Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 1, 2012

Research Article

ANTIHEPATOTOXIC ACTIVITY OF *SMILAX CHINA* ROOTS ON CCL_4 INDUCED HEPATIC DAMAGE IN RATS

B.G.SOLOMON RAJU*, GANGA RAO. BATTU, MANJU LATHA.Y.B* K.SRINIVAS***

***Sri Vasavi Institute of Pharmaceutical Sciences, Tadepalligudem, Andhra Pradesh, India, **University college of Pharmaceutical Sciences, Andhra University, Visakhapatnam. Email: gabrielsolomonraju@yahoo.co.in**

Received: 23 Sep 2011, Revised and Accepted: 19 Nov 2011

ABSTRACT

In the present study the Antihepatotoxic activity of the Ethanolic extract of *Smilax China* roots was evaluated using CCl_4 induced hepatotoxicity in albino rats. The degree of protection against liver toxicity was determined by measuring the serum biochemical parameters viz. SGPT (serum alkaline phosphatase), SGOT (serum glutamine oxaloacetate transaminase), SALP (serum alkaline phosphatase**),** ACP, ALP and Bilirubin (Direct and Total). In addition 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: *Smilax China*; Antihepatotoxic; CCl4

INTRODUCTION

Liver has a prominent role in the regulation of physiological processes. It is involved in varieties of vital functions such as metabolism, secretion and storage. Furthermore, detoxification of a variety of drugs and xenobiotics occurs in liver. Hence liver diseases are among the most serious heath ailments. They may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatosis (non inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver). Liver diseases are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride, paracetamol, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune disorder. So it has become very much necessary to protect the liver from all these agents.

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[1-4]

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.

*Smilax China (*Liliaceae) is distributed throughout the tropic and sub tropic parts of the world. Some pharmacological activities of *Smilax* spp. rhizome have been studied. Oral administration of the extract from *S. sarsaparilla* at the dose of 500 mg/kg reduced the paw edema induced by carrageen an in rats[5] . The methanol extract of rhizomes of *S. glabra* (100mg/kg, i.p.) reduced the blood glucose of normal mice and KK-Ay mice [6] .The aqueous extract (400, 800 mg/kg, p.o.) from rhizome of *S. glabra* inhibited the swelling of the adjuvant arthritis in rats[7].The ethyl acetate, butanol and aqueous extracted fractions from *S. china* root showed high levels of DPPH free radical scavenging activity[8]. The decoction of *S. china* (90 and 180 mg/kg, p.o.) could significantly inhibit inflammatory swelling on adjunctive arthritis mouse[9].

MATERIALS AND METHODS

The plant material and preparation of extracts:

The roots of *Smilax China* for the proposed study was purchased from a commercial source, at Visakhapatnam, 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 were extracted by using ethanol in soxhlet apparatus (Yield 14.52%). The preliminary phytochemical screening was carried out and revealed the presence of mainly glycosides, flavanoids, tannins and triterpenoids in EESCR.

The Experimental animals and acute toxicity studies:

The male albino rats weighing 170 – 200 g were used for the experimentation. They were housed in polyacrylic cages (38x28x10cm) with not more than four animals per cage. After randomization into various groups, animals were acclimatized for the period of 7 days under standard laboratory conditions (room temperature 27 ± 3^{0}c, relative humidity 65 ± 10%, with dark and light cycle12/12 hrs). All the animals were allowed free access to standard pellet diet (Hindustan liver, Kolkotta, India) and water was allowed *ad-labium* under strict hygienic condition. Ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) before initiating the experiments. The acute oral toxicity in male albino rats was determined. The animals were fasted over night prior to the dosing. Fixed dose (OECD Guideline No. 420) method of CPCSEA was adapted for toxicity studies.

CCl_4 induced hepatotoxicity [10, 11]

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 – EESCR (100 mg/kg, p.o.),

Group V – EESCR (250 mg/kg, p.o.) and

Group VI – EESCR (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[12].

Statistical analysis

The results were expressed as the mean ± standard error of mean (SEM).The results were analyzed for statistical significance by one way ANOVA followed by Dunnett's *post hoc* test of significance.

RESULTS

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.

Table 1: Effect of EESCR on biochemical parameters in CCl_4induced hepatoxicity

Group	SGPT IU/L	SGOT IU/	ALP IU/L	ACP IU/L	TB mg/dl	DB mg/dl
I	115.70 ± 3.251	117.39 ± 6.636	301.37 ± 1.823	29.46 ± 1.821	0.270± 0.002	0.281 ± 0.003
II	235.77 ± 9.423	356.57 ± 9.112	500.56 ±7.145	54.11 ± 0.749	0.631 ± 0.011	0.575 ± 0.013
III	139.60 ± 3.530***	162.91 ± 3.513***	339.42 ± 3.632***	37.69 ± .210***	0.407 ± 0.004***	0.368 ± 0.016***
IV	219.13±3.536 *	331.29±1.220*	489.56±6.640*	50.78±0.256*	0.498±0.002*	0.532±0.003*
V	219.21 ± 2.950**	327.30 ± 7.281**	468.87 ± 3.141***	49.13 ±0.234**	0.576 ± 0.005**	0.523 ± 0.003**
VI	158.39 ± 0.713***	200.31 ± 4.437***	357.92 ± 4.419***	41.96 ± .445***	0.459 ± 0.009***	0.345 ± 0.004***

