-0829) was prepared and stored in the

departmental herbarium for subsequent reference and verification. After collection, the bark was washed with water to remove adhering debris and foreign particles. The treated material was shade-dried at room temperature (25–30°C) for several days to preserve heat-sensitive phytoconstituents. After drying completely, the bark was pulverized using a mechanical grinder to produce a coarse powder and kept in airtight containers until use [9,10].

2.2 Preparation of Extract and Fractions

About 500 g of the powdered bark material was extracted using 80% methanol by cold maceration method. The extraction process was performed for 72 hours at room temperature with slight agitation to facilitate the solubilization of the solvent and efficient phytoconstituents extraction. The extract was filtered through Whatman No. 1 filter paper, and the solvent was removed under reduced pressure using a rotary vacuum evaporator at 40°C, leaving only crude methanolic extract as the solvent [11,12]. Using solvents of different polarity, the isolated methanolic extract was fractionated into ethanol, acetic acid, acetone, and ethyl acetate fractions. Phytochemicals were fractionated according to their solubility properties based on the fractionation procedure. Each solvent fraction was concentrated under reduced pressure and stored in airtight containers at 4°C until they were subjected to pharmacological evaluation.

2.3 Experimental Animals

Healthy adult male Wistar albino rats weighing between 150 and 200 g were used to carry out the experiment. Animals were acquired from the Central Animal House and maintained under standard laboratory conditions with temperature (22 ± 2 °C), relative humidity (55 ± 5 %), and a 12-hour light/dark cycle. Daily pellet diet and drinking water were freely supplied as part of the experimental protocol [14]. Animals were acclimatized to laboratory conditions for 7 days prior to study commencement. All animal experimentation was approved by the Institutional Animal Ethics Committee (IAEC Approval No. QISCP/IAEC/01/2023-24). In accordance with CPCSEA, Government of India, the study was performed according to guidelines.

2.4 Evaluation of Hepatoprotective Activity

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Carbon tetrachloride (CCl₄)-induced hepatotoxicity model was used to investigate hepatotoxicity in vivo in this study. This molecule is hepatotoxic due to the generation of free radicals and oxidative stress. The acute liver injury caused by it can increase serum liver enzyme levels in the presence of hepatocellular damage, all produced by such a carcinogen. Therefore, as previously demonstrated by this case study, it can lead to hepatocellular injury and sustained damage regardless of age and sex.

2.4.1 Experimental Design

The animals were divided into eleven randomized groups, each consisting of three rats (n = 3). All treatments were given orally once daily over seven consecutive days. On the seventh day, one hour after treatment, CCl₄ diluted with olive oil (1:1 v/v) was injected intraperitoneally at a dose of 1 mL/kg to induce hepatotoxicity [18].

2.4.2 Collection of Blood Samples

Twenty-four hours after CCl₄ administration, animals were anesthetized and blood was collected by retro-orbital puncture. The blood was allowed to clot at room temperature and then centrifuged at 3000 rpm for 15 minutes to separate the serum. The serum obtained was used for biochemical assessment of liver function markers [19].

2.4.3 Biochemical Estimation

Serum samples were evaluated for the following biochemical parameters using commercially available diagnostic kits as per manufacturer's recommendations:

- Serum Glutamate Pyruvate Transaminase (SGPT/ALT)
- Serum Glutamate Oxaloacetate Transaminase (SGOT/AST)
- Alkaline Phosphatase (ALP)

- Total Bilirubin (TB)

Elevated levels of these markers indicate liver damage, and decreased levels following treatment indicate hepatoprotective activity [20].

2.4.4 Histopathological Examination

Animals were sacrificed after blood collection, and the liver tissues were excised immediately and washed with normal saline to remove blood traces. Sections of liver tissue were fixed in 10% neutral buffered formalin for 24–48 hours. The fixed tissues were processed through graded alcohols, embedded in paraffin wax, and sliced into 4–5 μm thick sections using a rotary microtome. The sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for pathological changes such as hepatocyte necrosis, fatty degeneration, inflammatory cell infiltration, sinusoidal congestion, and restoration of normal hepatic architecture [21].

