

HEPATOPROTECTIVE ACTIVITY OF *ENICOSTEMMA AXILLARE* IN CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN ALBINO RATS

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ABSTRACT

The present study was conducted to evaluate the hepato-protective activity of pet ether, chloroform, methanol and water extract of aerial parts of *Enicostemma axillare* are evaluated in CCl₄-induced hepatotoxicity in albino rats. Silymarin (200mg/kg) was given as reference standard. The methanolic and water extracts of aerial parts of *Enicostemma axillare* have shown very significant hepatoprotection against CCl₄-induced hepatotoxicity in albino rats in reducing serum total bilirubin, direct bilirubin, SGPT and SGOT levels.

INTRODUCTION

Enicostemma axillare belongs to family Gentianaceae is a perennial herb found in through out India and common in costal areas. The plant is used in folk medicine to treat diabetes mellitus, rheumatism, abdominal ulcers, hernia, swelling, itching and insect poisoning (kirtikar and Basu, 1999) and also anti-inflammatory (Sadique *et al.*, 1987), hypoglycemic (Jyoti, *et al.*, 2000, Murli *et al.*, 2002; Jyoti *et al.*, 2003) and anticancer (Murli *et al.*, 2002) activities have been reported. These reported activities and many of the ethnobotanical uses of the plant are related to its hepatoprotective activity. Swertimarin, alkaloids, steroids, triterpenoids, saponins, flavonoids, xanthenes, and phenolic acid were isolated from the plant (Kavimani and Manisenthilkumar, 2001) many such compounds have protective effects due to pharmacological activities (Wargovich *et al.*, 2001) Liver disease remains one of the serious health problems. Herbs play a major role in the management of various liver disorders. A number of plants possess hepatoprotective property (Heba *et al.*, 2006). As a result the chemical reactions liver may generate several reactive species like free radicals. These reactive species form covalent bond with the lipids of the tissue. However, inbuilt protective mechanisms combat the hazardous reactions associated with the free radicals. Due to excessive exposure to hazardous chemicals, the free radicals generated will be so high that they overpower the natural defensive system leading to hepatic damage and cause jaundice, cirrhosis and fatty liver, which remain one of the serious health problems. Carbontetrachloride (CCl₄) is one such hazardous chemical which induces hepatopathy through membrane lipid

peroxidation by its free radical derivative. Excessive production of the reactive species manifests in tissue depletion, lipid peroxidation, plasma membrane damage etc., culminating into severe hepatic injury. With this scientific information, the present study was designed to assess the hepatoprotective activity of the different extract of aerial parts of *Enicostemma axillare* against CCl₄ induced liver damage.

MATERIALS AND METHODS

Plant material

The plant material used in this study was collected during month of January in Rajur (Sangamner) and authenticated by Botanical survey of India, Pune. A voucher specimen has been deposited in Botanical survey of India Pune (EA/ 745/2006)

Preparation of the extract

The shade dried aerial part of *Enicostemma axillare* was extracted with pet ether, chloroform and methanol successively by soxhlation method, water by maceration method, concentrated over water bath and evaporated under reduced pressure. The yields of extract were calculated

Animals

Albino rats (either sex) of Sprague dawley strain, weighing 150-200g were used. The animals were acclimatized to laboratory conditions (25°C) for 4 days and given pelleted animal feed (Hindustan Lever) and drinking water, Diagnostic reagent kits (Enzopak) were used for the estimation of serum SGPT and SGOT levels.

Toxicity studies

Acute toxicity study was performed for different extract according to the acute toxic classic method as per OECD guidelines (7), albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose of 100, 200 and 400 mg/kg and observed for 16 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If the mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose *i.e.* 400 mg/kg.

