

Hepatoprotective potential of *Cordia subcordata* Lam. against carbon tetra chloride (CCl₄)-induced hepatotoxicity in Wistar albino rats.

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ABSTRACT

Aim: To investigate the phytoconstituents, acute oral toxicity and hepatoprotective activity of ethanol (90%) extract of *Cordia subcordata* Lam. (EECS) using CCl₄ induced hepatotoxicity in male Wistar albino rats.

Methods: The EECS at doses of 100, 200 and 400mg/kg, p.o and the standard drug Liv.52 (40mg/kg, p.o) were administered for 7 days in CCl₄ intoxicated rats. The hepatoprotective activity was assessed by using various biochemical parameters like SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP), also total bilirubin and urea along with histopathological studies of liver tissue. The biochemical changes and histopathological studies were observed on 4th and 8th day.

Results: EECS at tested doses significantly decrease ($P < 0.001$) the elevated levels of the hepatic enzymes, total bilirubin and urea in a dose dependent manner after 3days whereas it's subsequent return towards near normal after 7days indicating the recovery of hepatic cells. In the liver sections of the rats treated with EECS extracts for 7 days, the normal cellular architecture was retained as compared to Liv.52, thereby furtherly confirming the potent hepatoprotective effect of EECS.

Conclusion: The EECS afforded significant protection against CCl₄ induced hepatocellular injury.

KEY WORDS: Hepatotoxicity, CCl₄, Hepatic Enzymes, *Cordia subcordata* Lam. Hepatoprotective, EECS

INRODUCTION

The liver regulates many important metabolic functions, detoxification, and secretory functions in the body. Hepatic injury is associated with distortion of these metabolic functions [1]. Thus, liver diseases remain one of the serious health problems and its disorders are numerous with no effective remedies. Despite, considerable progress in the treatment of liver diseases by oral hepatoprotective agents, search for newer drugs continues because the existing synthetic drugs have several limitations [2-4]. So, the search for new medicines is still ongoing. Because liver performs many vital functions in the human body and damage of liver causes unbearable problems. [5, 6]. Keeping this fact in view, the present study was undertaken to investigate the hepatoprotective

activity of *Cordia subcordata* Lam. leaves against carbon tetrachloride-induced hepatic damage in albino rats.

***Cordia subcordata* Lam.** Family: Boraginaceae) is a medium-sized spreading tree to 12m tall with grayish grooved flaking bark. Leaves alternate, petiolate, the petiole about half as long as blade, broadly ovate and entire, often wavy-margined, the apex obtuse to short-pointed, base rounded, the blade up to 20 cm long. Flowers showy, orange, trumpet -shaped, unscented and borne in small axillary or terminal clusters. Fruit a globose drupe up to 3 cm long, surrounded by the enlarged calyx. Flowers and fruit usually available throughout the year. The seeds float and are highly resistant to salt water, thus the species is common in

coastal areas. In Tahiti, the leaves are used in remedies for bronchitis and asthma where the leaves probably act as a purgative. The plant is also used in the treatment of hepatic infections, cirrhosis of the liver and inflammation of the lymph nodes. It is also used to treat albumin present in the urine. Cook Islanders use the leaves in remedies for abdominal swellings and urinary tract infections [7-9].

However, there are no ethnomedicinal information and scientific findings for the above said traditional claim for hepatic disorders. Therefore, to justify the traditional claims the present study was undertaken to find out if ethanol extract of *Cordia subcordata* Lam. leaves demonstrates the hepatoprotective activity against CCl₄-induced liver damage in rats. Hence, the present study was designed to verify the claims of the native practitioners.

MATERIALS AND METHODS

Plant collection

The Plant material of *Cordia subcordata* Lam. leaves was collected from Tirunelveli District, in the Month of August 2008. The plant was authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The voucher specimen (CHE-SA-CS-08) of the plant was deposited at the college for further reference.

Preparation of plant extract

The leaves of *Cordia subcordata* Lam. were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (80gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample.

Percentage yield of ethanolic extract of *Cordia subcordata* was found to be 16.5 % w/w.

