

Hepatoprotective Activity of *Enicostema Axillare* Against CCl₄ – Induced Hepatic Injury in Rats.

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Summary

To evaluate the hepatoprotective activity of ethanolic extracts of *Enicostema Axillare* against CCl₄ induced hepatic injury in rats.

In hepatotoxic rats, liver damage was studied by assessing parameters such as alkaline phosphate (ALP), GOT, GPT, Glucose, Cholesterol and total protein concentration in blood. Hepatic damage is evidenced by rise in the level of alkaline phosphate, GOT, GPT, Glucose, Cholesterol and total protein. Liver shows a tendency to attain near normalcy in animals co-administered with *Enicostema Axillare*. Values of normal rats for GOT, GPT, alkaline phosphate, serum glucose, serum cholesterol and total protein are 157.89±19.31, 92.24±4.46, 147.75±9.07, 146.44±11.55, 72.14±5.16, 5.65±0.6 in IU/L respectively. Where as in CCl₄ treated rats values rose to 247.06±14.26, 184.4±12.11, 171.33±11.39, 180.47±6.92, 73.61±2.59, 3.29±1.38 in IU/L respectively. Silymarin treated rats GPT, Alk-PO₄, serum-glucose, serum cholesterol and total protein levels values reduced to 179.83±15.91, 141.33±7.37, 190.9±18.15, 61.11±7.30, 6.43±0.70 in IU/L respectively. While in plant extract administered rats GOT, GPT, alkaline phosphate, Glucose, Cholesterol and total protein are 144.84±10.20, 124.34±14.20, 108.58±6.50, 187.11±11.6, 76.52±3.16, 4.45±0.38 in IU/L respectively attained near normal values. There is no significant change in bilirubin of normal rats (0.525±0.03), CCl₄ treated rats (0.49±0.03), Silymarin treated rats (0.53±0.03) and *Enicostema Axillare* treated rats (0.5±0.05). All these values are expressed as means ± S.D.

Enicostema Axillare has potent Hepatoprotective activity against CCl₄ induced liver damage in rats.

Keywords: Hepatoprotective; *Enicostema Axillare*; Silymarin, CCl₄

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Introduction

Enicostema Axillare belong to the family Gentianaceae. This is seasonal plant found in India. Local name of plant is Nai. The leaves, flowers and root parts of this plant has been traditionally used as a folk remedy for the treatment of Jaundice, blood purifier, lactagogue, in Maharashtra, India, especially by the tribal peoples of western ghats. Work on other plants of Gentianaceae family like *Swertia longifolia Boiss* [1] has been reported to possess clinically useful hepatoprotective activity but research on other family member having genus *Enicostema* have not done before this. At present, one of the plant-derived medicine approved for use in liver cirrhosis and alcoholic liver diseases is silymarin. There are number of studies which conclude the efficacy of silymarin in these conditions [4]. Silymarin is a mixture of flavonolignans from the fruits of *Silybum marianum* that has been known since ancient time and recommended in traditional European and Asian medicine mainly for the treatment of liver disorder [5]. Therefore in the present study silymarin was used as positive control to compare the efficacy of *Enicostema Axillare* against CCl₄-induced hepatotoxicity.

Materials and Methods

2.1. Plant

Enicostema Axillare plant is collected from the fields in and around Ahmednagar (District), Maharashtra, India and authenticated by the Botanical Survey of India, Pune. A voucher specimen is deposited in the Department of Chemistry in Sangamner College, Sangamner.

2.2. Extraction

Leaves and roots dried at 40°C and pulverized were extracted with 70% ethanol at room temperature for 72h and dried at 60°C to give a yellow coloured residue. A portion of the residue was dissolved in distilled water, filtered and dried to determine the amount of the water-soluble fraction in the residue. Prior to the experiment residue was dissolved in a saline/Cremophor (0.025%v/v) solution and diluted to desired concentration to give a water-soluble fraction (AFSC).

2.3. Animals

Wistar rats of either sex, weighing 150–250 g, were used. Animals were housed under controlled conditions of temperature (25±2°C) and photoperiod 12-h light/dark and fed with standard rodent pellet diet with tap water.

