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Antihepatotoxic Activity of *Hygrophila Spinosa* Roots on CCl_4 induced Hepatic Damage in Rats

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ABSTRACT

In the present study the Antihepatotoxic activity of the Ethanolic extract of *Hygrophila spinosa* roots was carried out using CCl_4 induced Antihepatotoxic in albino rats. The magnitude of protection against liver toxicity was estimated by measuring the serum biochemical parameters viz. SGPT, SGOT, SALP, ACP, ALP and Bilirubin (Direct and Total). In addition to this, morphological changes of liver like wet liver volume and wet liver weight were recorded. Further, histopathological examination of the liver was also studied. Silymarin at the dose of 25 mg/kg, p.o. was used as reference standard drug and it exhibited significant protection.

Keywords: *Hygrophila spinosa*, Antihepatotoxic, CCl_4.

INTRODUCTION

The liver is a key organ in the human body, regulating homeostasis and is a frequent target for a number of toxicants[1]. In spite of tremendous scientific advancement in the field of hepatology during recent years liver problems are on the rise. Regrettably there are only a few drugs with serious side effects available for the treatment of liver ailments[2]. In view of the undesirable side effects of synthetic agents, there is growing focus towards the therapeutic evaluation of medicinal plants using systemic research methodology.

In spite of the tremendous advances made in allopathic medicine, no effective antihepatotoxic medicine is available till date. Plant drugs are known to play a vital role in the management of liver diseases. In India, more than 87 medicinal plants are used in different combinations in the preparation of 33 patented herbal formulations[3-8].

In the traditional system of medicine there are numerous plants and polyherbal formulations have been used in liver diseases. But only a small portion of them have been pharmacologically evaluated for their efficacy. Still more number of medicinal plants is needed to be investigated for their antihepatotoxic effect.

Hygrophila spinosa is commonly found in water-logged areas throughout India[9]. The plant is used as a diuretic and for the treatment of rheumatism, jaundice, inflammation, pain, hepatic obstruction, gout, bacterial infection etc[10-14]. The aerial parts of the plant are reported to contain lupeol, stigmasterol and butelin while the seeds mainly contain fatty acids[15]. Its root contains an alkaloid named hygrosterol[16] while its flower contains apigenin 7-o-glucuronide[17]. However, no data were found regarding the pharmacological and phytochemical evaluation of the roots of the plant. The aim of the present study is to investigate the Antihepatotoxic properties of the Ethanol extract of the roots of *H. spinosa*

MATERIALS AND METHODS

The plant material and preparation of extracts

The roots of *Hygrophila spinosa* were collected from Bhimavaram, West Godavari District, Andhra Pradesh, India, and was authenticated by Professor K. Venkiah, Department of Botany , Andhra university, Visakhapatnam. A voucher specimen has been deposited at the museum of our college. After collection, the roots were washed thoroughly under running tap water, cut into pieces , shade dried at room temperature (24-26^{0}C) and ground into a coarse powder. The powdered roots was extracted by using Ethanol in soxhlet apparatus (Yield 12.68%). The preliminary phytochemical screening was carried out and revealed the presence of mainly flavanoids , tannins and triterpenoids in EEHSR**.**

The Experimental animals and acute toxicity studies

Wistar rats of both sexes, weighing 150 – 200g were used for the study. The animals were kept in polypropylene cages (38x28x10cm) with not more than four animals per cage in a room maintained under controlled laboratory conditions(room temperature 27 ± 3 ^{0}c, relative humidity 65 ± 10%, with dark and light

cycle12/12 hrs). The animals were fed with standard diet (Hindustan liver, Mumbai, India) and had free access to clean drinking water under strict hygienic condition. The animals were fasted over night prior to the dosing. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of University college of Pharmaceutical Sciences, Visakhapatnam, India.

Table-1: Effect of EEHSR on biochemical parameters in CCl_4 induced hepatoxicity

Group	SGPT IU/L	SGOT IU/	ALP IU/L	ACP IU/L	TB mg/dl	DB mg/dl
I	111.70 ± 3.151	107.39 ± 6.136	306.37 ± 1.823	39.46 ± 1.221	0.170± 0.022	0.271 ± 0.002
II	225.77 ± 9.063	376.57 ± 9.102	540.56 ±7.245	44.11 ± 0.739	0.631 ± 0.012	0.475 ± 0.023
III	129.60 ± 3.110***	169.91 ± 3.573***	332.42 ± 3.632***	34.29 ± .230***	0.307 ± 0.034***	0.268 ± 0.026***
IV	200.13±3.436 *	300.29±1.221*	459.56±6.620*	40.78±0.259 *	0.456±0.002*	0.432±0.002*
V	229.21 ± 2.150**	322.30 ± 7.181**	428.87 ± 3.161***	42.13 ±0.334**	0.576 ± 0.002**	0.543 ± 0.003**
VI	168.39 ± 0.813***	210.31 ± 4.237***	351.12 ± 4.423***	41.96 ± .125***	0.419 ± 0.008***	0.445 ± 0.005***

Values are mean ± SEM (n = 6);*P* < 0.05*, 0.01** and 0.001*** as compared to +ve control