Preliminary phytochemical screening

The phytochemical examination of ethanolic (90%) extract of *Cordia subcordata* Lam. leaves was performed by the standard methods [10].

Animals used

Wistar albino rats (150-220g) of either sex were obtained from the animal house in C.L. Baid Metha College of Pharmacy, Chennai. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref No. IAEC / XIII / 01 / CLBMCP / 2008 - 2009).

Acute Toxicity Study

The acute toxicity of 90% ethanolic extract of *Cordia subcordata* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, 1/20th (100mg/kg), 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study [11].

Carbon tetrachloride-induced hepatotoxicity in rats

The liver protective effect was evaluated using the carbon tetrachloride (CCl₄) model described by Visweswaram et al. [12]. Wistar albino rats (150-220gm) were divided into six groups of six rats each and were subjected to the following treatments: Group-I served as normal control received distilled water (1 ml/kg, p.o) for 7days. Group II -VI received

0.75 ml/kg CCl₄ administered orally as single dose. After 36 hours, Groups III-VI received EECS with doses of 100, 200 and 400mg/kg, p.o and the standard drug Liv.52 with dose of 40mg/kg, p.o, respectively once daily for 7days. The blood was collected by puncturing the retro-orbital sinus of three rats from each group on 4th day of treatment and 8th day after the treatment respectively. From the collected blood samples, serum was separated to assess various biochemical parameters.

Biochemical estimation

The separated serum was subjected to estimate SGOT and SGPT by Reitman and Frankel method, alkaline phosphatase (ALP) and acid phosphatase (ACP) by Kind and King method, bilirubin by Malloy and Evelyn method and urea by Bousquet method [13-16]. The rats were then sacrificed by bleeding and the liver was carefully dissected, cleaned of extraneous tissue, and part of the liver tissue was immediately processed for histopathological investigation.

Histopathological studies

The tissues of liver were fixed in 10% formalin and embedded in paraffin wax. Sections of 4-5 microns thickness were made using rotary microtome and stained with haematoxylin-eosin and histological observations were made under light microscope [17, 18].

Statistical analysis

The data were expressed as mean \pm standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

RESULTS

The results of preliminary phytochemical screening of the ethanolic extract of *Cordia subcordata Lam.* revealed that presence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, terpenoids and absence of saponins and steroids.

Acute toxicity study

Acute toxicity study in which the animals treated with the EECS at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

Effect of EECS on CCl₄ - induced hepatotoxicity

The results of EECS on carbon tetrachloride-induced hepatotoxicity were represented in **Table 1 and Table-2**. The CCl₄ only treated animals exhibited a significant increase ($P < 0.001$) the levels of SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP) and also total bilirubin and urea when compared to the normal control group on both 4th and 8th day, indicating hepatocellular damage.

The EECS at tested doses (group III-V) produced a significant reduction ($P < 0.001$) in the CCl₄-induced elevated levels of SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP), also total bilirubin and urea when compared to the CCl₄ only treated animals (group-II) after 3days of treatment and reduced furthermore to the normalcy on 8th day although the lowest dose (100 mg/kg) tested could produced significant reduction even after 3days of treatment (**Table 1**). Overall, EECS at tested doses significantly reduced the levels of hepatic enzymes, total bilirubin and urea in a dose dependent manner.

Table-1: EECS on CCl₄-induced alteration of hepatic enzymes, serum bilirubin and urea in rat liver after 3 days