2.4.5 Calculation of Percentage Protection

The hepatoprotective impact of the treatment was presented as percentage protection via the following formula:

$$\text{Percentage Protection (\%)} = \left[\frac{(\text{Toxic Control} - \text{Treatment})}{(\text{Toxic Control} - \text{Normal Control})} \right] \times 100$$

where values are defined as the mean of the SGPT, SGOT, ALP, or total bilirubin concentrations in the groups [22].

2.5 Statistical Analysis

All data were reported as Mean ± Standard Error of Mean (SEM). Statistical analysis was performed with one-way ANOVA and an appropriate post hoc test. The differences were considered statistically significant when p < 0.05 [23].

RESULTS

Table 1: Effect of different fractions of methanolic Bark Extract of *Euphorbia tirucalli* on the serum enzymatic activity of the CCl₄-induced hepatotoxicity

Group	Treatment	SGPT (IU/mL)	SGOT (IU/L)	ALP (IU/L)	TB (mg/dL)
I	Control	75.45 ± 0.85	76.98 ± 0.57	206.85 ± 2.15	0.54 ± 0.12
II	CCl ₄ + Olive Oil	174.32 ± 1.85	269.25 ± 1.25	338.25 ± 2.08	3.35 ± 0.17
III	Silymarin (100 mg/kg)	76.29 ± 0.54*	81.25 ± 1.53*	215.85 ± 1.25*	0.62 ± 0.01*
IV	Ethanolic Fraction (200 mg/kg)	134.25 ± 1.52*	176.34 ± 1.28*	295.36 ± 1.62*	2.15 ± 0.12*
V	Ethanolic Fraction (400 mg/kg)	127.25 ± 1.25*	165.25 ± 1.85*	284.34 ± 1.86*	1.89 ± 0.13*

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VI	Acetic Acid Fraction (200 mg/kg)	125.75 ± 1.65*	156.59 ± 1.86*	278.90 ± 1.35*	1.89 ± 0.23*
VII	Acetic Acid Fraction (400 mg/kg)	116.81 ± 1.23*	144.32 ± 1.32*	255.25 ± 1.29*	1.82 ± 0.14*
VIII	Acetone Fraction (200 mg/kg)	103.99 ± 1.74*	134.28 ± 1.65*	246.89 ± 2.17*	1.29 ± 0.12*
IX	Acetone Fraction (400 mg/kg)	100.27 ± 2.14*	128.70 ± 1.34*	237.35 ± 1.85*	1.23 ± 0.12*
X	Ethyl Acetate Fraction (200 mg/kg)	96.55 ± 1.22*	126.38 ± 0.54*	233.42 ± 1.82*	1.12 ± 0.22*
XI	Ethyl Acetate Fraction (400 mg/kg)	85.15 ± 1.28*	116.89 ± 1.51*	225.65 ± 1.17*	0.76 ± 0.05*

All the Values are expressed in mean ± SEM, N = 3 rats in each group, * $p < 0.05$, when compared with CCl₄-treated group.

Liver function biomarkers—SGPT, SGOT, ALP, and total bilirubin (TB)—of these various treatments were studied compared to different groups. In the control group (Group I) these enzymes were all normal, and this means that the liver functions normally. All measured parameters were markedly increased following CCl₄ exposure combined with olive oil (Group II) (e.g., SGPT: 174.32 ± 1.85 IU/mL; SGOT: 269.25 ± 1.25 IU/L; ALP: 338.25 ± 2.08 IU/L; and TB: 3.35 ± 0.17 mg/dL, compared with the control group), attesting to the notable hepatic injury induced by CCl₄ toxicity. Silymarin (Group III), a widely accepted hepatoprotective product, effectively reversed CCl₄-induced damage with similar enzyme and bilirubin levels (SGPT: 76.29 ± 0.54 IU/mL; SGOT: 81.25 ± 1.53 IU/L; ALP: 215.85 ± 1.25 IU/L; TB: 0.62 ± 0.01 mg/dL), with comparable levels to the control group, resulting in significant hepatoprotection. Hepatoprotection was dose-dependent with the

