RESEARCH PAPER

Accepted 30 January 2005
Revised 30 August 2004
Received 4 May 2004
Indian J. Pharm. Sci., 2005, 67(1): 84-88

Hepatoprotective Activity of Rhizomes of *Cyperus rotundus* Linn against Carbon tetrachloride-induced Hepatotoxicity

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Ethyl acetate extract and two crude fractions, solvent ether and ethyl acetate, of the rhizomes of *Cyperus rotundus* (Cyperaceae) were evaluated for hepatoprotective activity in rats by inducing liver damage by carbon tetrachloride. The ethyl acetate extract at an oral dose of 100 mg/kg exhibited a significant protective effect by lowering serum levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase and total bilirubin. These biochemical observations were supplemented by histopathological examination of liver sections. Silymarin was used as positive control.

Cyperus rotundus (Family-Cyperaceae) commonly known as *mustaka* is a pestiferous perennial weed and has an elaborate underground system consisting of tubers, rhizomes and roots[1]. It is one of the plants mentioned in the literature having claims of activity against liver disorders[2]. It contains a wide variety of phytoconstituents, which are useful in treatment of different ailments and include alkaloids, glycosides, essential oils and flavonoids (www.tiririca.com). The present study has been taken up to evaluate hepatoprotective activity of the rhizome extract of this plant in experimental animals against carbon tetrachloride (CCl_4)-induced hepatotoxicity.

MATERIALS AND METHODS

Rhizomes of *Cyperus rotundus* were colleted from an Ayurvedic drug shop in Vadodara, and their identity was confirmed by specimen species preserved in Food and Drugs Laboratory (Botany section). The rhizomes were air-dried, powdered and used for further studies. All the chemicals used for serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALKP) and total bilirubin (TB) determination were analytical grade of E. Merck, Mumbai and Qualigens, Mumbai.

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Preparation of extract:

The powdered rhizomes were extracted with petroleum ether (60-80°) to remove lipids and then again extracted with chloroform, ethyl acetate and methanol in soxhlet extractor. The solvents are distilled to concentrate the extracts and are dried in vacuum desiccator. The extractive yield of chloroform and methanol are found to be less and ethyl acetate was found to contain flavonoids, when detected by thi.. layer chromatography[3]. Hence ethyl acetate extract was selected for hepatoprotective screening. All the test suspensions (100 mg/ml) were prepared in the vehicle i.e., 5% w/v acacia mucilage and were administered in the dose of 100 mg/kg[4] orally.

Toxicity studies:

Wistar rats weighing 200-250 g of either sex, procured from Deep Bio-med animal supply, Ahmedabad, maintained under standard husbandry conditions (Temp 23±2°, relative humidity 55±10% and 12 h light dark cycle) were used for all sets of experiments in groups of six animals. Animals were allowed to take standard laboratory feed and tap water.

The ethyl acetate extract was administered to different groups of rats in doses ranging from 100-1000 mg/kg. There is no lethality in any of the groups. Rats, which received

extract in doses above 900 mg/kg exhibited ptosis. One tenth of the maximum dose of the extract tested for acute toxicity was selected for evaluation of hepatoprotective activity. i.e., 100 mg/kg[4].The experiments were performed after the experimental protocols approved by the Institutional Animal Ethics Committee, M. S. University of Baroda, Vadodara.

Effect on normal liver function:

The ethyl acetate extract at a selected dose was evaluated for its effect on normal liver function by studying serum biochemical parameters. Rats were divided into control and test groups, each group comprising of six animals. The control group received vehicle (5% acacia mucilage 1 ml/kg p.o.) at 0, 24 and 48 h intervals and the test group received the test sample (100 mg/kg p.o.) at 0, 24 and 48 h intervals. After 72 h of first dose administration, blood was collected by puncturing the retro orbital plexus and was allowed to clot at room temperature for 30 min. Serum was separated by centrifuging at 2500 rpm and was used for the determination of SGOT[5], SGPT[5]. Serum alkaline phosphatase was assayed by the phenyl phosphate method[6] and total bilirubin assay was carried out according to the method of Jendrassik and Grof[7].

