ISSN: 2309-5288 (Print)
ISSN: 2309-6152 (Online)
CODEN: JCLMC4

Journal of Coastal Life Medicine

Assessment of Hepatoprotective Potential of Ecbolium Linneanum Extract on Experimental Animals

Received: 14 February 2023, **Revised:** 18 March 2023, **Accepted:** 20 April 2023

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Keywords:
Ecbolium linneanum, Antioxidant, Xenobiotics, Hepatic Disease.

Abstract

The hepatoprotective potential of Ecbolium Linneanum extract was evaluated in experimental animals. Liver diseases pose a significant health burden worldwide, and the search for effective hepatoprotective agents is ongoing. In this study, we investigated the potential of Ecbolium Linneanum extract in protecting the liver against damage induced by various hepatotoxic agents.

Treatment with Ecbolium Linneanum extract resulted in a significant attenuation of liver damage induced by the hepatotoxic agents. Biochemical parameters such as serum levels of liver enzymes, bilirubin, and lipid peroxidation were restored to near-normal levels in the extract-treated groups compared to the control group. Histopathological examination of liver tissues further confirmed the protective effect of Ecbolium Linneanum extract, showing reduced hepatocellular necrosis, inflammation, and fatty infiltration. Furthermore, treatment with Ecbolium Linneanum extract improved liver function markers, including increased levels of antioxidant enzymes and enhanced hepatic glycogen content. The extract also exhibited significant anti-inflammatory activity, as evidenced by reduced levels of pro-inflammatory cytokines in the liver.

1. Introduction

One of the essential organs with multiple significant homeostatic duties is the liver. It is significant to the physiological system in its own right. One of the liver's main jobs is to help with digestion, lipid, protein, and carbohydrate metabolism as well as blood coagulation and immunomodulation.[1] The contemporary remedy of immunosuppressive and corticosteroid sellers simplest introduced approximately suggestive support. Synthetic or traditional pills used withinside the control of liver illnesses are once in a while insufficient and might have excessive unwanted property. Moreover, their remedy is related with danger of relapse and extreme undesirable side effects. In India, Ayurveda, and indigenous device of medication has a completely vintage way of life of treating liver disorder with plant materials, so maximum of the human beings shift to standard medicinal plants. Numbers of herbal merchandise of natural foundation are in use for the remedy of liver disorder [2-9]. *E. linneanum*, a member of the Acanthaceae family, is commonly known as Blue Fox Tail or Blue Justicia in English, Udajati in Hindi, and Nilambari in Tamil. According to Ethnomedical facts the *E. linneanum* has been used for treating diverse illnesses which include diabetes, anthelmintic, purgative, anti- inflammatory, anti-diabetic and in girl fertility. Moreover *E. linneanum* leaf and stem extracts possess "antioxidant and free radical scavenging activities". Therefore, to justify conventional claims, attempt was made to assess hepatoprotective impact of *E. linneanum* by using CCl_4 intoxicated rats [10-13].

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2. Material And Methods

Drugs and Chemicals:

All biochemical evaluation kits were obtained from "Erba Diagnostics Mannheim GmbH" Germany. All other solvents and chemicals used were of analytical grade.

Collection and extraction of plant materials:

The leaves of *Ecbolium linneanum (EL)* collected in July 2021 from local area. The plant was authenticated by renowned botanist. The plants were dried in the shade, powdered and stored in closed containers for further study.

Test animal:

A healthy Wistar albino rats of 150-200 g used for the study. Animals were fed on standard pellet diet, *ad libitum*, and maintained on a 12 hour light / dark cycle. The animals were divided into 6 groups, each consisting of 6 rats. All animal studies were conducted after the research protocol was approved by the IAEC-CPCSEA.

Acute toxicity test:

The acute toxicity evaluation of *EL* leaf ethanolic extracts was conducted on Wistar albino mice in accordance with the guidelines established by the OECD. Various doses of the extracts were administered orally, and it was determined that all extracts were well-tolerated up to a dose of 2000 mg/kg.

EXPERIMENTAL PROTOCOL

Preparation of Test Solution

For oral administration, the ethanol and aqueous fractions of the EL plant were mixed with 1% w/v Carboxy methyl cellulose (CMC) suspension.

