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SHORT COMMUNICATION

Protective Effect of Leaf Extract of *Ficus hispida* Linn. Against Paracetamol-induced Hepatotoxicity in Rats

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The methanol extract of the leaves of *Ficus hispida* Linn. (Moraceae) was evaluated for hepatoprotective activity in rats by inducing acute liver damage by paracetamol (750 mg/kg, p.o.). The extract at an oral dose of 400 mg/kg exhibited a significant protective effect by lowering the serum levels of transaminase (SGOT and SGPT), bilirubin and alkaline phosphatase (ALP). These biochemical observations were supplemented by histopathological examination of liver sections. The activity of extract was also comparable to that of Liv-52 a known hepatoprotective formulation. Copyright © 2000 John Wiley & Sons, Ltd.

Keywords: Ficus hispida; leaf extract; hepatoprotective; paracetamol; hepatic damage; rats.

INTRODUCTION

Ficus hispida Linn. (Family - Moraceae) is a moderate sized tree found throughout India and the Andaman Islands in damp localities, and flowers and fruits practically throughout the year. All part of the plant have been used but the leaves are of particular interest from a medicinal point of view (Nadkarni, 1976), of as an antidiarrhoeal, hepatoprotective, antitussive, antipyretic, astringent, antiinflammatory, vulnerary, haemostatic and, anti-ulcer, among others (Nadkarni, 1976; Rastogi and Mehrotra, 1993).

The present study was undertaken to evaluate the antihepatotoxic activity of the leaf extract of this plant and is reported here.

MATERIALS AND METHODS

Plant material. The leaves of *Ficus hispida* Linn. (Moraceae) were collected from Hetyasole, West Bengal, India in August 1997. A voucher specimen was deposited in the Central National Herbarium, Botanical Survey of India, Shibpur, Howrah (CNH/7-3/(20)Tech.II/95/619). The leaves were collected and dried under shade, pulverized in a mechanical grinder and stored in a closed container for further use.

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Preparation of extract. The powdered leaves were extracted with petroleum ether (60°–80°C) which was discarded and then again extracted with methanol in a Soxhlet extractor. On evaporation of methanol from the methanol extract *in vacuo*, a greenish coloured residue was obtained (yield 4.7% (w/w) with respect to the dry starting material) and was stored in a desiccator. For pharmacological experiments a weighed amount of the dried extract was suspended in a 2% v/v aqueous Tween 80 solution.

Phytochemical screening. On preliminary screening the methanol extract showed positive reaction for, alkaloids tannins and saponins (Kokate, 1988).

Test animals. Albino Wistar rats of either sex weighing 200 –250 g supplied by B.N. Ghosh Co., Calcutta, India, were placed in cages with wire-net floors in a controlled room temperature $22° \pm 2°$C and provided with food and water *ad libitum*. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water throughout. All studies were carried out using six rats in each group.

Chemicals used. Serum glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), bilirubin and alkaline phosphatase (ALP) were determined using kits from Span Diagnostics Ltd., Surat, India. All other reagents were of analytical grade. Liv-52 (The Himalaya Drug Co., Bangalore, India) was used as standard liver tonic.

Paracetamol induced hepatotoxicity. Paracetamol intoxication in rats is an experimental model widely used to study necrosis and steatosis of the liver (Dwivedi *et al.*,

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Table 1. Effect of *F. hispida* leaf extract on serum biochemical parameters during paracetamol-induced acute liver damage in rats

Parameter	Group I control	Group II paracetamol (750 mg/kg)	Group III leaf extract (400 mg/kg) + paracetamol	Group IV liver tonic (5 mL/kg) + paracetamol
SGOT (IU/L)	49.7 ± 1.3	141.2 ± 1.0[a]	53.9 ± 1.3[c]	55.9 ± 1.2[b]
SGPT (IU/L)	35.4 ± 1.0	110.0 ± 1.4[a]	39.7 ± 1.3[c]	41.7 ± 1.2[a]
Alkaline phosphatase (U/L)	60.0 ± 1.1	142.8 ± 1.0[a]	63.9 ± 1.2[c]	63.4 ± 1.3[c]
Bilirubin (g/L)	1.9 ± 0.01	7.5 ± 0.05[a]	2.6 ± 0.03[a]	2.3 ± 0.02[a]

Significance vs control group, n = 6 animals per group.
[a] $p < 0.001$.
[b] $p < 0.01$.
[c] $p < 0.05$.