methanolic bark extract fractions in all tests studied. For these, the ethanolic fraction (Groups IV and V) at 200 mg/kg and 400 mg/kg showed an amelioration of elevated liver enzymes and bilirubin, but at the toxic control level, although the level was significantly higher than those found with silymarin. Likewise, acetic acid fractions (Groups VI and VII) improved liver function markers significantly with higher doses. The acetone fraction (Groups VIII and IX) decreased SGPT, SGOT, ALP, and TB further to a level closer to normal, while the ethyl acetate fraction (Groups X and XI) exerted the most potent hepatoprotective activity among the tested extracts, especially at 400 mg/kg, when the enzyme concentrations were near to normal, as compared to the normal control. Taken together, these data indicate that all fractions of methanolic bark extract of *Euphorbia tirucalli* are hepatoprotective against CCl₄-induced liver injury, with the ethyl acetate fraction at 400 mg/kg having the strongest protective effect. The findings suggest several therapeutic properties of these extracts for hepatic injury management.

Figure 2: Effect of different fractions of methanolic Bark Extract of *Euphorbia tirucalli* on the serum enzymatic activity of the CCl₄-induced hepatotoxicity

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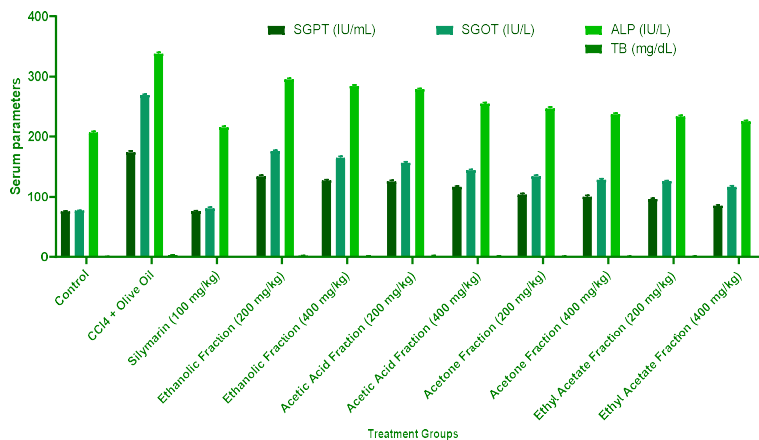


Table 2: Percentage Protection due to treatment of different fractions of methanolic Bark Extract of *Euphorbia tirucalli*

Group	Treatment	SGPT Protection (%)	SGOT Protection (%)	ALP Protection (%)	TB Protection (%)
III	Silymarin (100 mg/kg)	99.14	94.76	90.75	79.02
IV	Ethanolic Fraction (200 mg/kg)	41.83	51.44	32.62	40.44
V	Ethanolic Fraction (400 mg/kg)	50.02	57.87	39.89	46.53
VI	Acetic Acid Fraction (200 mg/kg)	52.50	61.42	43.62	46.53
VII	Acetic Acid Fraction (400 mg/kg)	61.25	67.56	61.37	49.42
VIII	Acetone Fraction (200 mg/kg)	71.03	73.77	55.19	64.63
IX	Acetone Fraction (400 mg/kg)	74.17	75.93	59.55	66.85
X	Ethyl Acetate Fraction (200 mg/kg)	78.32	76.58	62.95	70.73
XI	Ethyl Acetate Fraction (400 mg/kg)	88.21	81.70	67.17	86.83

Values of percentage protection of liver enzymes (SGPT, SGOT, ALP) and total bilirubin (TB) show that the functional and functionalized fractions may be hepatoprotective to CCl₄-induced liver injury. As anticipated, silymarin (Group III) as a representative hepatoprotection standard was found to be superior in protection with almost complete normalization (99.14% of SGPT, 94.76% of SGOT, 90.75% of ALP, 79.02% of TB, respectively). The ethyl acetate fraction is the most promising dose-dependent hepatoprotection from the different fractions. At group XI (400 mg/kg), it conferred considerable protection in all parameters with 88.21% for SGPT, and 81.70% for SGOT, 67.17% for ALP, and 86.83% for TB. Ethyl acetate fraction achieved significantly more liver enzyme normalization at 200 mg/kg (Group X) when compared to toxic control. Group VIII and IX acetone fraction gave good