Design of Treatment	Biochemical parameters					
	SGOT(U/ml)	SGPT(U/ml)	ALP (KA Units)	ACP(KA Units)	Bilirubin(mg/dl)	Urea(mg/dl)
Group-I: Normal control (DW-1 ml/kg; p.o)	49.31 ± 1.16	60.61 ± 0.06	17.60 ± 0.92	4.02 ± 0.17	0.82 ± 0.03	36.41 ± 1.53
Group-II: CCl ₄ (0.75 ml/kg; p.o)	186.20 ± 1.35* ^c	154.20 ± 1.35* ^c	46.00 ± 1.02* ^c	5.93 ± 0.03* ^c	3.06 ± 0.04* ^c	96.82 ± 1.26* ^c
Group-III:EECS (100 mg/kg; p.o)	124.46 ± 1.46*	132.46 ± 0.46*	38.42 ± 1.72*	5.23 ± 0.01*	1.61 ± 0.03*	78.24 ± 1.15*
Group-IV:EECS (200 mg/kg; p.o)	74.03 ± 1.41*	97.03 ± 1.41*	29.80 ± 1.85*	4.58 ± 0.16*	1.24 ± 0.04*	57.12 ± 1.13*
Group-V:EECS (400mg/kg; p.o)	66.40 ± 1.72*	71.40 ± 1.02*	27.02 ± 0.05*	4.26 ± 0.03*	1.12 ± 0.02*	48.60 ± 0.92*
Group-VI: Liv.52 (40 mg/kg; p.o)	59.74 ± 1.32	65.74 ± 0.92*	25.82 ± 1.85*	4.11 ± 0.02*	1.01 ± 0.03*	42.06 ± 0.94*

Values are Mean ± SEM of 6 animals each in a group.

^c P<0.001, when compared group I Vs group-II,

*P<0.001, when compared group II Vs group III, IV, V and VI

EECS = ethanol (90%) extract of *Cordia subcordata*, CCl₄ = Carbon tetrachloride

DW=distilled water

Table-2: EECS on CCl₄-induced alteration of hepatic enzymes, serum bilirubin and urea in rat liver after 7 days

Design of Treatment	Biochemical parameters					
	SGOT(U/ml)	SGPT(U/ml)	ALP (KA Units)	ACP(KA Units)	Bilirubin(mg/dl)	Urea(mg/dl)
Group-I: Normal control (DW-1 ml/kg; p.o)	49.31 ± 1.16	60.61 ± 0.06	17.60 ± 0.92	4.02 ± 0.17	0.82 ± 0.03	36.41 ± 1.53
Group-II: CCl ₄ (0.75 ml/kg; p.o)	159.20 ± 1.03* ^c	114.14 ± 1.41* ^c	46.12 ± 0.91* ^c	4.02 ± 0.13* ^c	2.12 ± 0.04* ^c	89.17 ± 0.54* ^c
Group-III: EECS (100 mg/kg; p.o)	108.46 ± 1.15*	95.13 ± 0.25*	28.13 ± 0.04*	3.61 ± 0.04*	1.40 ± 0.05*	67.24 ± 1.15*
Group-IV: EECS (200 mg/kg; p.o)	65.20 ± 0.91*	73.07 ± 0.62*	21.01 ± 0.64*	3.26 ± 0.14*	1.18 ± 0.02*	48.12 ± 1.13*
Group-V: EECS (400 mg/kg; p.o)	57.17 ± 1.24*	64.02 ± 0.04*	17.04 ± 0.02*	3.15 ± 0.02*	1.00 ± 0.01*	39.60 ± 1.43*
Group-VI: Liv.52 (40 mg/kg; p.o)	52.13 ± 0.81*	61.12 ± 0.43*	15.34 ± 0.92*	3.09 ± 0.03*	0.90 ± 0.02*	34.46 ± 0.62*

Values are Mean ± SEM of 6 animals each in a group. ^c P<0.001, when compared group I Vs group-II,

*P<0.001, when compared group II Vs group I, III, IV, V and VI

EECS= ethanol (90%) extract of *Cordia subcordata*, CCl₄ = Carbon tetrachloride. DW=distilled water

After 7 days, the hepatic enzymes levels were almost restored to the normal after treating with EECS at the dose of 400mg/kg, p.o.

A standard drug, Liv.52 at a dose of 40 mg/kg (group-VI) administered orally produced a significant reduction (p<0.001) compared to CCl₄ only treated animals (group-II) on both 4th and 8th day and these protective effects almost close to EECS 400mg/kg, p.o.

