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Hepatoprotective Effect *of Oroxylum Indicum* Bark Extracts against Chemically-Induced Hepatic Damage In Rats

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Abstract: Liver diseases remain a significant global health challenge, with limited effective therapeutic options available in modern medicine. The present study aimed to evaluate the hepatoprotective potential of ethanolic bark extracts of *Oroxylum indicum* against carbon tetrachloride (CCl_4)-induced hepatic damage in Wistar albino rats. The plant extract was prepared using a percolation method and subjected to phytochemical screening, which revealed the presence of bioactive constituents such as alkaloids, steroids, and saponins. Experimental animals were divided into six groups, including normal control, toxic control, standard (silymarin-treated), and three test groups receiving different doses (100, 200, and 400 mg/kg) of *Oroxylum indicum* extract. Hepatotoxicity was induced using CCl_4, and the protective effects of the extract were assessed through biochemical parameters including serum AST, ALT, ALP, GGT, and bilirubin levels, along with histopathological examination of liver tissues.Results demonstrated that CCl_4 administration significantly elevated liver enzyme levels and caused severe hepatic damage. Treatment with *Oroxylum indicum* extract resulted in a dose-dependent reduction in serum biomarkers and improvement in liver histoarchitecture. Notably, the highest dose (400 mg/kg) exhibited hepatoprotective effects comparable to the standard drug silymarin. The findings suggest that *Oroxylum indicum* bark extract possesses significant hepatoprotective activity, likely attributed to its phytochemical constituents and antioxidant properties. This study supports the traditional use of the plant and highlights its potential as a natural therapeutic agent for the management of liver disorders.

Keywords: *Oroxylum indicum*, Hepatoprotective activity, Carbon tetrachloride (CCl_4), Liver injury, Ethanolic bark extract, Wistar albino rats.

1. INTRODUCTION

Liver diseases represent a major global health concern, contributing significantly to morbidity and mortality worldwide. The liver plays a central role in regulating metabolic homeostasis, detoxification, protein synthesis, and biochemical transformations essential for survival. Due to its strategic position in receiving blood from the gastrointestinal tract, the liver is particularly vulnerable to damage from xenobiotics, toxins, drugs, and environmental pollutants [1]. Hepatic disorders such as cirrhosis, hepatitis, fatty liver disease, and drug-induced liver injury are increasingly prevalent, especially in developing countries where healthcare resources are limited [2]. Among the various hepatotoxic agents, carbon tetrachloride (CCl_4) is widely used in experimental models to induce liver injury. Its metabolism by cytochrome P450 enzymes generates reactive free radicals, leading to lipid peroxidation, oxidative stress, and cellular damage [3]. These processes ultimately result in hepatocellular necrosis, inflammation, and fibrosis. Biochemical markers such as alanine

transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and bilirubin are commonly used to assess liver function and the extent of hepatic damage [4].

Despite advances in modern medicine, effective therapeutic options for liver diseases remain limited. Many conventional drugs used for treating liver disorders are associated with adverse effects and inconsistent efficacy [5]. Consequently, there is a growing interest in exploring plant-based medicines as alternative therapeutic agents. According to the World Health Organization, approximately 80% of the global population relies on traditional herbal medicine for primary healthcare needs [6]. Medicinal plants are rich sources of bioactive compounds such as flavonoids, alkaloids, glycosides, and phenolic compounds, which exhibit antioxidant, anti-inflammatory, and hepatoprotective properties [7]. *Oroxylum indicum* (L.) Kurz, commonly known as the Indian trumpet tree, is a medicinal plant belonging to the family Bignoniaceae. It is widely distributed across India, Sri Lanka, China, and Southeast Asia. The plant has been extensively used in traditional systems of medicine such as Ayurveda and Unani for the treatment of various ailments including inflammation, respiratory disorders, and liver diseases [8]. Different parts of the plant, particularly the bark, are known to contain biologically active compounds such as flavonoids (baicalein), alkaloids, tannins, and saponins, which contribute to its pharmacological activities [9]. Previous studies have demonstrated that *Oroxylum indicum* possesses a wide range of therapeutic properties including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and hepatoprotective effects [10]. The hepatoprotective activity of this plant is primarily attributed to its ability to scavenge free radicals, inhibit lipid peroxidation, and enhance the antioxidant defense system of the liver [11]. These mechanisms help in preserving the structural integrity of hepatocytes and improving liver function. In recent years, there has been increasing scientific interest in validating the traditional uses of medicinal plants through experimental studies. However, comprehensive evaluation of the hepatoprotective potential of *Oroxylum indicum* bark extracts, particularly against chemically-induced liver damage, remains limited. Therefore, systematic investigation using appropriate animal models is essential to establish its efficacy and safety. The present study aims to evaluate the hepatoprotective effect of ethanolic bark extracts of *Oroxylum indicum* against carbon tetrachloride-induced hepatic damage in Wistar albino rats. The study focuses on assessing biochemical parameters and histopathological changes to determine the protective role of the plant extract on liver function.

