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SHORT COMMUNICATION

Hepatoprotective Activity of *Ficus racemosa* Leaf Extract on Liver Damage Caused by Carbon Tetrachloride in Rats

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An extract of the leaves of *Ficus racemosa* Linn. (Moraceae) was evaluated for hepatoprotective activity in rats by inducing chronic liver damage by subcutaneous injection of 50% v/v carbon tetrachloride in liquid paraffin at a dose of 3 mL/kg on alternate days for a period of 4 weeks. The biochemical parameters SGOT, SGPT, serum bilirubin and alkaline phosphatase were estimated to assess the liver function. The activity of extract was also comparable to a standard liver tonic (Neutrosec).

Keywords: Ficus racemosa leaf extract; hepatoprotective; carbon tetrachloride; hepatic damage; rats.

INTRODUCTION

Ficus racemosa Linn. Syn. *Ficus glomerata* Roxb. (Fam. Moraceae) is a moderate to large spreading tree found in the greater part of India, commonly known as 'Jagyadumur' (Bengali), 'Gular' (Hindi) and 'Udumbara' (Sanskrit) (Anonymous, 1952). All parts of this plant are medicinally important in the traditional system of medicine in India and have been used extensively in biliary disorders, jaundice, dysentery, diabetes, diarrhoea and as an antiinflammatory (Chopra *et al.*, 1958; Kirtikar and Basu, 1975; Nadkarni *et al.*, 1976). The antidiabetic activity of the leaves of this plant has been reported from our laboratory (Mandal *et al.*, 1997). Neutrosec (Tablet India Ltd, Madras, India) is used as a standard liver tonic, each 15 mL of Neutrosec containing methionine USP 100 mg, choline dihydrogen citrate NF XIII 100 mg, vitamin B-complex and vitamin E acetate.

The present study was therefore undertaken to evaluate the hepatoprotective activity of the leaf extract of this plant.

MATERIALS AND METHODS

Plant material. The leaves of *Ficus racemosa* were collected from Hetyasole, Bankura district of West Bengal, India, during July and August. Taxonomical identification of the plant (Reference No. CNH/7-3(20)Tech.II/95/239) was performed by Botanical Survey of India, Shibpur, Howrah.

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Preparation of extract. The dried powdered leaves were extracted with petroleum ether (B.P. 60°–80°C) in a soxhlet extractor. On evaporation of the petroleum ether *in vacuo*, a greenish coloured residue was obtained (yield 6.43% (w/w) and stored in a desiccator. For pharmacological experiments, a weighed amount of the dried extract was suspended in a 2% (w/v) aqueous Tween 80 solution.

Phytochemical screening. On preliminary screening the extract showed the positive Liebermann–Burchard reaction (Liebermann, 1885) and a positive Noller test (Noller *et al.*, 1942) for triterpenoids, which was confirmed by thin layer chromatography.

Test animals. Adult albino rats (Wistar strain) weighing 200–250 g were used. The animals were housed in standard metal cages and provided with food and water *ad libitum*.

Chemical used. Carbon tetrachloride used for this study was obtained from (E. Merck (India) Ltd, Mumbai). The serum SGOT, SGPT, bilirubin, alkaline phosphatase of the rats were determined by using kits from Span Diagnostic Ltd, Surat, India. All other reagents were of analytical grade.

Carbon tetrachloride-induced hepatotoxicity. Carbon tetrachloride (CCl_4) intoxication in rats is an experimental model widely used to study necrosis and steatosis of the liver (Recnagel 1983; Lin *et al.*, 1996). Liver cell damage was induced by subcutaneous injection of 50% CCl_4 in liquid paraffin (3 mL/kg) (Chun-ching and Wei-chih, 1996). Animals were randomized into four groups of six rats each. The first group received only normal saline 1.0 mL/kg (p.o.). The second group of animals

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Table 1. Effect of extract of *F. racemosa* leaf on serum biochemical parameters during carbon tetrachloride (CCl_4) induced chronic liver damage in rats ($n = 6$)