1991). Animals were randomized into four groups of six rats each. Group I was given normal saline 1 mL/kg (p.o.). group III and group IV were given 400 mg/kg (p.o.) of leaf extract and Liv-52, (5 mg/kg, p.o.) respectively for a period of 7 days. On 7 day paracetamol suspension (750 mg/kg, p.o.) was given to group II animals and also to group III and group IV animals (Mandal *et al.*, 1998).

Assay of serum GOT and GPT activities. Thirty six hours after the last administration of paracetamol, rats of each group were anaesthetized with ether and the 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 the determination of glutamic oxaloacetate transaminase (GOT) and glutamic pyruvate transaminase (GPT) (Reitman and Frankel, 1957).

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

Histopathological examination of hepatocytes. Each rat was laparotomized to obtain the liver immediately after collecting the blood under ether anaesthesia. Small fragments of the rat liver were fixed in a 10% formalin solution dehydrated with ethanol solution from 50% to 100%, embedded in paraffin and cut into 5 μm thick sections which were stained using haemotoxylin–eosin

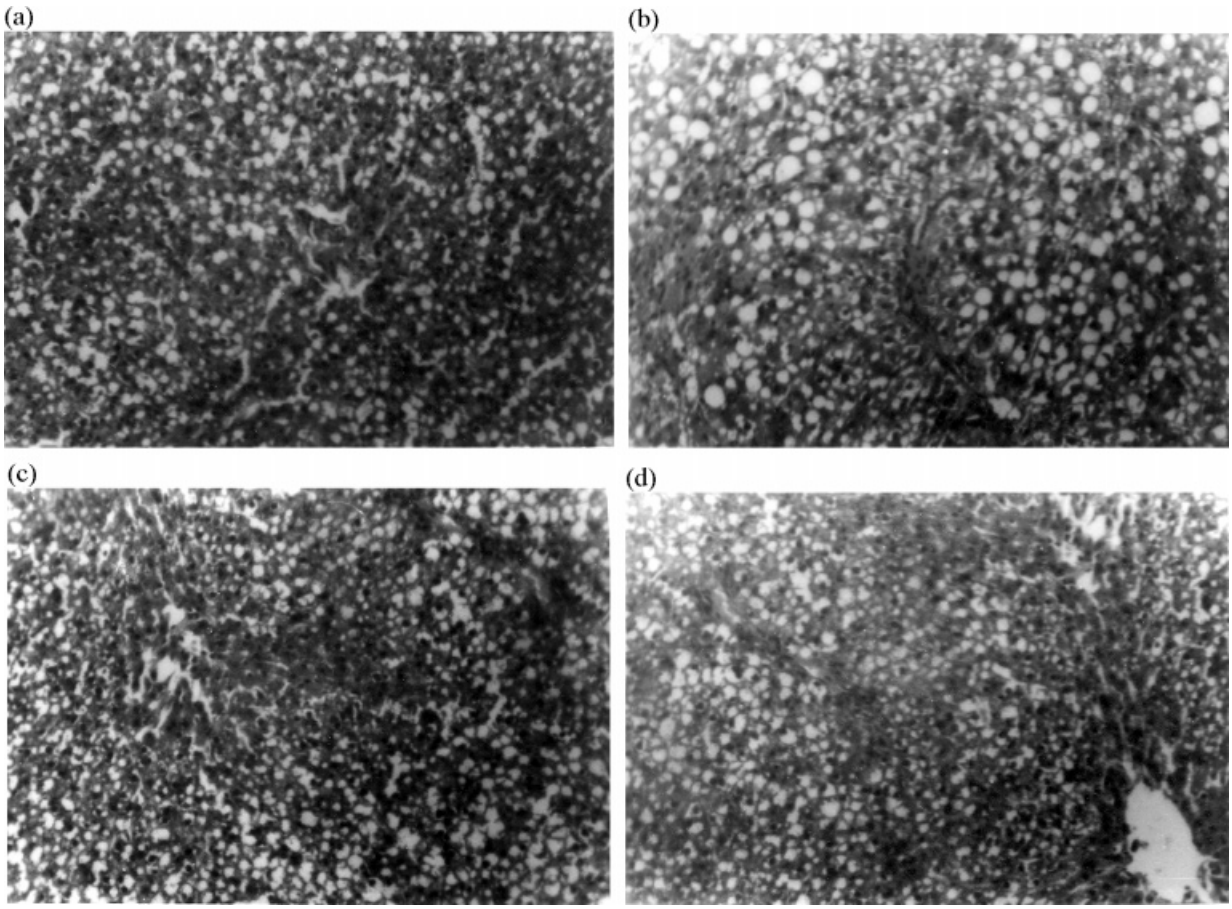

Figure 1. Liver section taken from rats: (a) vehicle control group; (b) paracetamol (750 ml/kg); (c) paracetamol + *F. hispida* leaf extract (400 mg/kg); (d) paracetamol + liver tonic (5 mL/kg). Haematoxylin - eosin stain, 100x.

dye for photomicroscopic observation (Gray, 1964), including necrosis, steatosis and fatty change of hepatic cells.

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

RESULTS

Acute administration of paracetamol caused a marked hepatocellular injury in rats which was clearly evident from the significant elevation in activities of serum GOT, GPT, ALP and bilirubin used as reliable markers of hepatotoxicity (Table 1). There was an increase in serum GOT, GPT, ALP and bilirubin, 36 h after the acute paracetamol regimen compared with that of the control group. The