Standard Drug:

100 mg/kg body weight of Silymarin was prepared by using distilled water in 1% w/v CMC and administered by oral route.

Group – I:	Distilled water (o) 1 × 7d.
Group –II:	CCl_4& ethanol Control: CCl_4 (i.p, 0.5 ml/kg) single dose & ethanol (3.67 mg/kg, twice daily, p.o.), on 7th day in Groups III, IV, V and VI.
Groop - III:	Standard Silymarin
Group –IV:	Low dose of ethanolic extract & CCl_4 extract
Group –V:	Medium dose of ethanolic extract & CCl_4 extract
Group –VI:	High dose of ethanolic extract & CCl_4 extract

Single dose of pentobarbitone (45 mg/kg i.p.) was given to rats of all groups on the last day, two hours after ethanol &CCl_4 injection. The time between loss of the righting reflex and its recovery was taken as duration of pentobarbitone-induced sleep time.

Biochemical Parameter Estimations:

Anesthesia was induced using diethyl ether as the anesthetic agent. Animals were euthanized 24 hours after the final treatment. Blood samples were collected and serum was obtained by centrifuging at 10,000 rpm for 10 minutes. Biochemical analyses, including (SGOT, SGPT, ALP, Gamma GT, total bilirubin, and direct bilirubin) were conducted. Livers were excised immediately and examined for histopathological studies[16-37].

Histopathological studies of the liver in CCL_4induced hepatotoxicity

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Normal control group: The liver samples exhibited the typical lobular structure with hepatocytes organized in individual cords. Hepatocytes displayed centrally positioned nuclei, and sporadic binucleate cells were observed. The sinusoidal cells were juxtaposed with the nuclei of Kupffer cells.

Toxicant group: Hepatocytes exhibited fatty changes and few show granular degeneration with loss of nuclear architecture. There is also moderate chronic inflammatory infiltrate.

Standard group (Silymarin+CCL_4): No necrosis and no degeneration that indicates prevention of hepatic damage

Low dose Extract + CCL_4: The sections from the liver show mild Inflammation, focal granular degeneration.

Medium dose Extract + CCL_4: Sections shows mild Inflammation

High dose Extract +CCL_4: The sections from liver reveal normal hepatocytes indicating protective effect.

- **Histopathological studies : Ethanol-induced hepatotoxicity**

Normal control group: There is normal lobular architecture of the liver with hepatocyte arranged in single cords.

Toxicant group: The hepatocytes exhibited microvesicular fatty alterations, accompanied by a significant presence of hepatocytes demonstrating hydropic degeneration and disruption of nuclear architecture.

Standard group (Silymarin+ Ethanol): No centrilobular necrosis or severe hydropic degeneration

Low dose of Extract + Ethanol: Sections show Inflammation and focal areas of granular degeneration of hepatocytes.

Medium dose Extract + Ethanol: The sections from the liver, shows mild Inflammation and liver cells appear normal.

High dose Extract + ethanol: Normal hepatocytes with normal lobular architecture.

Table.1. Effect of various treatments on CCL_4 induced rats

Group	SGOT	SGPT	ALP	GGT	TB	DB
Normal Group	205.06±6.91	66.8±3.89	184.7±2.95	0.59±0.015	205.06±6.91	0.17±0.02
CCl_4Treated Group	335.8±3.43	122.86±2.87	495.1±2.98	1.96±0.12	2.49±0.31	0.86±0.021
Standard Group	239.17±2.19	69.7±2.91	211.6±5.9	0.69±0.02	0.81±0.10	0.28±0.25
EL (100 mg/kg)	269±3.7**	81.03±2.09**	251±2.68***	0.87±0.04**	1.09±0.27*	0.47±0.03**
EL (200 mg/kg)	247.1±1.68**	72.9±2.68**	237.1±2.94***	0.77±0.02**	0.89±0.02**	0.41±0.011**
EL (400 mg/kg)	281.2±4.51**	83.1±2.57**	269±2.97***	0.89±0.06**	1.12±0.09*	0.5±0.010**