2.4. Induction of hepatic injury

Hepatic injury was induced in rats by subcutaneous administration of a single dose of 0.3 ml/kg CCl₄ mixed with 0.5ml liq. Paraffin on the 7th day, 2 h after the last treatment [6]. Animals were grouped as follows:

Normal Control group: Treated with vehicle (0.5ml,liq.paraffin i.p.) on first day. Followed by 2ml D/W daily for 7 days.

CCl₄ control group: Treated with 0.7ml/kg CCl₄ in 0.5ml. Liq.paraffin i.p. Followed by 2.0ml D/M water oral dose daily for 7 days.

CCl₄ Recovery group: Treated with 0.7ml/kg CCl₄ in 0.5ml. Liq.paraffin i.p on first day, Followed by 2.0ml D/M water oral dose daily for 7 days.

Silymarin control group: Treated with 0.7ml/kg CCl₄ in 0.5ml. Liq.paraffin i.p. and 0.007g/kg silymarin daily for 7 days.

Plant extract group: Treated with 0.7ml/kg CCl₄ in 0.5ml. Liq.paraffin i.p. and 0.66g/kg plant extract dose daily for 7 days.

On day 9, 48 h after CCl₄ administration, blood sample of each animal was taken from abdominal aorta under pentobarbitone anesthesia (35 mg/kg i.p.) and serum cholesterol [7], GOT, GPT, Bilirubin [8], serum glucose, total protein [9] and alkaline phosphates [10] were evaluated.

2.5. Statistical analysis

All values are expressed as means \pm S.D. The results were calculated and subjected to analysis of variance (ANOVA) followed by Student's t-test. P values of $N>0.05$ were considered significant [11].

Results

Food consumption and weight gain

We observed that there was significant decrease in body weight of CCl₄ treated group as compared to normal control group. Treatment of rats with silymarin and plant extract showed significant increase in body weight as compared to CCl₄ treated group (Table 1).

Table 1 Effect of *Enicostema Axillare* ethanolic extract on body weight

Groups	Body weights of rats (g) [#]						
	1 st Day	2 nd Day	3 rd Day	4 th Day	5 th Day	6 th day	7 th Day
Normal control	160.9 \pm 3.2	161.33 \pm 3.3	161.5 \pm 3.5	162 \pm 3.6	162.9 \pm 3.2	163.9 \pm 4.2	164.5 \pm 2.6
CCl ₄ control	155.1 \pm 4.2**	146.2 \pm 4.6*	140.2 \pm 5.0**	135.0 \pm 5.0*	130 \pm 5.1**	127.5 \pm 4.5*	122.9 \pm 5.0**
CCl ₄ recovery	154.1 \pm 5.0*	156.5 \pm 4.5**	158.5 \pm 4.8***	158.9 \pm 5.0*	159.2 \pm 5.2**	160.1 \pm 3.2**	160.2 \pm 4.3**
Silymarin control	158.1 \pm 3.2***	160.2 \pm 9.0***	161.1 \pm 6.2**	161.5 \pm 7.5***	162.0 \pm 8.0***	162.5 \pm 3.2***	163.0 \pm 3.6***
Plant extract control	161.1 \pm 2.5**	159.22 \pm 2.2**	160.0 \pm 3.0**	160.8 \pm 5.2**	161.1 \pm 3.5**	161.0 \pm 2.6**	162.2 \pm 4.2***

N=6 [#]Values are expressed as mean of \pm S.D.***P <0.001, **P <0.01, *P <0.05 in comparison to Normal control group..

Serum marker enzymes

All the marker enzymes, viz., AST, ALT, ALP and GGT registered enhanced activity in CCl₄-treated rats as compared to control group (Table 2). In MEC co-administered group, the levels of these enzymes were found retrieving towards normalcy.

