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HEPATOPROTECTION BY *ELEPHANTOPUS SCABER* LINN. IN CCl_4-INDUCED LIVER INJURY

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Abstract : The efficacy of the medicinal plant Elephantopus scaber Linn. (Asteraceae), to prevent carbon teterachloride (CCI_4)-induced chronic liver dysfunction in the rats was examined by determining different biochemical markers in serum and tissues. In serum, liver function marker enzymes like aspartate aminotrasferase (AST), alanine aminotrasferase (ALT), alkaline phosphatase (ALP) and also protein were evaluated. The concentrations of total lipid, cholesterol and phospholipids were studied in serum and the different tissues. The concentration of serum triglycerides was also studied. The biochemical changes induced by CCI_4 in different tissues particularly in the liver tissue improved following treatment with *E. scaber* Linn. The results suggest the hepatoprotective effect of this medicinal plant.

Key words: *Elephantopus scaber* carbon tetrachloride hepatoprotective effect medicinal plant

INTRODUCTION

The liver, which occupies the pivotal position in body, plays an essential role in drug and xenobiotic metabolism and in maintaining the biological equilibrium of the organism. The role played by this organ in the removal of substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign (xenobiotic) compounds culminating in liver dysfunction. Despite the tremendous strides in modern medicine, there is hardly any drug that stimulates liver function, offers protection to the liver from damage or helps regeneration of hepatic cells. However, many herbal formulations are available for liver disorders on the Indian market based on Ayurvedic principles (1, 2). Polyherbal drugs are frequently considered to be less toxic and free from side effects than synthetic drugs. Medicinal plants like *Andrographis paniculata, Boerhaavia diffusa, Eclipta alba, Hibiscus rosasinensis, Phyllanthus amarus, Phyllanthus debilis, Vitex negundo* etc. are well-known for their hepatoprotective effects (3-8).

Elephantopus scaber Linn. of the family Asteraceae, is a perennial herb. It is light,

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cooling, astringent, diuretic and good for the heart (9). It is used in the treatment of wound, chapped lips, gonorrhea, rheumatism, tetanus, arthritis, dysentery, filariasis, heart trouble, liver ailments, colic pain and diarrhoea (9, 10). Recently it has also been used to cure hepatitis (11). *E. scaber* is reported to possess anti-inflammatory and anti-bacterial properties (12-13). The plant part used varies depending upon the disease to be treated (i. e. entire plant, bark, leaf and root) (10). The root of this plant is used in the treatment of liver disorders (14). In the present study, we have attempted to investigate the antihepatotoxic potential of the roots of *E. scaber* Linn. on liver damage induced by CCl_4 in albino rats.

METHODS

E. scaber Linn. was collected from Kottayam district, Kerala. The roots of the plants were cut, washed and dried at 45°C for two days and powdered. This powdered plant part was used for the experiment.

Male albino rats of Sprague Dawley strain weighing between 120 g to 150 g were used for the experimental purpose. They were housed in polypropylene cages and given standard pellet diet (M/s Hindustan Lever Ltd., Bombay, India). Water was given *ad libitum*. The animals were divided into three groups of six rats each. Group I rats served as normal control. Group II rats received a dose of 0. 1 ml of CCl_4 in groundnut oil (1:1v/v) per 100 g body weight through an intragastric tube twice a week (i. e. every first day and fourth day) for a period of two months. Group III consisted of rats, which in addition to CCl_4, received a dose of 1000 mg/kg body weight of *E. scaber* root powder suspended in water daily in the morning for 60 days. The dose of the plant was ascertained by a pilot study over a range of doses varying from 250 mg/kg body weight to 1500 mg/kg body weight.

At the end of the experimental period, rats were deprived of food overnight and sacrificed by decapitation. Blood was collected by excising the jugular vein. It was allowed to clot and then centrifuged at 3000 rpm for 20 min. The serum samples were collected and left standing on ice until required. The tissues (liver, kidney, heart and lungs) were excised and transferred into ice-cold containers for biochemical estimations.