Hepatoprotective activity

The animals were divided into seven groups comprising of six albino rats in each group using randomization technique and treated with the extract for seven days to assess the hepatoprotective potential of the plant. The first group (vehicle control) received vehicle for all the seven days. The second group was kept as toxin control and given only the CCl₄ treatment. The third group received pet ether extract in the dose of 200mg/kg p.o. and the fourth group received the chloroform extract in the dose of 200mg/kg p.o. fifth group received the methanolic extract in the dose of 200mg/kg p.o. sixth group received the water extract in the dose of 200mg/kg p.o. and The seventh group received Silymarin in the dose of 200mg/kg, p.o. as a reference material for the study. All the animals except the vehicle control received CCl₄ on 16th day of the treatment. The animals were sacrificed by cervical dislocation after 48 hrs of CCl₄ administration. The blood samples were collected by cardiac puncture in heparinized microfuge tubes. The blood samples thus collected were immediately centrifuged at 2200rpm for 15 minutes. When serum clearly separated out, the serum was analyzed for SGPT and SGOT levels using enzopak reagent kits by the method proposed by Reitman and Frankel (10, 11). The results thus obtained were subjected to statistical analysis using *student t-test* and analysis of variance (Table 1)

RESULTS AND DISCUSSION

The present studies were performed to assess the hepatoprotective activity in rats against carbon tetrachloride as hepatotoxin to prove its claims in folklore practice against liver disorders. Carbon tetrachloride-induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plants extracts and drugs. The extent of hepatic damage is assessed by histological evaluation and the level of various biochemical parameters in circulation. Highly reactive trichloro free radical formation, which attacks polyunsaturated fatty acids of the endoplasmic reticulum, is responsible for the hepatotoxicity of CCl₄. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals. From the Table 1 it was evident that all extracts were able to reduce all the elevated biochemical parameters due to the hepatotoxin intoxication. The levels of total proteins and albumin were reduced due to the CCl₄ induced hepatotoxicity. The reduction is attributed to the initial damage produced and localised in the endoplasmic reticulum which results in the loss of P450

Table 1: Effect of different extracts of *Enicostemma axillare* aerial parts on CCl₄-induced hepatotoxicity

Groups	Total bilirubin ^a (mg/dL)	Direct bilirubin ^a (mg/dL)	SGPT (Units/mL) ^a	SGOT (Units/mL) ^a
Control	0.70 ± 0.03	0.66 ± 0.12	81.23 ± 1.62	195.25 ± 1.62
CCl ₄	1.20 ± 0.07	2.11 ± 0.14	269.50 ± 44.70	349.23 ± 17.30
Pet ether extract (200mg/kg)	0.93 ± 0.03	1.32 ± 0.16	110.34 ± 36.27	240.32 ± 13.56
Chloroform extract (200mg/kg)	0.90 ± 0.02	1.12 ± 0.20	130.13 ± 7.21	208.56 ± 19.10
Methanolic extract (200mg/kg)	1.16 ± 0.12	0.67 ± 0.50	84.79 ± 7.90	198.10 ± 30.39
Water extract (200mg/kg)	0.71 ± 0.13*	0.65 ± 0.44*	80.75 ± 37.93*	196.06 ± 14.01*
Silymarin (200mg/kg)	0.79 ± 0.04*	0.71 ± 12*	89.32 ± 13.49*	199.35 ± 21.01*

^a Values of mean ± S.E.M. (n=6), * p < 0.01 vs. control, Student 't' test

leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver. Reduction in the levels of SGOT and SGPT towards the normal value is an indication of regeneration process. The protein and albumin levels were also raised suggesting the stabilization of endoplasmic reticulum leading to protein synthesis. The protective effect exhibited by extracted at dose level of 200 mg/kg was comparable with the standard drug silymarin. The histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxin intoxication. In the liver sections of the rats treated with extracted and intoxicated with CCl₄; rats treated with water extract and intoxicated with CCl₄ the normal cellular architecture was retained as compared to silymarin, there by confirming the protective effect of the extract. In accordance with these results, it may be hypothesized that tannin, saponins and flavonoids, which are present in extracts, could be considered responsible for the hepatoprotective activity.

The methanolic and water extracts of aerial parts of *Enicostemma axillare* have shown very significant hepatoprotection against CCl₄-induced hepatotoxicity in albino rats in reducing serum total bilirubin, direct bilirubin, SGPT and SGOT levels. Liver section of *Enicostemma axillare* treated animal group clearly showed normal hepatic cells and central vein thereby confirming hepatoprotective activity.

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