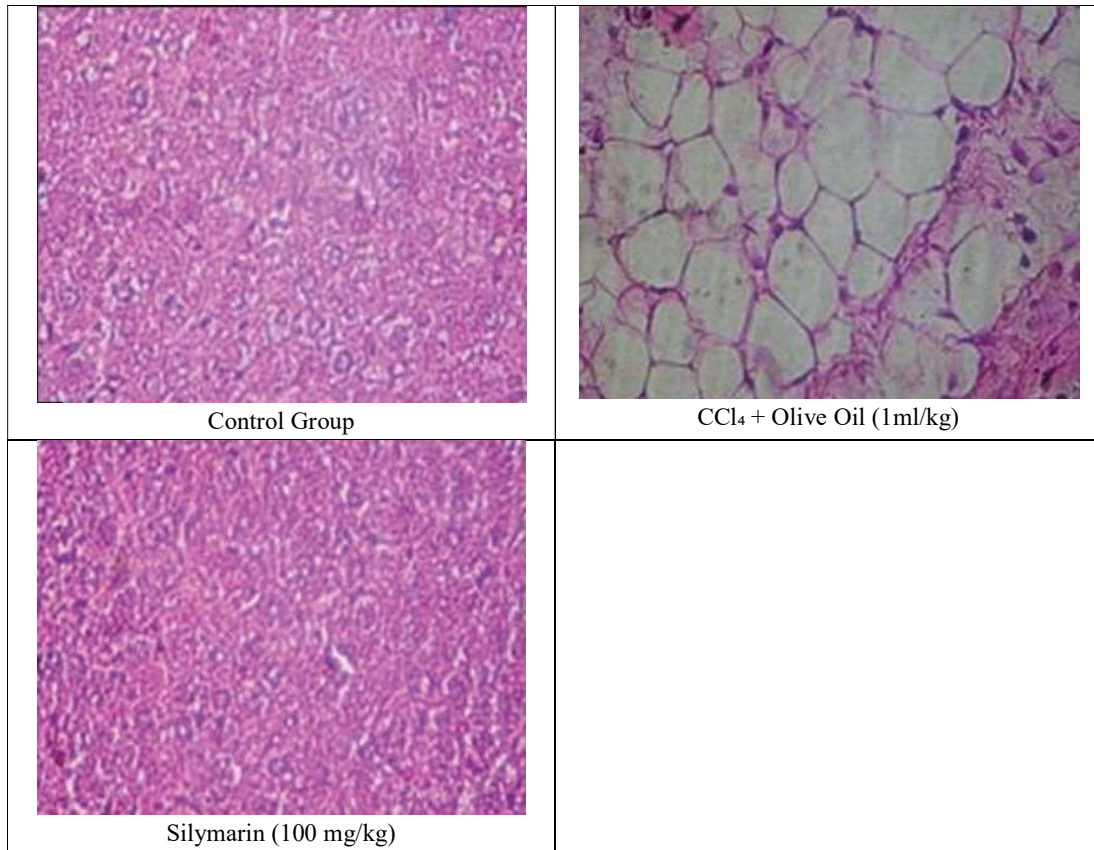
hepatoprotective effect with the % protection between 55% and 76% on several indicators and the protective activity continued to increase with increasing doses. The acetic acid and ethanolic fractions had moderate protection and higher dose compared to lower dose was beneficial. Dose and enzyme-mediated protective activity were between about 30% and 67% in all experiments. Taken together, the results suggest that, while all fractions tested exert some degree of hepatoprotection against CCl₄-induced toxicity, those from ethyl acetate and acetone fractions demonstrated improved protection. The dose-dependent improvement in percentage protection indicates that the bioactive compounds that perform hepatoprotection are more concentrated or more bioavailable present in the fractions.