Activities of serum enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) (15) and alkaline phosphatase (ALP) (16) were assayed. The protein content of serum was also estimated (17). The concentrations of total lipid (18), phospholipids (19), cholesterol (20) and triglycerides (21) were estimated in serum and tissues.

The protective effect of *E. scaber* roots was evaluated by comparing the above-mentioned biochemical parameters of group II with group I and group III with group II. Results were expressed as the mean ± SEM. Student's 't'-test was used to assess statistical significance.

RESULTS

The activities of serum enzymes and the concentrations of total protein, total lipid, cholesterol, phospholipids and triglycerides are presented in Table I. A marked elevation in the activities if the enzymes AST, ALT, ALP and in the concentrations of total lipid, cholesterol, phospholipids, triglycerides and decrease in protein content was observed in the CCl_4 treated group II rats compared to normal control. In rats, which received both *E. scaber* and CCl_4 the activities of the liver function marker enzymes and the concentrations of total protein, total lipid, phospholipids, triglycerides and cholesterol were maintained at near normal levels.

Table II shows the concentration of total lipid, cholesterol and phospholipids in tissues such as liver, kidney, heart and lungs. Significant increase in the concentration of total lipid and cholesterol was observed in the tissues of group II rats. Remarkable increase in the concentration of phospholipids was noticed in all the tissues studied except liver and lungs of the group II rats. In liver and lungs, the level of phospholipids was found to decrease. Co-administration of *E. scaber* root powder to group III rats significantly ($P<0.01$) prevented the CCl_4 induced alterations in the lipid profile. However, no significant change in the concentration of phospholipids in the liver of the above group was observed.

TABLE I: Effect of *E. scaber* on the activities of enzymes and the concentration of total protein, total lipid, phospholipids, triglycerides and cholesterol in serum.

	Group		
Parameters	*I–Normal control*	*II–CCl_4 treated*	*III–CCl_4 + E. scaber*
AST	16.66±0.42	29.17±1.02*	16.66±0.86†
ALT	27.78±0.70	69.44±2.45*	43.33±2.21†
ALP	88.75±2.24	390.50±13.73*	159.75±8.00†
Total protein	5.16±0.13	4.02±0.14*	4.68±0.24†
Total lipid	256.76±6.45	342.95±12.00*	249.18±12.46†
Phospholipids	150.00±3.77	187.50±6.58*	150.00±7.67†
Triglycerides	5.70±0.14	8.70±0.31*	5.90±0.30†
Cholesterol	50.00±1.26	66.67±2.33*	53.33±2.72†

Group II has been compared with Group I
Group III has been compared with Group II
*$P<0.01$
†$P<0.01$
(Values expressed in IU/L serum for AST, ALT, ALP, g/100 ml serum for total protein and mg/100 ml serum for other parameters)
(Values are mean ± SEM of 6 animals in each group)

TABLE II : Effect of *E. scaber* on various biochemical parameters in different tissues.

Parameters	*Group*	*Liver*	*Kidney*	*Heart*	*Lungs*
Total lipid	I : Pairfed control	6012.90±149.84	2153.84±53.42	1846.15±46.15	1969.22±49.23
	II : CCl_4 treated	8017.20±280.60*	2769.23±97.20*	2153.84±75.39*	2297.43±80.18*
	III : CCl_4 + *E. scaber*	6347.14±222.15†	1846.15±92.31†	1350.77±67.54†	2461.53±123.08†
Phospholipids	I : Pairfed control	2230.50±55.76	2240.91±56.02	2018.80±50.29	1066.50±26.66
	II : CCl_4 treated	1584.82±55.47*	5890.26±206.16*	12108.0±419.91*	986.50±34.53*
	III : CCl_4 + *E. scaber*	1526.28±76.32NS	2667.79±133.39†	3027.00±30.27†	1279.75±63.99†
Cholesterol	I : Pairfed control	533.33±13.40	533.33±13.28	365.42±9.14	346.66±8.67
	II : CCl_4 treated	719.72±7.20*	666.66±23.33*	602.94±21.10*	266.66±9.33*
	III : CCl_4 + *E. scaber*	533.33±27.20†	466.66±23.33†	324.90±16.25†	359.99±18.00†