1.1 Oroxylum indicum

Taxonomical Classification:

Kingdom: Plantae (Plants)

Division: Magnoliophyta (Angiospermes, flowering plants)

Class: Magnoliopsida (Dicotylédones)

Order: Lamiales

Family: Bignoniaceae

Genus: Oroxylum

Species: *Oroxylum indicum*

Used Part: Bark

Aim:

To Hepatoprotective Effect of *Oroxylum indicum* Bark Extracts Against Chemically-Induced Hepatic Damage in Rats

Objective:

1. Due to its medicinal properties, the bark extract derived from Oroxylum indicum is highly valuable.
2. This potent herbal remedy offers wide-ranging advantages for health, featuring characteristics that combat inflammation and pain, and also aid digestion.
3. The process involved ensuring and verifying the genuineness, along with handling, the substances collected from Oroxylum indicum Bark.
4. The method includes purifying, drying, and grinding the Oroxylum indicum Bark Extracts into a fine powder.
5. Oroxylum indicum Bark Extracts Provide Liver Protection: Counteracting Liver Injury Caused by Chemicals in Rats.
6. The research centers on analyzing the studies performed regarding the Protective Liver Action of the Oroxylum indicum Bark Extracts.

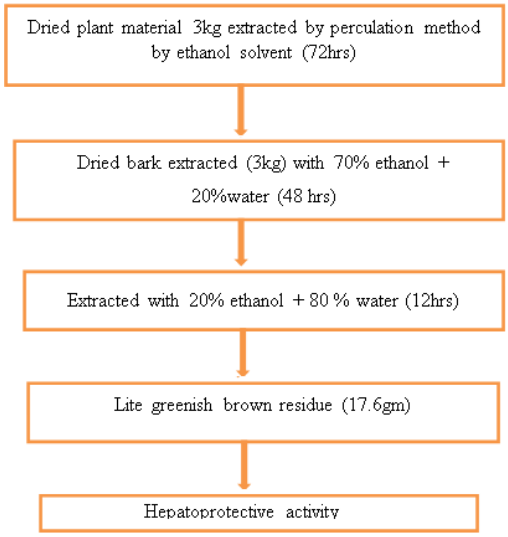
2. Materials And Methods

2.1 Plant Material and Preparation

The bark of *Oroxylum indicum* was collected, authenticated, and thoroughly washed with running water to remove dirt and impurities. The cleaned bark was shade-dried at room temperature for approximately 20 days, followed by brief sun drying. The dried material was pulverized into coarse powder, sieved (mesh no. 60), and stored in airtight containers for further analysis.

2.2 Extraction of Plant Material

Approximately 3 kg of powdered bark was subjected to extraction using the percolation method. Initially, extraction was carried out with ethanol for 72 hours at room temperature. This was followed by successive extraction using 70% ethanol and 30% distilled water for 50 hours, and then with 20% ethanol and 80% distilled water for 12 hours. The combined extracts were filtered and concentrated under reduced pressure to obtain a dry extract (yield ~9.8% w/w). A light greenish-brown residue (17.6 g) was obtained and used for further studies.



2.3 Phytochemical Screening

The ethanolic extract of *Oroxylum indicum* bark was subjected to qualitative phytochemical analysis to detect the presence of bioactive constituents such as alkaloids, steroids, saponins, flavonoids, glycosides, tannins, and proteins using standard procedures.