Parameter	Group I Control	Group II CCl_4 (3 mL/kg)	Group III Leaf extract (400 mg/kg) + CCl_4	Group IV Liver tonic (5 mL/kg) + CCl_4
SGOT (IU/L)	49.2 ± 1.1	161.3 ± 1.3^a	58.4 ± 1.1^b	53.4 ± 1.1^b
SGPT (IU/L)	31.3 ± 1.2	11.3 ± 1.2^a	41.2 ± 1.1^a	38.3 ± 1.0^a
Alkaline phosphatase (U/L)	56.1 ± 1.3	169.8 ± 1.1^a	65.7 ± 1.2^a	61.9 ± 1.1^b
Bilirubin (g/L)	1.2 ± 0.07	4.1 ± 0.04^a	2.5 ± 0.03^a	2.1 ± 0.03^a

Experimental groups were compared with control.
[a] $p < 0.001$,
[b] $p < 0.01$.

were given CCl_4. The third group received CCl_4 and leaf extract (400 mg/kg, p.o.), and the fourth group received CCl_4 and liver tonic (5 mL/kg). All the animals were given treatment on alternate days for a period of 4 weeks.

Assay of serum GOT and GPT activities. All rats were killed and blood was withdrawn from the carotid artery and centrifuged at 2000 × **g** at 4 °C for 10 min to separate the serum. The serum obtained was used for determina-