Group II has been compared with Group I
Group III has been compared with Group II
*P<0.01
†P<0.01
NS – Not significant
(Values expressed as mg/100 g tissue)
(Values are mean ± SEM of 6 animals in each group)

DISCUSSION

Carbon tetrachloride is a commonly used standard hepatotoxin (22). It is converted by the liver drug metabolizing enzyme system into CCl_3 radical which attacks unsaturated fatty acids of membranes in the presence of oxygen to give lipid peroxides. Consequently, the functional integrity of hepatic mitochondria is altered. All these events ultimately lead to liver damage (23). The enzymes AST, ALT and ALP are found in higher concentration in the cytoplasm (24). In hepatic dysfunction, these enzymes leak into the bloodstream. So the extent of liver injury can be assessed by estimating the level of these cytoplasmic enzymes released into the circulation (25). Hepatotoxins impair the capacity of the liver to synthesize albumin (26). So the protein content of serum decreases in such cases. In the medicinal herb treated group, the protein level of serum was almost normal. This is a clear indication of the improvement of the functional integrity of the liver cells. In the CCl_4-treated rats, there was a significant increase in the activities of AST, ALT and ALP. In our study, *E. Scaber* root powder administration to the rats with the hepatotoxin caused a decrease in the activities of these enzymes. This elucidates the protective efficacy of *E. scaber* on CCl_4-induced liver damage.

Treatment of rats with CCl_4 also causes

centrilobular necrosis which results in the accumulation of fat in liver and kidney. Fat from the peripheral adipose tissue is translocated to the liver and kidney leading to its accumulation during toxicity. Hepatotoxins like CCl_4 and ethyl alcohol interfere with hepatic phospholipid synthesis (27, 28). This is also evident from the decreased concentration of phospholipids in the liver of the CCl_4-treated group as seen in this study. The changes in the lipid profile caused by CCl_4 were almost completely restored with *E. scaber* treatment.

From the results, it can be concluded that the root powder of *E. scaber* prevents hepatic injury induced by CCl_4 in rats by neutralizing the oxidative stress. Further studies are required for its potential use as a hepatoprotective drug in clinical practice.