Values are mean ± SEM (n = 8); $P < 0.05$*, 0.01** and 0.001*** as compared to +ve control

Table 2: Effect of EESCR on morphological parameters in CCl_4induced hepatoxicity

Group	Liver wt. in g /100 g b.w.	Liver volume in ml / 100 g b.w.
I	3.118 ±0.042	3.173 ±0.023
II	4.135 ±0.021	4.585 ±0.037
III	3.082 ±0.313***	3.135 ± 0.026***
IV	4.041 ±0.036*	4.200 ± 0.037*
V	4.005 ±0.048**	4.245 ± 0.040**
VI	3.235 ±0.084***	3.367±0.066***

Values are mean ± SEM (n = 8); $p < 0.05$*, 0.01** and 0.001*** as compared to +ve control

DISCUSSION

Liver injury induced by CCl_4 is a commonly used model for the evaluation of Antihepatotoxic agents[13, 14]. 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 $CCl^{\bullet}_3$ free radical, which in turn alters the structure and function of liver cells [15-16]. The results of the present study reveal that Methanolic extract of *Smilax China* 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[17-20]. Thus, it appears that the hepatoprotection offered by *Smilax China roots* extract may be due to its flavonoid content.

REFERANCES

1. Handa SS, Sharma A, Chakraborty KK: Natural products and plants as liver protecting drugs. Fitoterapia 1989; 57: 307-51.
2. Hikino H, Kiso Y: Natural products for liver diseases. Economic and Medicinal Plant Research. Vol.II, London: Academic Press; 1988, 39-72p.
3. Evans WC: An overview of drugs having antihepatotoxic and oral hypoglycaemic activities. Trease and Evans Pharmacognosy. 14thed. UK: W. D. Saunders company
4. Sharma A, Shing RT, Sehgal V, Handa SS: Antihepatotoxic activity of some Fitoterapia 1991; 62: 131-8.
5. Ageel, A.M., Mossa, J.S., al-Yahya, M.A., al-Said, M.S.and Tariq, M. 1989. Experimental studies onFukunaga, T., Miura, T., Furuta, K. and Kato, A. 1997.
6. Fukunaga, T., Miura, T., Furuta, K. and Kato, A. 1997.Hypoglycemic effect of the rhizomes of *Smilax glabra* in normal and diabetic mice. Biol. Pharm.Bull., 20: 44-46.
7. Jiang, J. and Xu, Q. 2003. Immunomodulatory activityof the aqueous extract from rhizome of *Smilaxglabra* in the later phase of adjuvant-inducedarthritis in rats. J. Ethnopharmacol., 85: 53-59.
8. Lee, S.E., Ju, E.M. and Kim, J.H. 2001. Free radicalscavenging and antioxidant enzyme fortifyingactivities of extracts from *Smilax china* root.Exp. Mol. Med., 33: 263-268.
9. Lu, Y., Chen, D., Deng, J. and Tian, L. 2003. Effect of*Smilax china* on adjunctive arthritis mouse.Zhong Yao Cai., 26: 344-346. [Chinese, Abstract]

10. Manoj B, Aqueel K: Protective effect of *Lawsonia Alba* Lam., against CCl4 induced hepatic damage in albino rats. Indian J Expt Biol 2003; 41: 85-7.
11. Gupta M, Mazumder UK, Kumar TS, Periyasamy, Gomathi, Kumar RS. Antioxidant and hepatoprotective effects of *Bauhinia racemosa* against paracetamol and carbon tetrachloride induced liver damage in rats. Iranian J Pharmacol Therapeutics 2004; 3: 12-20.
12. Luna LG: Manual of histology and staining methods of Armed Forces institute of Pathology, 3rd ed. New York, McGraw Hill Book Co., 1986; 1p.
13. Oyaizu M: Studies on product of browing reaction preparation from glucose amine. Jap J Nutrition 1986; 44: 307-9.
14. Halliwell B, Gutteridge MCJ: Formation of a thiobarbituric acid reactive substance from deoxy ribose in the presence of Iron salts. FEBS Letters 1981; 128(2): 347-52.
15. Slater TF: Biochemical mechanism of liver injury. London; Academic press 1965: 1p.
16. Plea GI, Hewitt WR. Toxicology of the liver. Boyer TD; Raven Press Zakim D 1982: 103p.
17. Singh B, Saxena AK, Chandan BK, Suri OP, Suri KA, Sathi NK: Hepatoprotective activity of verbenalin on experimental liver damage in rodents. Fitoterapia 1998; 60: 135.
18. Bhat AD, Bhat S: Indigenous drugs for liver diseases. Indian J Gastroenterol 1996; 15: 63-7.
19. Rajesh Kumar, Sushil Kumar, Arjun Patra, Jayalakshi.S, Hepato protective activity of arial parts of *Plumbago zeylanica* against carbon tetrachloride- induced hepatotoxicity in Rats, IJPPS,Vol.1,Supp l,Nov-Dec.2009
20. Brijesh K.Tiwari, R.L Khosa Evaluation of hepatoprotective and antioxidant activity of *Berberis asiatica* against experimentally induced Liver injury in Rats, IJPPS. Vol.2 Supp.1. 2010