"Values are expressed as mean ± S.E.M. *P<0.05, **P<0.01, *** P<0.001 when compared with the toxicant control groups (one-way ANOVA followed by Dunnett's't' test)"

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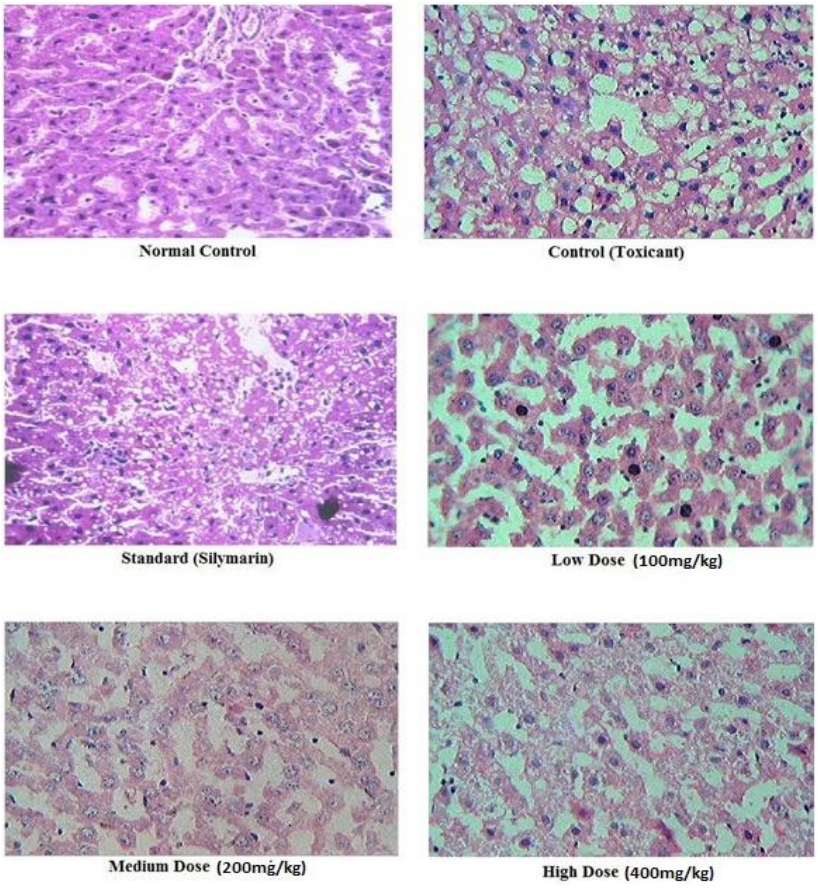
Table.2. Effect of various treatments on Ethanol induced rats

Group	SGOT	SGPT	ALP	GGT	TB	DB
Normal Group	179.06±4.81	71.6±3.09	107.22±2.39	0.63±0.03	0.29±0.02	0.20±0.02
Ethanol Treated Group	237.01±3.43	121.06±2.16	207.53±1.82	1.69±0.15	1.74±0.04	1.75±0.007
Standard Group	228.12±2.09	62.5±2.71	105.68±1.51**	0.59±0.07	0.35±0.07**	0.34±0.01**
El (100 mg/kg)	257±2.07**	79.07±2.19**	201.7±1.21*	0.78±0.03**	1.6±0.024*	1.58±0.024
El (200 mg/kg)	239.1±1.85**	70.09±2.38**	146.16±1.06**	0.71±0.09**	1.20±0.03**	1.13±0.034**
El (400 mg/kg)	241.2±2.31**	81.16±1.97**	126.58±0.69**	0.84±0.07**	0.87±0.01**	0.77±0.04**

"Values are expressed as mean ± S.E.M. *P<0.05, **P<0.01, *** P<0.001 when compared with the toxicant control groups (one-way ANOVA followed by Dunnett's't' test)"