Table 2 Effect of *Enicostema Axillare* ethanolic extract on CCl₄-induced liver damage in rats

Groups	BILI	GOT	GPT	Alk- PO ₄	Serum Glucose (mg/dl)	Serum cholesterol (mg/dl)	Total protein
Normal control	0.525 ± 0.03	157.89± 19.31	92.24± 4.46	147.75± 9.07	146.44± 11.55	72.14± 5.16	5.65± 0.6
CCl ₄ control	0.49± 0.03	247.06± 14.26**	184.4± 12.11***	171.33± 11.39	180.47± 6.92	73.61± 2.59	3.29± 1.38**
CCl ₄ Recovery	0.53± 0.02	217.98± 27.35*	155.83± 12.61***	171.33± 20.69	222.73± 14.8*	71.79± 6.2	2.26± 0.20***
Silymarin control	0.53± 0.03	248.75± 20.18**	179.83± 15.91***	141.33± 7.37***	190.9± 18.15	61.11± 7.30	6.43± 0.70
Plant extract control	0.55± 0.05	148.84± 6.20*	135.34± 6.20*	128.58± 5.50***	195.11± 8.6**	76.52± 3.16	5.00± 0.40*

N=6, Values are expressed as mean of ± S.D. ***P < 0.001, **P < 0.01, *P < 0.05 in comparison to Normal control group.

Other biochemical parameters:

The total protein concentration of the serum and liver was lesser in Group II animals, when compared with normal control. (Tables 3) and it attained an almost normal value in group III rats. The level of total lipids, triglycerides and cholesterol in serum as well as liver recorded significant increment in CCl₄- administered rats as compared to those of group I. All these biochemical changes showed signs of returning towards the normalcy in-group III animals. There was a significant decline in the concentration of phospholipids in liver tissues of CCl₄-treated rats as compared to normal control. In group III animals phospholipid concentration attained normalcy.

Table 3 Effect of *Enicostema Axillare* ethanolic extract on liver weight and volume

Groups	Liver Weight [#] (gm)	Liver Volume [#] (ml)
Normal control	6.32± 0.05	7.95± 0.05
CCl ₄ control	7.752± 0.04 **	11.01± 0.07***
CCl ₄ Recovery	7.269± 0.06**	9.05± 0.07***
Silymarin control	6.63± 0.09***	8.45± 0.05**
Plant extract control	6.07± 0.10**	7.75± 0.12**

N=6 [#]Values are expressed as mean of ± S.D.***P <0.001, **P <0.01, *P <0.05 in comparison to Normal control group.

Discussion

Carbon tetrachloride is one of the most commonly used hepatotoxin. It is well documented that carbontetrachloride is biotransformed under the action of cytochrome P-450 in the microsomal compartment of liver to trichlomethyl radical which readily reacts with molecular oxygen to form trichloromethyloeroxy radical [12].

This free radical in the presence of oxygen may cause peroxidation of lipid on target cell resulting in extensive damage [13]. Administration of CCl₄ (1.0 ml s.c.) to rats produced hepatotoxicity showed by significant increase in the serum levels of GOT, GPT and alkaline phosphate in comparison to control group. Also total protein levels were significantly decreased to 3.29g/dl in CCl₄ control groups from the level of 5.65g/dl in normal control group as shown in the Table 2. Ethanolic extract of *Enicostema Axillare* given at dose 1000mg/kg not only prevented the rise in serum level of GOT, GPT, alkaline phosphates but also improved serum lipid profile. The results are well comparable with silymarin (standard drug) treated group [14].

Conclusion

Based on the present findings, it can be concluded that the probable mechanism by which the *Enicostema Axillare* leaves exerts its protective action against CCl₄-induced hepatocellular metabolic alterations could be by the stimulation of hepatic regeneration through an improved synthesis of proteins, or due to its ability to block the bioactivation of CCl₄ by inhibiting the P 450 2E1 activity and/or its accelerated detoxification and the potential to minimise the deleterious effects of free radicals including the peroxy radicals and its antioxidant activity in combination with the inhibition of lipid peroxidation, thereby the *Enicostema Axillare* leaves can be ranked as hepatoprotective agent by the combined synergistic effect of its constituents and micronutrients rather than to any single factor through free radicals scavenging activity. Further work is going on to isolate the active components, which are responsible for hepatoprotection.

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