2.4 Experimental Animals

Healthy Wistar albino rats (170–225 g) of either sex were used. The animals were maintained under standard laboratory conditions (25 ± 2°C, 12-hour light/dark cycle) with free access to standard diet and water. All experimental procedures were conducted in accordance with standard ethical guidelines for animal care.

2.5 Induction of Hepatotoxicity

Hepatotoxicity was induced using carbon tetrachloride (CCl_4). A mixture of CCl_4 (25%) with olive oil was administered orally to induce liver damage in experimental animals.

2.6 Experimental Design

The animals were randomly divided into six groups (n = 6 per group):

- **Group I:** Normal control (distilled water, 6 mL/kg)
- **Group II:** Toxic control (CCl_4, 3 mL/kg/day, p.o.)
- **Group III:** Standard group (CCl_4 + silymarin 25 mg/kg, i.p.)
- **Group IV:**CCl_4 + *Oroxylum indicum* extract (100 mg/kg)
- **Group V:**CCl_4 + *Oroxylum indicum* extract (200 mg/kg)
- **Group VI:**CCl_4 + *Oroxylum indicum* extract (400 mg/kg)

2.7 Biochemical Analysis

At the end of the experimental period, blood samples were collected via cardiac puncture and allowed to clot. Serum was separated by centrifugation and stored at −20°C until analysis. The following biochemical parameters were estimated:

- Aspartate transaminase (AST)
- Alanine transaminase (ALT)
- Alkaline phosphatase (ALP)

- Gamma-glutamyl transferase (GGT)
- Total bilirubin
- Total protein

Standard protocols were followed for enzyme assays using spectrophotometric methods.

2.8 Histopathological Studies

Liver tissues were excised, washed with saline, and fixed in 10% formalin. The tissues were embedded in paraffin, sectioned (5 µm thickness), and stained with hematoxylin and eosin. The sections were examined under a microscope for histopathological changes such as necrosis, fatty degeneration, and cellular architecture.

2.9 Statistical analysis

The result of hepatoprotective activity is expressed as mean "Mean ± SEM from five animals in each group. Results were statistically analyzed using one-way ANOVA followed by Newman Keuls multiple range test for individual comparisons: $p<0.01$ was considered significant. GraphPadInStat version 3.00 of GraphPadSoftware Inc. (sandiego, CA), was used for statistical analysis.

3. RESULTS

Phytpchemical Analysis

S.No	Phytoconstituents	Presence
1.	Tannins	-
2.	Alkaloids	+
3.	Steroids	+
4.	Glycosides	-
5.	Flavonoids	+
6.	Polyphenols	+
7.	Carbohydrates	-
8.	Saponins	+

+ indicates presence ,- indicates absence

EVALUATION OF BIO CHEMICAL PARAMETER

Serum Marker Enzyme

S.NO	AST (UNITS/L)	ALT (UNITS/L)	GGT (UNITS/L)	ALP (UNITS/L)	BILIRUBIN (UNITS/L)
GROUP 1	33.23±1.45	62.75±1.2	65.4±2.32	117.1±1.8	0.32±0.02
GROUP 2	80.75±2.65	81.02±2.3	78.42±1.33	249.6±4.4	1.42±0.2
GROUP 3	56.78±4.6	65.01±0.1	58.15±1.2	142.1±2.8	0.74±0.02
GROUP 4	80.33±2.9	73.91±1.3	69.02±01	205.5±4.7	1.05±0.05
GROUP 5	57.98±1.9	65.23±2.1	45.23±1.02	241±5.7	0.73±0.04
GROUP 6	51.25±2.5	53.23±2.3	36.12±2.5	195.25±5.3	0.72±0.04

Group 1: Normal control rats treated with distilled water

Group 2: Rats treated with CCL4 (3ml/kg/day) by p.o.