Effect of EECS on histopathological change:

Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic

cells, sinusoidal spaces and central vein on both 4th and 8th day (**Fig.1a and 1b**).

Disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration were observed (on both 4th and 8th day) in CCl₄ intoxicated rats (**Fig.2a and 2b**).

The liver sections (on both 4th and 8th day) of the group-V rats treated with EECS (400mg/kg, p.o) showed a sign of protection as it was evident by the moderate accumulation of fatty lobules, absence of necrosis and vacuoles (**Fig. 3a and 3b**). Almost similar sign of protection was shown in the liver sections of Liv.52 at a dose of 40 mg/kg treated rats (**Fig. 4a and 4b**).

Fig. 1 (a): Normal control treated group on 4th day (100x)

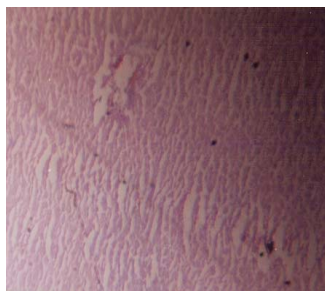


Fig. 1(b): normal control treated group on 8th day (100x)

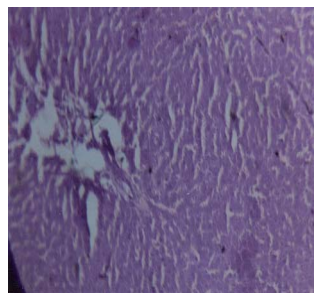


Fig. 2(a): CCl₄ treated group on 4th day (100x)

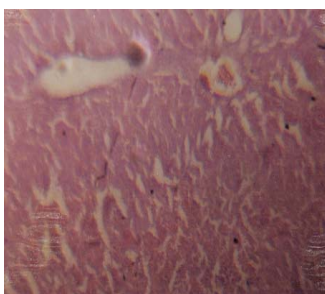


Fig. 2(b): CCl₄ treated group on 8th day (100x)

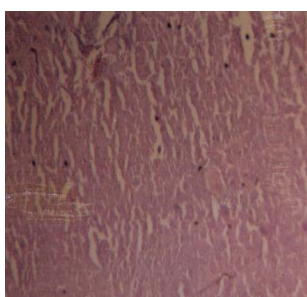


Fig. 3 (a): CCl₄ supplemented with EECS 400 treated group on 4th day (100x)

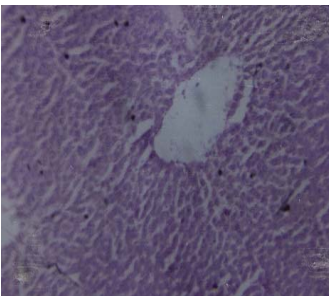


Fig. 3 (b): CCl₄ supplemented with EECS 400 treated group on 8th day (100x)

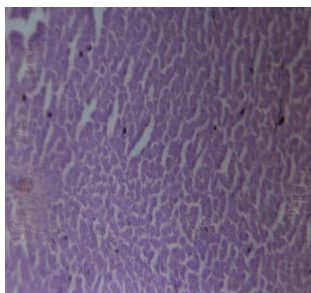


Fig. 4(a): CCl₄ with Liv.52 (40mg/kg, p.o) treated group on 4th day (100x)

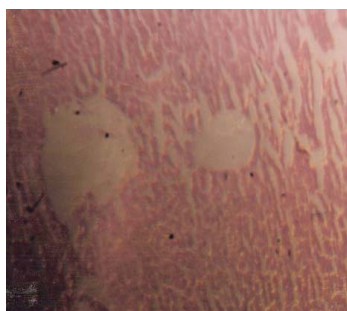
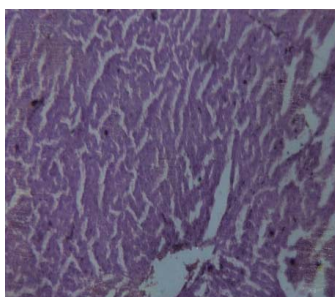


Fig. 4(b): CCl₄ with Liv.52 (40mg/kg, p.o) treated group on 8th day (100x)



DISCUSSION AND CONCLUSION

The present studies were performed to assess the hepatoprotective activity of ethanol (90%) extract of *Cordia subcordata* leaves in rats against carbon tetrachloride as hepatotoxin to prove its claims in folklore practice against liver disorders.