Carbon tetrachloride- induced hepatotoxicity in rats[8]:

Rats were divided into five groups of six each, control, hepatotoxin, test and positive control groups. The control group has received oral vehicle treatment at 0, 24 and 48 h. The animals in hepatotoxin-treated group received vehicle followed by carbon tetrachloride (in liquid paraffin 1:1 ratio, i. p.) at a dose of 1 ml/kg at 0 and 24 h, while at 48 h, these animals received only vehicle. The test groups have received the first dose of extracts at 0 h, second dose of extracts at 24 h, which was followed by a dose of carbon tetrachloride and at 48 h the third dose of extracts. The positive control group has received the first dose of silymarin (200 mg/kg)[9] at 0 h, at 24 h, second dose of silymarin followed by a dose of carbon tetrachloride and at 48 h the third dose of silymarin. After 72 h blood was collected from all the groups, allowed to clot for the separation of serum. The separated serum was utilized for estimation of SGOT, SGPT, ALKP and TB by reported methods as described in the previous section.

Histopathological studies:

One animal from the treated groups showing maximal activity as indicated by improved biochemical parameters from each test, positive control, hepatotoxin and control groups were utilized for this purpose. The animals were sacrificed and the abdomen was cut open to remove the liver. 5 mm thick pieces of the liver were fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12 h, then embedded in paraffin using conventional methods[10] and cut into 5 μm thick sections and stained using haematoxylin-eosin dye and finally mounted in di phenyl xylene. Then the sections were observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

Statistical analysis:

The mean values±SEM are calculated for each parameter. Percentage reduction against the hepatotoxin by test samples were calculated by considering enzyme level difference between the hepatotoxin treated and the control group as 100% levels of reduction. For determining the significant inter group difference each parameter was analyzed separately and one-way analysis of variance (ANOVA)[11] was carried out Then the individual comparisons of the group mean values were done using Dunnet's Procedure[11].

RESULTS

In the study of the effect of the ethyl acetate extract of *Cyperus rotundus* on normal liver function, it was found to be non-toxic at the selected dose (100 mg/kg p.o.) since the parameters SGOT, SGPT, ALKP and TB are with in the limits like that of control (Table 1). Carbon tetrachloride intoxication in normal rats elevated the levels of SGOT, SGPT, ALKP and TB significantly indicating acute hepato cellular damage and biliary obstruction. The rats treated with ethyl acetate extract of *Cyperus rotundus* showed a significant reduction in all the four-biochemical parameters elevated by carbon tetrachloride (Table 2) and the rats treated with ethyl acetate extract of *Cyperus rotundus* showed a significant reduction in SGOT, SGPT, ALKP and TB levels elevated by carbon tetrachloride (Table 3). This reduction in biochemical parameters exhibited by ethyl acetate extract is similar when compared with that of positive control.

The histological profile of the rat treated with ethyl acetate extract has showed no visible changes confirming the safety of the extract at selected dose regimen (fig.1). Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (fig. 2). Disarrangement of normal hepatic cells with intense centrilobular ne-

TABLE 1: EFFECT OF ETHYL ACETATE EXTRACT OF *CYPERUS ROTUNDUS* ON NORMAL LIVER FUNCTION

GROUP	Bio chemical parameters±SEM			
	SGOT (U/ml)	SGPT (U/ml)	ALKP (U/ml)	TB (mg/dl)
Control	128.6±7.18	100.0±7.16	85.28±2.19	0.36±0.02
Ethyl acetate extract	122.0±7.28	81.20±12.24	83.60±2.27	0.42±0.02
F calculated	0.35	0.35	0.26	2.23
5 % Allowance	28.26	81.48	8.08	0.09

F critical=4.96 ($p<0.05$)

TABLE 2: EFFECT OF ETHYL ACETATE EXTRACT OF *CYPERUS ROTUNDUS* ON CCL_4-INDUCED-HEPATOTOXICITY

GROUP	Bio chemical parameters ±SEM			
	SGOT (U/ml)	SGPT (U/ml)	ALKP (U/ml)	TB (mg/dl)
Control	132.5±7.79	98.50±6.03	87.18±2.61	0.38±0.03
Carbon tetrachloride	358.8±20.39	243.7±4.86	246.70±8.44	1.12±0.08
Ethyl acetate extract	180.5±8.08*	119.0±7.18*	102.96±3.13*	0.44±0.03*
Ether fraction	289.5±18.26*	189.8±12.10*	178.10±8.01	0.74±0.06*
Ethyl acetate fraction	206.6±5.31*	148.2±8.67*	124.10±2.41	0.80±0.03*
Silymarin	167.0±9.51*	91.20±5.77*	91.36±3.04*	0.35±0.02*
F Calculated	43.76	37.08	138.57	41.14
5 % Allowance	48.30	36.39	19.96	0.18