Table-2: Effect of EEHSR on morphological parameters in CCl_4 induced hepatoxicity

Group	Liver wt. in g /100 g b.w.	Liver volume in ml / 100 g b.w.
I	3.008 ±0.042	3.076 ±0.023
II	4.023 ±0.021	4.079 ±0.037
III	3.123 ±0.313***	3.025 ± 0.026***
IV	4.221 ±0.036*	4.000 ± 0.037*
V	4.911 ±0.048**	4.975 ± 0.040**
VI	3.415 ±0.084***	3.877± 0.066***

Values are mean ± SEM (n = 6) ; < 0.05*, 0.01** and 0.001*** as compared to +ve control

CCl_4 induced hepatotoxicity

Albino rats weighing 170 – 200 g were divided into six groups of each containing eight(n=8) animals.

Group I – Negative control (received vehicle, distilled water 1 ml/kg, p.o.),
Group II – Positive control (CCl_4 1ml/kg, i.p.),
Group III – Standard (Silymarin 25 mg/kg, p.o.),
Group IV – EEHSR (100 mg/kg, p.o.),
Group V – EEHSR (250 mg/kg, p.o.) and
Group VI – EEHSR (500 mg/kg, p.o.)

Animals were treated as shown above for a period of 10 days. At the end of every 72 hrs. i.e. 4 th day, 7 th day and 10th day CCl_4 (30% in liquid paraffin 1 ml/kg, i.p.) was administered to all groups other than group I. Group III received standard drug silymarin25 mg/kg p.o. once a day and CCl_4 as mentioned above. Whereas group IV, V and VI were treated with test extract dose of (100, 250 and 500 mg/kg, p.o.) respectively. During this period of treatment, the rats were maintained under normal diet and water. The biochemical parameters were determined after 24 hrs. After the last dose of CCl_4 i.e. on 11thday. All the animals were sacrificed by cervical dislocation for the study of liver biochemical parameters. Blood was collected by carotid bleeding under mild ether anesthesia using disposable syringe and needle. Blood was allowed to clot at room temperature for 30 min. then subjected to

centrifugation (3000 rpm for 15 min.) and estimation of biochemical parameters namely SGPT, SGOT, ALP, ACP, Bilirubin (Total and Direct). The liver was dissected out and subjected for morphological study such as wet liver weight and wet liver volume. The volume of wet liver was measured by displacement method and further the livers were placed in 10% formalin solution for histopathological study[18].

Statistical analysis

The results obtained were subjected to statistical analysis using ANOVA followed by Turkey-Kramer Multiple Comparison Test.

RESULTS AND DISCUSSION

Administration of CCl_4 resulted in a significant rise in the levels of SGPT, SGOT, ALP, ACP and Bilirubin (Total and Direct) when compared to the vehicle treated group (Group-I). Pre-treatment with test extract significantly reduced the elevated levels of biochemical parameters in dose dependent manner. The results indicated that the effect of test extract on biochemical markers was found to be less potent than the reference standard, Silymarin. The results are shown in (Table-1)

Intoxication of rats with CCl_4 resulted in enlargement of liver which was pale reddish brown. Rats subjected to the CCl_4 challenge developed significant increase in the morphological parameters like wet liver weight and wet liver volume when compared to negative control group (Table-2). Oral administration of the test extract exhibited dose dependent significant reduction in the morphological parameters. Treatment with reference standard, silymarin (25 mg/kg, p.o.) also reversed increased morphological parameters significantly. Organ protective potency of the test extract at the dose of 500 mg/kg was found closer to that of standard. Histopathological profile of liver in CCl_4 (Group-II) intoxicated rats shown the fatty degeneration of hepatocytes, hepatic cell necrosis, portal tract fibrosis and presence of fatty cyst. The sinusoids of liver were congested and the central vein of globule was constricted. Administration of test extract at the dose of 500 mg/kg shown a significant recovery in the hepatic architecture. The sinusoids are recovered, the globule was normal and hepatocytes are improved. However, there was an improvement in the hepatic architecture observed in rats treated with 100 mg/kg and 250 mg/kg of test extract.

Liver injury induced by CCl_4 is a commonly used model for the evaluation of Antihepatotoxic Agents[19,20]. Administration of CCl_4 elevated the serum levels of SGOT, SGPT, ALP, ACP and bilirubin (total and direct) significantly due to its enzymatic activation of $\dot{C}Cl_3$ free radical, which in turn alters the structure and function of liver cells[21]. The results of the present study reveal that Ethanolic extract of ***Hygrophila spinosa*** roots (100,250 and 500 mg / kg, p.o.) exhibited protective action against CCl_4 induced liver damage in a dose related fashion. The amelioration of liver toxicity by the test extract was evident from its significant effect on serum enzyme levels and morphological parameters. These findings were further supported by histopathological observations.

Further, preliminary photochemical investigation revealed that the extract showed presence of flavonoids, tannins, alkaloids, saponins and glycosides. The literature has already documented the Antihepatotoxic value of flavonoids[21]. Thus, it appears that the hepatoprotection offered by ***Hygrophila spinosa*** extract may be due to its flavonoid content.

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