It is well documented that carbon tetrachloride-induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of drugs or medicinal plants' extracts, by in vivo and in vitro techniques [19-21]. Carbon tetrachloride (CCl₄) is a potent hepatotoxin producing centrilobular hepatic necrosis. It is accumulated in hepatic parenchyma cells and metabolized to CCl₃ by liver cytochrome P450-dependent monooxygenases [22].

Usually, the extent of hepatic damage is assessed by histopathological evaluation and the level of hepatic enzymes ALT, AST and ALP release in circulation [23]. The administration of CCl₄ resulted in a significant increase in the serum SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP) and also total bilirubin and urea within 36 hours [24, 25]. The rise in serum levels of AST, ALT, ALP and ACP has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into circulation after cellular damages [26].

In our study, the biochemical changes were observed after each 3 and 7 days. Thereby, it was found that, the administration of EECS at doses of 100, 200 and 400mg/kg, p.o for 3 days resulted in significantly decreases the CCl₄-induced elevated levels of the hepatic enzymes SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase (ACP) in a dose dependent manner. These results indicating the production of structural integrity of hepatocytic cell

membrane or regeneration of damaged liver cells by the extracts. Whereas, the EECS extracts at tested doses decreases the CCl₄-induced elevated level of hepatic enzymes in rats, and its subsequent return towards near normalcy after 7days. Reduction in the levels of SGOT and SGPT towards the normal value is an indication of regeneration process. Reduction of ALP levels with concurrent depletion of raised bilirubin level suggests the stability of the biliary function during injury with CCl₄.

Bilirubin is the conventional indicator of liver diseases [27]. The rise in the levels of serum bilirubin is the most sensitive and confirms the intensity of jaundice [28]. These biochemical restorations may be due to the inhibitory effects on cytochrome P450 or/and promotion of its glucuronidation [29]. The marked elevation of bilirubin and urea level in the serum of group II CCl₄ intoxicated rats were significantly decreased in the groups III-V EECS treated animals after 3days. Whereas, after 7 days of treatment, bilirubin and urea level in the serum CCl₄ intoxicated rats subsequently return towards near normalcy in the groups III-V EECS treated animals. These results further substantiate *Cordia subcordata* as a potent hepatoprotective agent.

It has been reported that Liv.52 protects liver from the hepatotoxicity of carbon tetrachloride [30, 31]. An appreciable protective effect was observed even after 3 days compared with 7 days treatment using marketed product (Liv.52). The extent of production by extracts appeared to depend on the duration of treatment. Overall, these results suggest that the EECS could protect the liver against damage induced by CCl₄ when comparable with Liv.52.

The attributivity of the observed alterations of SGOT, SGPT, alkaline phosphatase (ALP) and acid phosphatase

(ACP), serum ALT were confirmed by histopathological studies of liver sections which reveal that the normal liver architecture was disturbed by hepatotoxin (CCl₄) intoxication. In the liver sections of the rats treated with EECS extract for 7 days, the normal cellular architecture was retained as compared to Liv.52, thereby further confirming the potent hepatoprotective effect of *Cordia subcordata* leaves.

Further research is needed to isolate and purify the active principle involved in hepatoprotection of this plant as well as to confirm the mechanisms responsible for hepatoprotective activity. The present finding provides scientific evidence to the ethnomedicinal use of *Cordia subcordata* in treating hepatic disorders.

Acknowledgement

Authors are sincerely thankful to Dr. S. Venkatraman, M.Sc., M.D., Ph.D., Director and Mr.P.Muralidharan, M.Pharm. (Ph.D), Department of Pharmacology, C.L. Baid Metha Foundation for Pharmaceutical Education and Research, for their contribution and facilities provided regarding our Research work.

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