Figure 3: Percentage Protection due to treatment of different fractions of methanolic Bark Extract of *Euphorbia tirucalli*

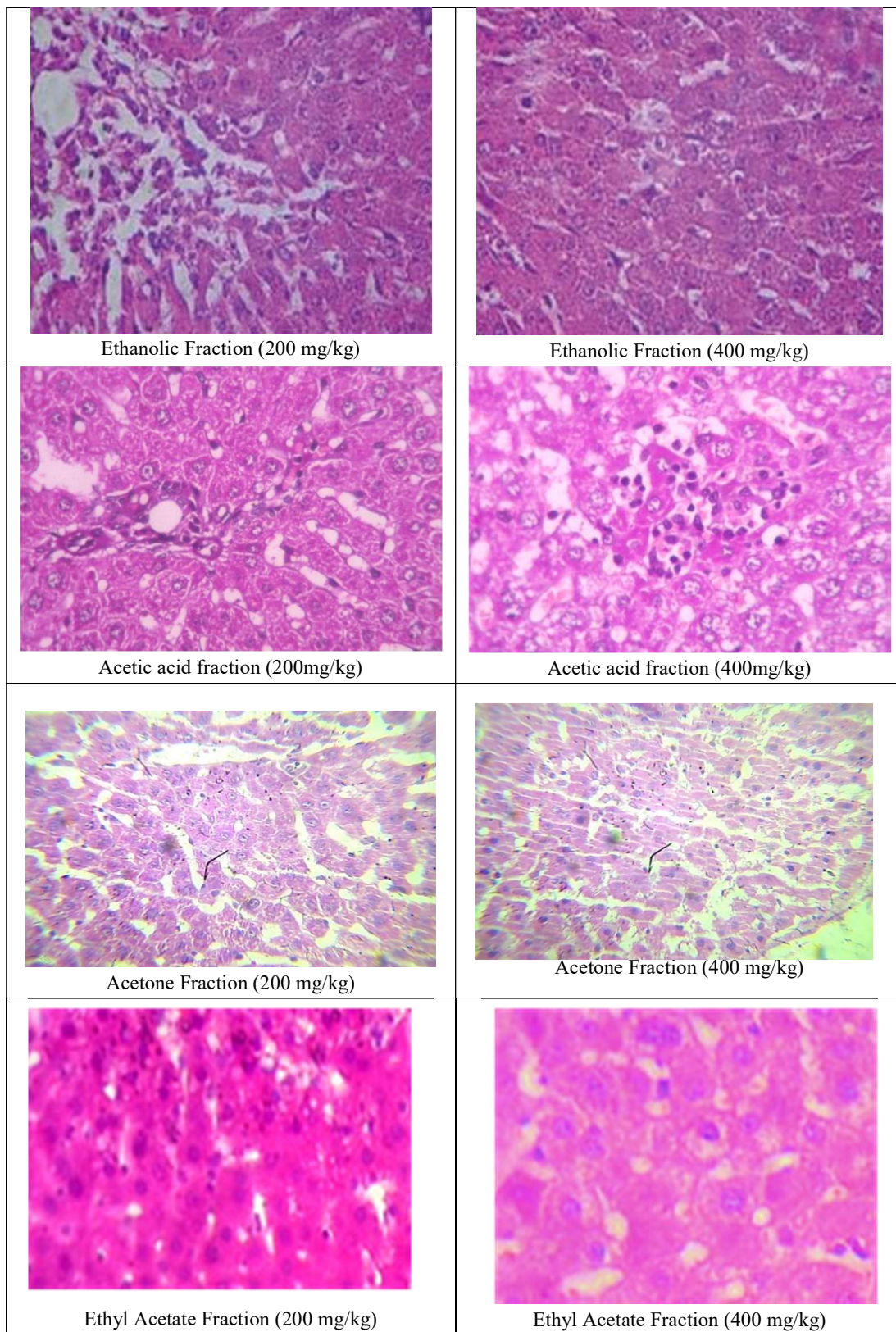
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Figure 4: Histopathology of different fractions of methanolic Bark Extract of *Euphorbia tirucalli*