*Significant reduction compared to carbon tetrachloride. F critical=2.53 ($P<0.05$)

crosis and vacuolization of periportal vein are observed in ccl_4-intoxicated liver (fig. 3). The liver sections of the rat treated with ethyl acetate extract and intoxicated with ccl_4 (fig. 4), rat treated with silymarin and intoxicated with ccl_4 (fig. 5) showed less vacuole formation and absence of necrosis and overall no visible changes observed, supplementing the protective effect of the extract.

DISCUSSION

The hepatotoxicity of CCl_4 has been reported to be due to the formation of the highly reactive trichloro free radical, which attacks polyunsaturated fatty acids. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals[12]. The CCl_4-induced liver peroxidation was inhibited significantly by *Cyperus rotundus* rhizome extract, which confirms the protective action of the ethyl acetate extract of *Cyperus rotundus* against experimentally-induced liver damage in rats. SGOT, SGPT, ALKP, TB are the most sensitive tests employed in the diagnosis of hepatic disease[13]. The elevated levels of these parameters were significantly reduced by the treatment of *Cyperus*

TABLE 3: PERCENT REDUCTION OF BIOCHEMICAL PARAMETERS BY ETHYL ACETATE EXTRACT AND SILYMARIN.

GROUP	SGOT	SGPT	ALKP	TB
Ethyl acetate extract	78.7	85.8	90.1	91.8
Silymarin	84.7	105.1	97.3	104.1

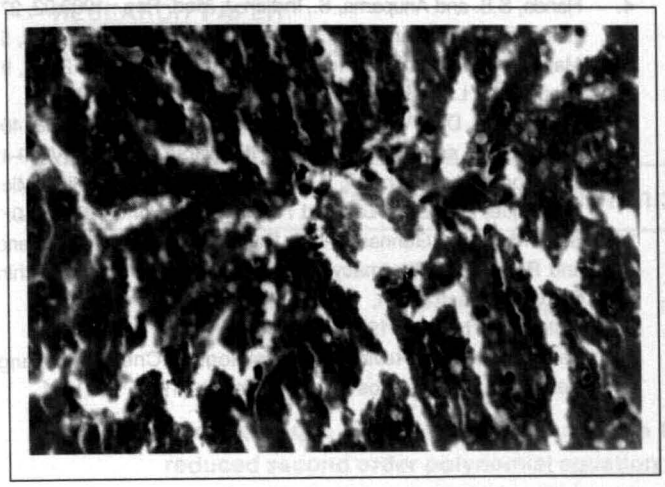

Fig. 1: Liver section of the rat treated with ethyl acetate extract.

Effect of ethyl acetate extract of *Cyperus rotundus* on normal liver function. Magnification 400 X, Haematoxylin-eosin stain.

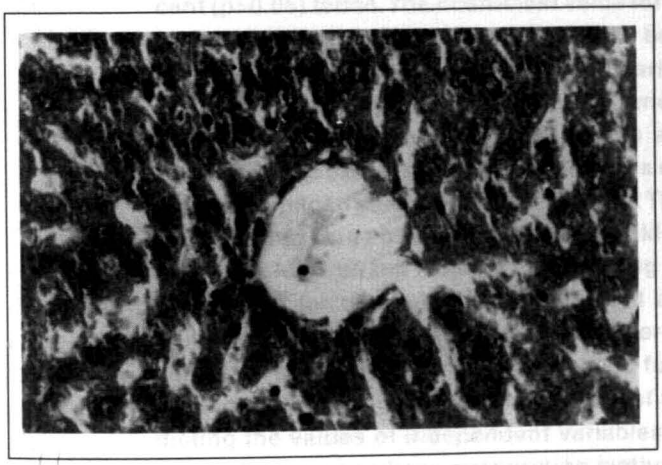

Fig. 2: Normal rat liver section.

