



Hepato-protective activity of *Enicostemma axillare* in paracetamol induced hepato-toxicity in albino rats

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Abstract

The present study was conducted to evaluate the hepato-protective activity of water extract of aerial parts of *Enicostemma axillare* in paracetamol-induced hepatotoxicity in albino rats. Silymarin (200mg/kg) was given as reference standard. The water extract of arial parts of *Enicostemma axillare* have shown very significant hepatoprotection against paracetamol -induced hepatotoxicity in albino rats in reducing serum total bilirubin, SALP, SGPT, SGOT levels and liver homogenates LPO, SOD, CAT, GPX, GST and GSH levels.

Key words: *Enicostemma axillare*, hepatotoxicity, paracetamol and Silymarin

Introduction

Enicostemma axillare belongs to family Gentianaceae. It is a perennial herb found through out India and is common in coastal 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), anti-inflammatory (Sadique et al., 1987), hypoglycemic (Jyoti, et al., 2000, Murili, et al., 2002, Jyoti, et al., 2003) and anticancer (Murili, et al., 2002) activities have been reported. These reported activities and many of the ethnobotanical used of the plant related to its hepatoprotective activity. Swertimarin, alkaloids, steroids, triterpenoids, saponins, flavonoids, xanthones, and phenolic acid were isolated from the plant (Kavimani, S. and Manisenthilkumar, 2000). Many such compounds have protective effects due to their 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). The present study was designed with an aim to assess the hepatoprotective activity of the water extract of arial parts of *Enicostemma axillare*, against paracetamol induced liver damage.

Material and methods

Plant material

The plant material used in this study was collected during the month of October in Rajur, Dist-Ahmednagar (MH), India and authenticated from Department of Botanical Survey of India, Pune (India).

Preparation of the Extract

The shade dried arial part of *Enicostemma axillare* was extracted with water by maceration at room temperature. The yield of extract was calculated.

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Animals

Albino rats (either sex) of Sprague dawley strain, weighing 150-200g were used. Diagnostic reagent kits (Enzopak) were used for the estimation of serum SALP, SGPT and SGOT levels and assay procedure was used for the estimation of liver homogenates LPO, SOD, CAT, GPX, GST and GSH. (Handa, 1986, *et al.*)

Toxicity studies

Acute toxicity study was performed for water extract according to the acute toxic classic method as per OECD guidelines, (Ashok, 2001, *et al.*). Albino rats were used for acute toxicity study.

Hepatoprotective Activity

The animals were divided into four groups comprising of six albino rats in each group using randomization technique and treated with the extract for seven days to assess the hepato-protective 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 paracetamol treatment. The third group received water extract in the dose of 200mg/kg p.o. and the fourth group received the Silymarin in the dose of 200mg/kg p.o. as a reference material for the study. All the animals except the vehicle control received paracetamol on 7th day of the treatment. 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. The animals were sacrificed by cervical dislocation after 48 hours of paracetamol administration. The livers were dissected out immediately, washed with ice cold saline and 10% homogenates in 1.15%(w/v) KCl were prepared. The homogenates were centrifuged at 7000xg for 10 min at 4°C and the supernatants were used for the assays of LPO, SOD, CAT, GPX, GST and GSH.

Results and discussion

The present studies were performed to assess the hepatoprotective activity in rats against paracetamol as hepatotoxin to prove its claims in folklore practice against liver disorders. Paracetamol-induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plant 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 paracetamol. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals. From the Table I it was evident that the extract was 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 paracetamol induced hepatotoxicity. The reduction is attributed to the initial damage produced and localised in the endoplasmic reticulum which results in the loss of P450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver. Reduction in the levels of SALP, 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 paracetamol; rats treated with water extract and intoxicated with paracetamol 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 water extract of aerial parts of *Enicostemma axillare* has shown very significant hepatoprotection against paracetamol -induced hepatotoxicity in albino rats in reducing serum total bilirubin, SALP, SGPT and SGOT levels. It is also found that treatment with water extract of plant has brought down the elevated level of LPO and also significantly enhanced the reduced levels of SOD, CAT, GPX, GST and GSH .

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Table: 1 Effect of water extract of *Enicostemma axillare* aerial parts on paracetamol-induced hepatotoxicity (Serum parameters)

Sr. No.	Groups	Total Bilirubin (mg/dl)	SALP (Units/ml)	SGPT (Units/ml)	SGOT (Units/ml)
1.	Control	0.75± 0.04	231.1±1.11	81.50 ± 1.32	196.2.53± 1.62
2.	Paracetamol	1.40 ± 0.07	435.28±24.50	375.20 ± 34.64	355.88 ± 25.49
3.	Water Extract (200mg/kg)	0.79 ± 0.14	232.09±24.42	82.77 ± 62.67	199.06 ± 30.31
4.	Silymarin (200mg/kg)	0.80 ± 0.04	233.22±23.09	83.43± 06.23	199.33±12.65

Values of mean ± S.E.M. (n=6)

Table: 2 Effect of water extract of *Enicostemma axillare* aerial parts on paracetamol-induced hepatotoxicity (Liver homogenates)

Sr. No.	Groups	LPO nmoles/mg of protein	SOD Units/mg protein	CAT Units/mg protein	GPX ($\mu\text{g}/\text{mg}$)	GST $\mu\text{g}/\text{mg}$ of protein	GSH $\mu\text{g}/\text{mg}$ of protein
1.	Control	0.44 \pm 0.03	109.1 \pm 9.5	24.7 \pm 1.2	3.30 \pm 0.01	1.55 \pm 0.14	0.33 \pm 0.01
2.	Paracetamol	0.38 \pm 0.03	25.20 \pm 3.70	9.0 \pm 0.66	1.45 \pm 0.03	0.51 \pm 0.09	0.01 \pm 0.03
3.	Water Extract (200mg/kg)	0.42 \pm 1.22	105.11 \pm 1.55	22.13 \pm 0.11	3.01 \pm 0.06	1.30 \pm 0.08	0.29 \pm 0.01
4.	Silymarin (200mg/kg)	0.43 \pm 0.01	109.33 \pm 5.33	23.11 \pm 1.1	3.15 \pm 0.03	1.48 \pm 0.11	0.32 \pm 0.01

Values of mean \pm S.E.M. (n=6)