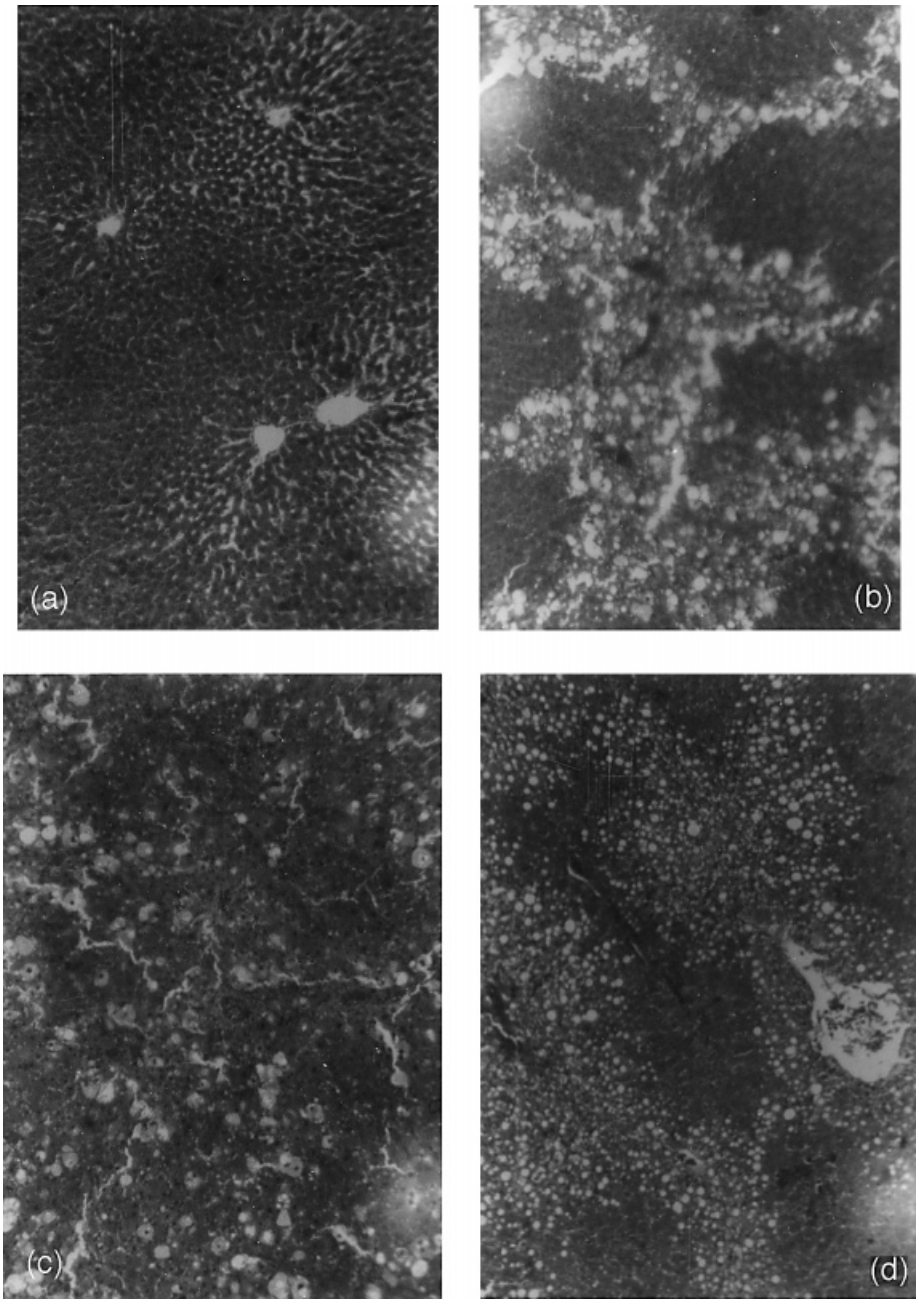

Figure 1. Liver section taken from rats. **(a)** Vehicle control group; **(b)** CCl_4 (3 mL/kg); **(c)** CCl_4 + extract (400 mg/kg); **(d)** CCl_4 + liver tonic (5 mg/kg). (HE stain, 65 x).

tion of glutamic oxaloacetate transminase (GOT) and glutamic pyruvate transminase (GPT) (Reitman and Frankel, 1957).

Assay of serum bilirubin and serum alkaline phosphatase. Serum bilirubin was estimated following the method of Malloy and Evelyn (1937) and serum alkaline phosphatase was estimated by following that of Kind and Kings (1954).

Histopathological examination of hepatocytes. Each rat was laparotomized to obtain the liver immediately after collecting blood under ether anaesthesia. Fragments of the rat liver were fixed in 10% formalin solution, dehydrated with ethanol solution from 50% to 100%, embedded in paraffin and cut into 5 μm sections and stained using haematoxylin–eosin dye for photomicroscopic observation of necrosis, steatosis and fatty change of hepatic cells.

Statistical analysis. The data are expressed as mean ± SEM and the statistical significance was evaluated by the student's *t*-test (Woodson, 1987).

RESULTS AND DISCUSSION

Rats subjected to the CCl_4 regimen alone developed significant hepatocellular damage as evident from a significant elevation in serum activities of SGOT, SGPT, alkaline phosphatase and bilirubin concentration (Table 1). Oral administration of *F. racemosa* leaf extract (400 mg/kg, p.o.) exhibited a significant reduction in the CCl_4 induced increase in the levels of SGOT, SGPT, alkaline phosphatase and serum bilirubin. Treatment with Neutrosec (a popular liver tonic) also reversed the hepatotoxicity significantly.

At the end of 4 weeks a 3.95% mortality was observed in the CCl_4 treated group and the autopsy showed congested and enlarged liver, sometimes associated with intestinal bleeding and inflammation. However, no mortality was observed with either the control or *F. racemosa* leaf extract treated groups. Continuous administration of extract of *F. racemosa* prior to and concurrent with CCl_4 treatment, reversed to varying degrees the enzyme alterations induced by CCl_4 (Table 1). The activity of this plant extract was compared with that of a standard hepatoprotective drug, Neutrosec, and was comparable in all the parameters tested. However, Neutrosec provided better hepatoprotection in terms of the inhibition of elevated serum activities of these enzymes as well as serum bilirubin concentration by CCl_4.

Histopathological profiles of the liver from CCl_4 treated rats revealed intense centrilobular necrosis, steatosis and often swelling of the hepatic cytoplasm. The protective effect of *F. racemosa* leaf extract was confirmed by histopathological examination of the liver section. Administration of extract to the experimental animals (400 mg/kg, p.o.) showed a significant improvement of the hepatocellular architecture over the CCl_4 treated control group, as evident from a considerable reduction in necrosis and fatty changes. A liver section of rats the treated with Neutrosec showed significant signs of amelioration of CCl_4 evoked liver injury which was evident from the presence of normal hepatic pods, and the absence of necrosis and steatosis. The extract at 400 mg/kg and liver tonic at 5 mL/kg showed a significant protective effect against liver injury which is evident from the histopathological examination (Fig. 1). Thus the present study confirms the liver protective action of the petroleum ether extract of *F. racemosa* against experimentally induced liver damage in rats, which was comparable to that of a standard hepatoprotective drug, Neutrosec. *F. racemosa* leaf extract contains steroids and triterpenoids; Thabrew and Hughes (1996) have reported that plants containing these compounds can control liver diseases. Further studies relating to the separation of the active component(s) responsible for this activity, as well as further confirmation of its hepatoprotective effect and mechanism of action, are under way in our laboratory.

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