Group 3: CCL_4 induced hepatotoxicity rats treated with of silymarin (25mg/kg) by i.p

Group 4: CCL_4 induced hepatotoxicity rats treated with ethanolic extract of *Oroxylum indicum* (100mg/kg)

Group 5: CCL_4 induced hepatotoxicity rats treated with ethanolic extract of *Oroxylum indicum* (200mg/kg)

Group 6: CCL_4 induced hepatotoxicity rats treated with ethanolic extract of *Oroxylum indicu*

(400mg/kg)

Comparison study:

Group 1. is a normal control, treated with distilled water.

Group 2. is a negative control, trated with CCL_4.

Group 3.is a standard drug compared with Group 1. Group 3 enzyme unit value almost equal to group 1.

Group 4 compared with group 1, the value shows group 4 units increased then group 1.

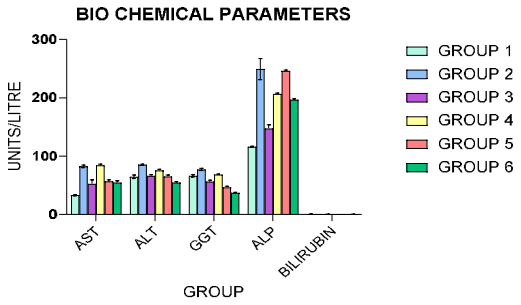
Group 5 compared with group 1, the value shows group 5 units increased then group 1.

And decreased then group 4.

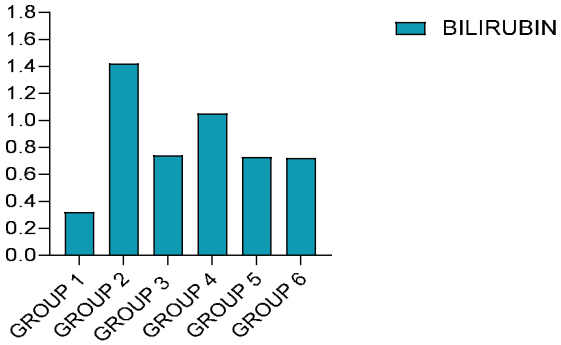
Group 6 compared with group 1, the value shows group 6 units increased then group 1.

And decreased then group 5.

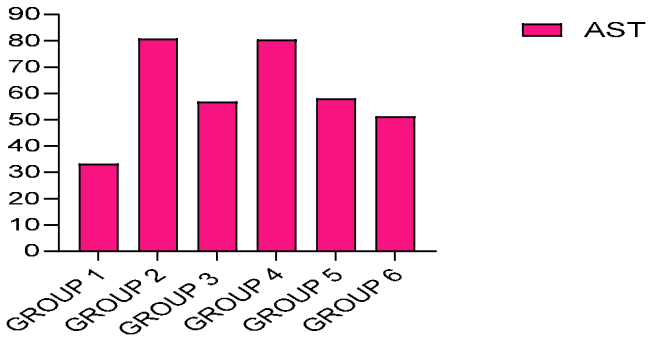
And the group 3 standard drug compared with Group 4,5,6. But the group 6 units similar to group 3.