Histopathological Studies

1. Ethanol -induced hepatotoxicity



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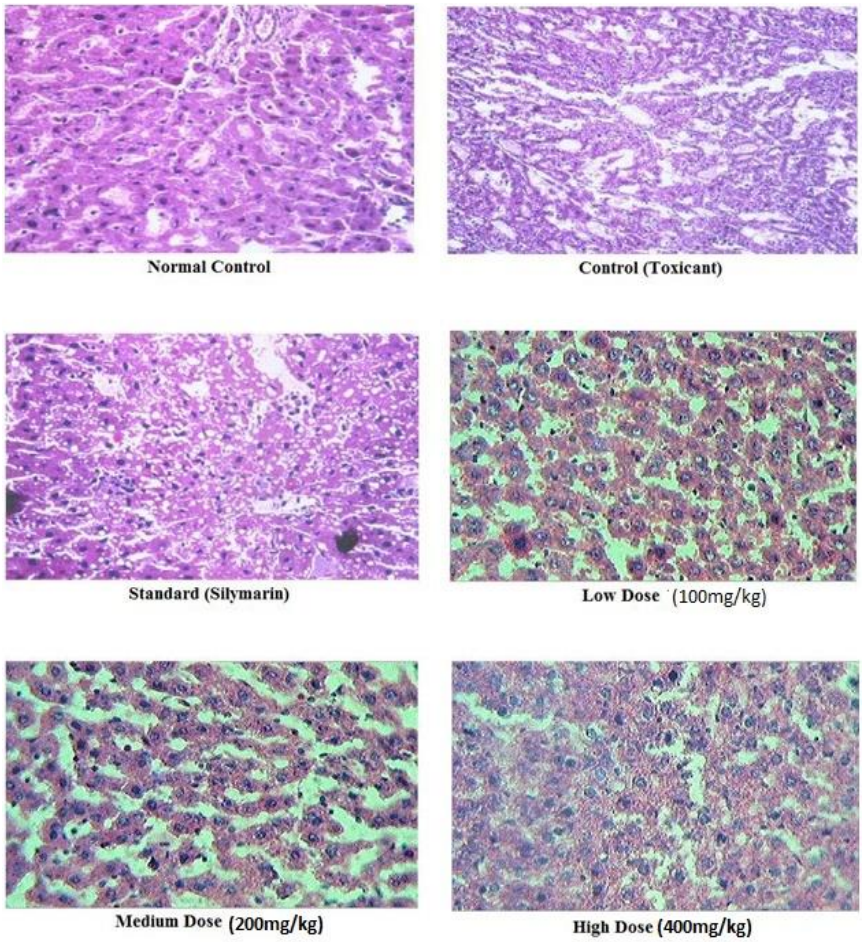
2. CCL_4 -induced hepatotoxicity



3. Discussion

Experimental animals given ethanol and CCl_4 showed increased production of lipoperoxides, conjugated diens, and malondialdehyde (MDA), as well as decreased levels of antioxidants. Increased level of biochemical parameters is an indicator of liver injury. Reactive oxygen species (ROS) produced by Kupffer cells are a major contributor to oxidative stress, which is one of the main factors in the aetiology of injury. Either alcohol or CCl4 activates Kupffer cells by acting on endotoxin, which is released by specific gram-negative bacteria in the intestine and causes ROS and pro-inflammatory cytokines (TNF alpha, IL1), both of which can cause liver damage[14]. Water lodging in hepatocyte contributes to bulging resulting in increased total liver weight and volume[15]. Administration of hepato toxicants induces hepatotoxicity, causes cellular enzymes like alanine transaminase, aspartate transaminase, and alkaline phospatase present in liver cells leak into serum, resulting in increased concentrations[16]. Treatment with ethanolic extracts from *Ecbolium linneanum* leaves restores biochemical enzymes and brings down to normal as compared to standard. The most significant result found at highest dose.. The histopathological studies for both models showed minimal degradation and fatty changes with withholding of regular lobular design confirms ethanolic extracts from *Ecbolium linneanum* leaves hepatoprotective function.

4. Conclusion

The significant hepatoprotective activity of ethanolic extracts from Ecbolium linneanum leaves is supported by the observed improvements in serum marker enzyme levels, physical parameters, histopathological studies, and the presence of phytoconstituents. These findings affirm the traditional Ayurvedic claim of Ecbolium linneanum as an effective hepatoprotective agent.

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ISSN: 2309-6152 (Online)
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