REFERENCES

1. Prathibha D, Aruna K, Ravindra K, Sadashiv M, Subhash P, Varuthe AT. Effect of mandur bhasma on lipolytic activities of liver, kidney and adipose tissue of albino rat during CCl_4-induced hepatic injury. *J Biosci* 1986; 10: 227–234.
2. Latha U, Rajesh MG, Latha MS. Hepatoprotective effect of an ayurvedic medicine. *Indian Drugs* 1999; 36: 470–473.
3. Rana AC, Avadhoot Y. Hepatoprotective effects of andrographis paniculata against carbon terachloride-induced liver damage. *Arch Pharm Res* 1991; 14: 93–95.
4. Rawat AK, Mehrotra S, Tripathi SC, Shome U. Hepatoprotective activity of Boerhaavia diffusa L. Roots - a popular Indian ethnomedicine *J Ethnopharmacol* 1997; 56: 61–66.
5. Saxena AK, singh B, Anand KK. Hepatoprotective effects of Eclipta alba on subcellular levels in rats. *J Ethnopharmacol* 1993; 40: 155-161.
6. Frederick OO, Ighofimoni AU, Julie OO. Prevention of carbon tetrachloride-induced hepatotoxicity in the rat by H. rosasinensis anthocyanin extract administered in ethanol. *Toxicol* 1998; 131: 93–98.
7. Sane RT, Kuber VV, Mary SC, Menon S. Hepatoprotection by Phyllanthus amarus and Phyllanthus debilis in CCl_4-induced liver dysfunction. *Curr Sci* 1995; 68: 1243–1246.
8. Avadhoot Y, Rana Ac. Heptoprotective effect of Vitex negundo against carbon tetrachloride-induced liver damage. *Arch Pharm Res* 1991; 14: 96-98.
9. Sivarajan VV, Indira B. In Ayurvedic Drugs and their plant Sources. Oxford and IBH Publishing Co., New Delhi 1994; 153–154.
10. Hammer MLA, Johns EA. Tapping an Amazonian plethora: four medicinal plants of Marajo Island, Para (Brazil). *J Ehtnopharmacol* 1993; 40: 53–75.
11. Lin C, Kan W. Medicinal plants used for the treatment of hepatitis in Taiwan. *Am J Clin Med* 1990; 18: 35–43.
12. Tsai CC, Lin CC. Anti-inflammatory effects of Taiwan folk medicine, Teng-Khia-U on carrageenan and adjuvant-induced paw edema in rats. *J Ethnopharmacol* 1999; 64:85–89.
13. Chen CP, Lin CC, Namba T. Screening of Taiwanese crude drugs for antibacterial activity against streptococcus mutans. *J Ethnopharmacol* 1989; 27: 285–295.
14. Rao R. Ethnobotany of Meghalaya: Medicinal plants used by Khasi and Garo tribes. *Eco Bot* 1981; 35: 4–9.
15. Mohun AF, Cook IJY. Simple methods for measuring serum level of the glutamic oxalacetic and glutamic pyruvic transaminases in routine laboratories. *J Clin Path* 1957; 10: 394–399.

16. Kind PRN, king EJ. Elimination of plasma phosphate by determination of hydrolysed phenol with aminoantipyrine. *J Clin Path* 1954; 7: 322–326.

17. Gornal AG, Bandawill CJ, David MM. Determination of proteins by means of the biuret reaction. *J Biol Chem* 1949; 177: 751–766.

18. Fring CS, Dunn R. A colorimetric method of determination of total lipids based on sulfophosphate-vanillin reaction. *Am J Clin Path* 1970; 4: 53–89.

19. Varley H. In: *Practical Clinical Biochemistry*. CBS Publishers and Distributors, New Delhi 1988; 319–320.

20. Abell LL, Levy BB, Kendall FE. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J Biol Chem* 1952; 195: 357–366.

21. Van Hendle E, Zilversmith DB. Determination of serum triglycerides. *J Lab Clin Med* 1957; 50: 152.

22. Janbaz KH, Gilani AH. Potentiation of paracetamol and carbon tetrachloride-induced hepatotoxicity in rodents by the food additive vanillin. *Food Chem Toxicol* 1999; 37: 603–607.

23. Brattin WJ, Glende EA, Recknagel RO. Pathological mechanisms in CCI_4 hepatotoxicity. *J Free Radic Biol Med* 1985; 1: 27–38.

24. Wells FE Tests in liver and biliary tract disease. In Gowenlock AH ed., varley's Practical Clinical Biochemistry. London Heinemann medical Books, 1988: 744–755.

25. Chenoweth, MB, Habe CL. The Smaller halogenated aliphatic hydrocarbon. *Ann Rev Pharmacol* 1962; 2: 363–398.

26. Dubey GP, Agrawal A, Dixit SP. Effect of Liv-52 on different biochemical parameters in alcoholic cirrhosis. *Antiseptic* 1994; 91: 205–208.

27. Recknagel RO. Carbon Tertrachloride hepatotoxicity. *Pharmacol Rev* 1967; 19: 145–208.

28. Kushnerova NF, Fomenko SE, Polozhentseva MI, Bulanov AE. Effect of natural complexes of biologically active substances on liver regeneration in alcohol poisoning. *Vopr Med Khim* 1995; 41: 20–23.