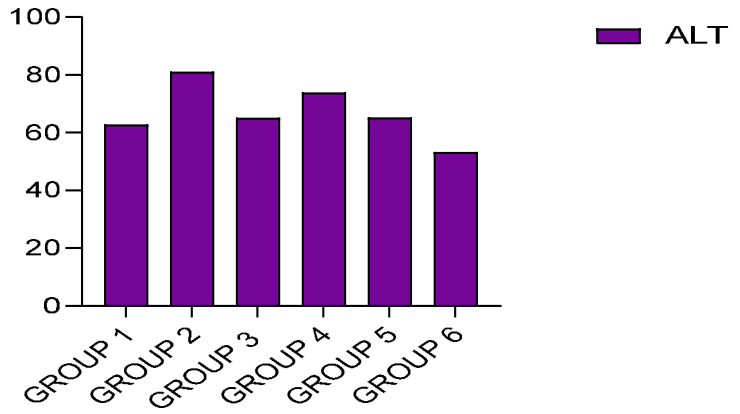
BIO MARKER ENZYME



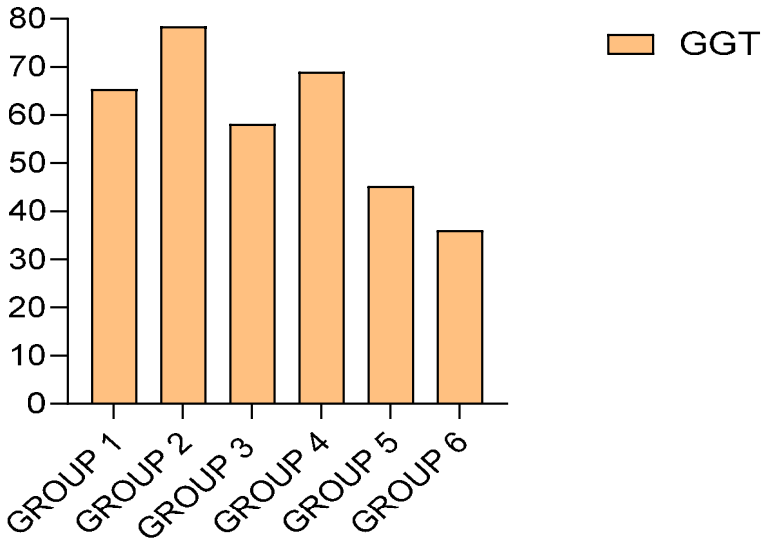
(img.-Enzyme mark of Bilirubin)



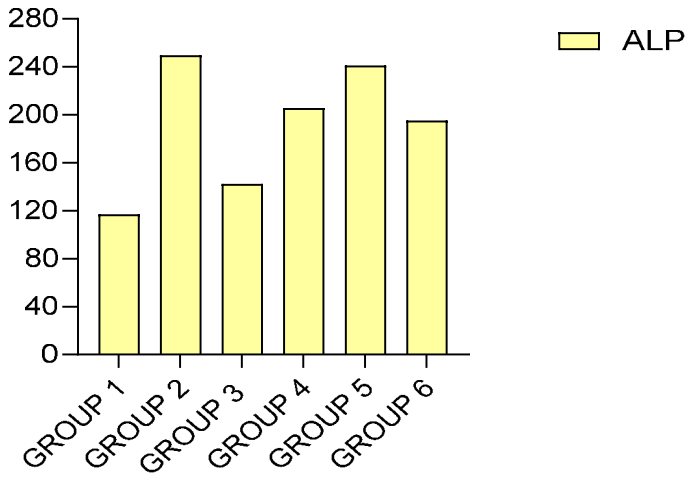
(img- Enzyme mark of AST)



(img-Enzyme mark of ALT)

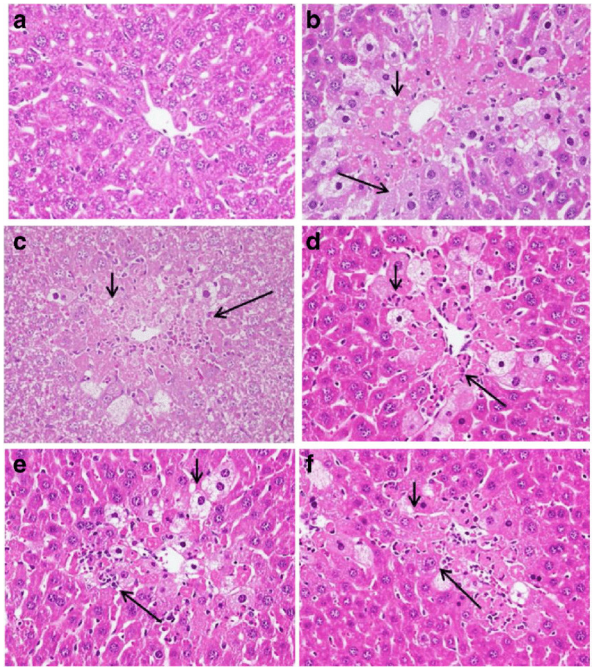


(img-Enzyme mark of GGT)



(img-Enzyme mark of ALP)

HISTOPATHOLOGICAL RESULT



(img.1-microscopical liver structure)

Hepatic histological analyses of *Oroxylum indicum*and silymarin on CCl 4 -induced acute liver damage in mice. Liver tissues were subjected to hematoxylin and eosin staining (400×). (a) Control group; (b) animals treated with 25% CCl 4 ; displayed cell necrosis (long arrow) and vacuole formation (short arrow) (c) animals pretreated with silymarin (200 mg/kg) and then treated with CCl 4 ; (d–f) animals pretreated with *Oroxylum indicum*(100,200,400gm/kg) and then treated with CCl 4.