Magnification 400 X, Haematoxylin-eosin stain.

rotundus rhizome extract. Among the ether and ethyl acetate fractions, which are also tested for hepatoprotective effect, both the fractions have shown significant reduction in only in three parameters out of four, elevated by carbon tetrachloride, significantly. It can be concluded from this investigation that rhizomes of *Cyperus rotundus* possess hepatoprotective activity. Further detailed studies may, however confirm the utility profile of this drug.

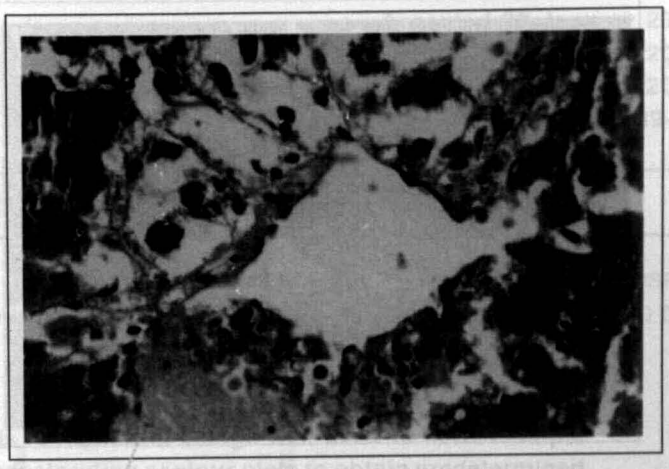

Fig. 3: Liver section of the rat treated with CCl_4.

Magnification 400 X, Haematoxylin-eosin stain.

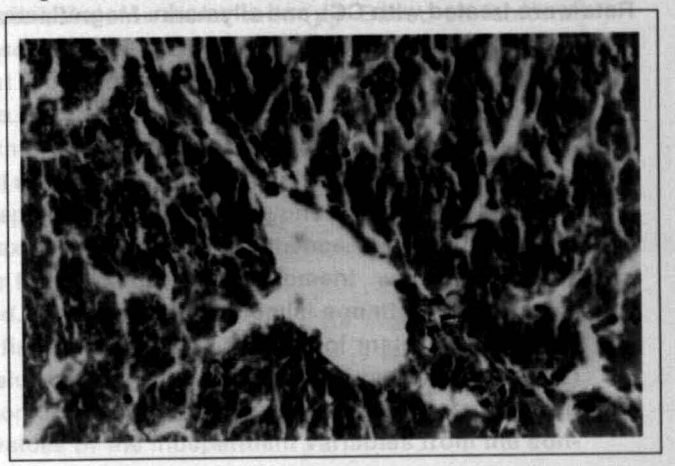

Fig. 4: Liver section of the rat treated with ethyl acetate extract and CCl_4.

Rats were treated with CCl_4 and ethyl acetate extract of *Cyperus rotundus.* Magnification 400 X, Haematoxylin-eosin stain.

ACKNOWLEDGEMENTS

The authors are thankful to the authority of Maharaja Sayaji Rao University of Baroda for providing all facilities. We thank the University Grant Commission, New Delhi for providing Junior Research Fellowship.

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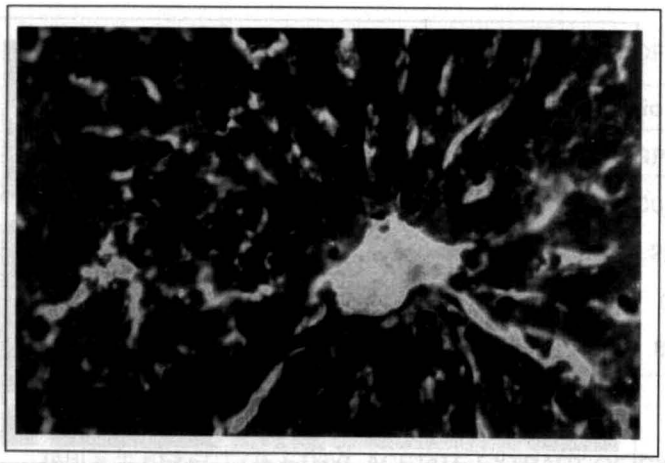

Fig. 5: Liver section of the rat treated with silymarin and CCl_4.

Rats were treated with CCl_4 and silymarin. Magnification 400 X, Haematoxylin-eosin stain.

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