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Analysis for cells showing liver tissue appeared acceptable with the biochemical studies indicating

the extent of hepatic injury and recovery after treatment. Liver sections from the normal controls

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demonstrated the usual hepatic histology with healthy hepatocytes distributed uniformly, open central veins, sinusoidal spaces intact, and no histological alterations were perceived. The CCl₄ and olive oil-treated group also presented clinically significant liver damage with extensive hepatocyte necrosis, cellular swelling, fat degeneration, infiltration by inflammatory cells as well as disruption of normal liver architecture. Such characteristics are suggestive of acute toxic injury. The liver morphology was greatly modified in the silymarin-treated group, including cellular integrity restoration, minimal necrosis and reduced inflammation consistent with silymarin protection. Within the ethanolic and acetic acid fraction groups, moderate histological recovery was recorded, with lesser necrosis and inflammatory infiltration compared with the toxic control group and with a marked improvement at increasing doses. The acetone fraction treatment showed a significantly higher histological resolution, with not only less liver organ damage but also more preserved liver architecture. At 400 mg/kg dose, the ethyl acetate fraction showed the greatest extent of hepatoprotection as the liver tissue showed nearly normal shape, little necrosis, and infrequently inflammatory cells. These histopathological findings lend powerful support to the biochemical evidences, suggesting that the different fractions of *Euphorbia tirucalli* bark extract were able to provide the dose-dependent treatment against CCl₄ driven liver injury, with ethyl acetate being the most efficacious.

DISCUSSION

The current study aimed to investigate the hepatoprotective effect of various solvent fractions derived from the methanolic bark extract of *Euphorbia tirucalli* on Wistar rats affected by liver injury via carbon tetrachloride (CCl₄). The results of the review show that treatment with CCl₄ resulted in substantial hepatic injury with increased serum levels of SGPT, SGOT, ALP, and total bilirubin and significant histopathological changes to liver tissue. The different fractions of *Euphorbia tirucalli* significantly reduced these modifications and showed a protective effect on hepatic function. Carbon tetrachloride is generally used as an experimental hepatotoxic agent because its metabolism produces extremely reactive free radicals leading to lipid peroxidation and oxidative damage to hepatocytes. These reactive intermediates

lyse cell membranes, undermine enzyme systems, and ultimately induce hepatocellular necrosis and inflammation. The potent elevation of serum transaminases and bilirubin found in the toxic control group suggests induction of liver injury is successful and is in correspondence with previous reports mentioning the hepatotoxicity due to CCl₄ [24,25]. Serum transaminases such as SGPT and SGOT represent established markers of hepatocellular integrity. Damaged liver cell membranes have leakage of those intracellular enzymes into the bloodstream, raising serum concentrations. In the current review, treatment with alternate fractions of *Euphorbia tirucalli* markedly decreased SGPT and SGOT scores in addition to the toxic control. This decrease may indicate stabilization of hepatocyte membranes and retention of cellular geometry. Similar findings have also been documented for plant-based hepatoprotective agents that are loaded with antioxidant phytochemicals [26]. Alkaline phosphatase and total bilirubin are biochemically important indices of biliary and hepatic excretory capacity. High levels of this measure usually mean loss of bile flow and hepatic dysfunction. The reduction of ALP and bilirubin levels in the extract fractions clearly confirmed an improvement in hepatic secretory mechanisms and restoration of liver physiology. Potential of the extract to reestablish these biochemical parameters could be due its ability to protect hepatocytes from damage and encourage cellular resiliency [27]. Ethyl acetate fraction scored the highest on hepatoprotective activity among the fractions tested, specifically within 400 mg/kg. This segment elicited significant reductions in serum enzyme concentration and bilirubin concentrations, and values were close to those found in the normal and silymarin-treated fractions. The improved activity of the ethyl acetate fraction should be based on the extraction from ethyl acetate to moderately polar phytoconstituents, e.g. flavonoids, phenolic compounds, and other antioxidant compounds efficiently. These compounds are reported to have free-radical scavenging, anti-inflammatory, and membrane-stabilizing elements responsible for hepatoprotection [28,29]. The percentage protection analysis also backed up the biochemical results. An even dose-response was observed in all fractions, higher doses providing more protection than lower doses. Such dose-dependent activity also indicates that the hepatoprotective effect is tightly associated with the concentration of active phytochemicals