Description

The group 1 is a normal control, histopathology report shows the liver tissue cells are normal and no cell destruction or cell injury.

The group 2 is a CCL_4 treated, histopathology report shows the liver tissue cells are abnormal and cell destruction or cell injury.

The group 3 is standard drug treated, histopathology report shows the liver tissue cells are normal and cell destruction or cell injury reduced when compared to group 2.

The group 4 is 100mg/kg *Oroxylum indicum*extract treated, histopathology report shows the liver tissue cells are normal and cell destruction or cell injury reduced when compared to group 2.

The group 5 is 200mg/kg *Oroxylum indicum*extract treated, histopathology report shows the liver tissue cells are normal and cell destruction or cell injury reduced when compared to group 2.

The group 6 is 400mg/kg *Oroxylum indicum*extract treated, histopathology report shows the liver tissue cells are normal and cell destruction or cell injury reduced when compared to group 2.

The group 4,5,6 is a ethanolic extract of *Oroxylum indicum*, the three groups are compared to group 3 standard silymarin. The report shows group 6 400mg/kg of *Oroxylum indicum*extract similar to group 3standard.

The liver tissue cells of group 6 similar to group 3. And decrease the liver cell damage.

4. DISCUSSIONS

In the present study, the protective effects of *Oroxylum indicum*ethanolic extract of against CCL_4 induced liver toxicity on rats were evaluated. The result showed that the ccl4 significantly increase the liver weight. The present study also showed that CCL_4induced liver cirrhosisthat should be considered as microsomal enzyme induction leading to increase storage of lipids peroxisome proliferation, and hyperfunction of the liver. Further the study demonstrated that *Oroxylum indicum*extract administration decreased the body and liver weight of the rats as well as reduction in the range of steatohepatitis.The state of liver function could be evaluated by blood assays, describing its functionality and its link with the biliary tract. *Oroxylum indicum*groups indicated that the extract impaired hepatocellular or secretory function of the liver in a dose dependent manner. The five biomarkers of liver damage are AST , ALT , ASP, GGT, bilirubin indicates the liver injury and the ratio of AST and ALT may be employed in disease diagnosis. An AST/ALT ratio greater than 1 suggests myocardial infaraction while, more than 2 is indicative of alcoholic hepatitis or cirrhosis and ranges from 1.2 to 1.4 is an indication of the abnormal functioning of the liver. In the present study, the extract relieved hepatic damage, as revealed by the results of blood chemistry analysis and histopathological assesment; the significant alterations in the indicators of liver damage(ALT, ALP, AST,GGT,bilirubin)and steatosis ratio. The *Oroxylum indicum*extract contains flavonoids which are known to have insulin-like properties and also an inhibitory effect on the lipogenase enzyme. Flavonoids improved cell viability and inhibited cellular leakage of hepatocyte AST and ALT. further, its been shown that flavonoids can decrease the insulin resistance and insulin requierment in adipose and muscle tissue. Besides flavonoids, the plant contain alkaloid, glycoside, steroids saponin that have hypolipidemic properties. Alkaloids reduce carbohydrate absorption and metabolism. Thus, this component of the extract could reduce the absorption of CCL_4 and also interrupt the CCL_4 metabolization to lipids. Saponin is other component of the extract not only regulates lipid metabolism but also improve hypertriglyceridemia. Moreover, polyphenolic and flavonoids compounds of the plant having antioxidant properties are another explanation for antihepatosteatosis of the plant by blocking the second hit of the progression of the disease.

5. CONCLUSION

Oroxylum indicum extract administered improves the signs of liver in wister albino rats. The beneficial effects of *Oroxylum indicum* extract could be seen both in histopathological features and hepatic serum markers of the rats. At histopathological level, the hepatosteatosis was dimished in *Oroxylum indicum* extract groups in a dose dependent manner.

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