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found in the fractions. The ethyl acetate fraction showed the highest percentage protection of the extracted products tested, suggesting its superior effect against hepatic damage attributed to CCl₄. Additional histopathological study corroborated the protective nature of the extract fractions. Liver sections obtained from the toxic control group showed marked pathological alterations with hepatocellular degeneration, necrosis, infiltration of inflammatory cells, and abnormal hepatic architecture. In contrast, the response of animals to extract fractions showed varying degrees of structural recovery. The ethyl acetate fraction (especially 400 mg/kg) was found to have almost normal hepatic architecture and a low inflammatory infiltration of tissue and less necrotic tissue. This observation is consistent with biochemical and confirms hepatoprotective effect of extract [30]. The hepatoprotective activity observed in this study may have been partially mediated by the phytochemical constituents obtained from *Euphorbia tirucalli*. Previous phytochemical studies have reported the presence of flavonoids, phenolic compounds, terpenoids, and sterols in this crop. Many of these compounds are potent antioxidants capable of neutralizing reactive oxygen species, inhibiting lipid peroxidation, and enhancing the original antioxidant defense mechanisms. Through these methods, the extract may protect liver cells from oxidative damage and promote tissue regeneration [31,32]. Results in summary have shown that the solvent fractions of the methanolic bark extract of *Euphorbia tirucalli* have effective hepatoprotective effect against CCl₄-driven hepatic injury. According to this, by far the ethyl acetate fraction with the best protection effect was found to be compared with that of the usual hepatoprotective drug silymarin. These findings indicate that *Euphorbia tirucalli* might develop as a natural ingredient for the preparation of new hepatoprotective drugs. The extraction and characterization of the bioactive compounds on which the studied activity is due and their exact mechanism of action is to be investigated into further for elucidating it.

CONCLUSION

In the current study, different fractions of solvent from the methanolic bark extract of *Euphorbia tirucalli* tested to exhibit a strong hepatoprotective activity against harmful effects of carbon tetrachloride (CCl₄) on liver tissues in Wistar rats. CCl₄ toxicity resulted in severe hepatic injury, with

serum concentrations of SGPT, SGOT, ALP, and total bilirubin elevated, while marked histopathological changes occurred in liver tissue. Treatment with the different fractions of *Euphorbia tirucalli* attenuated these biochemical and histological changes in a dose-dependent manner. Serum reduction of liver enzymes and bilirubin showed the normal hepatic function and protect hepatocytes in form of toxic insults. The hepatoprotective effects were also confirmed by histopathological observations with decreased necrosis, reduced inflammatory cell infiltration and improvement of hepatic architecture in treated animals. The ethyl acetate fraction proved to have the better hepatoprotective action given at 400 mg/kg among the fractions studied. This fraction showed the greatest percentage protection and demonstrated biochemical and histological recovery similar to that seen with the conventional hepatoprotective agent, silymarin. Bioactive phytoconstituents, flavonoids, phenolic compounds, among other antioxidants, scavenging free radicals and preventing oxidative damage, may account for the enhanced ethyl acetate fraction activity. It is concluded from our study that *Euphorbia tirucalli* bark can potentially offer natural hepatoprotective compounds. The protective effects that were observed could be mediated through antioxidant and membrane stabilizing effects in response to CCl₄ oxidative stress and hepatocellular injury. Finally, in the present report, the ethyl acetate fraction in the methanolic bark extract of *Euphorbia tirucalli* was found to be a more effective hepatoprotective than the other extracts; and the ethyl acetate fraction may be considered for additional phytochemical and pharmacological studies. The isolation, characterization and mechanistic examination of the active constituents in the present study could have a contribution in the design of attractive plant-based therapies for liver diseases as well as could be used as guide drug formulation.

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