effects of *F. hispida* methanol extract at 400 mg/kg reduced the increase in serum activities caused by paracetamol, which was found to be statistically significant when compared with that of the control group. The activity exhibited by the plant extract was compared with Liv-52, the standard hepatoprotective formulation. Liv-52 provided a better inhibition of the elevated GOT, GPT, ALP and bilirubin induced by paracetamol. The activity exhibited by 400 mg/kg of extract was similar to that of the Liv-52.

The histopathological profiles showed a hepatoprotective activity of the leaf extract of *Ficus hispida*, whereas the paracetamol-treated animals showed centrilobular necrosis in the liver including infiltration of lymphocytes kupffer cells, fatty changes and ballooning degeneration (Fig. 1). The inflammation of hepatocytes was less severe in the extract treated, as well as the Liv-52 treated groups. Paracetamol produces hepatotoxicity by altering liver microsomal membranes in experimental animals (Hiroshi *et al.*, 1987).

DISCUSSION

Paracetamol (acetaminophen), a widely used antipyretic - analgesic drug produces acute liver damage in overdose. The covalent binding of N-acetyl-P-benzoquinoneimine, an oxidation product of paracetamol, to sulphydryl groups of protein results in cell necrosis and lipid peroxidation induced by depletions of glutathione in the liver (Wendel *et al.*, 1987).

Paracetamol-induced lipid peroxidation was inhibited significantly in *F. hispida* leaf extract and Liv-52 (liver tonic) treated groups.

Thus the study confirms the protective action of the methanol extract of *F. hispida* against experimentally induced liver damage in rats, which was comparable to that of a standard hepatoprotective drug Liv-52. SGOT, SGPT, ALP and serum bilirubin are the most sensitive tests employed in the diagnosis of hepatic disease (Harper, 1961). The elevated levels of these parameters were significantly reduced by the treatment of *F. hispida* leaf extract as well as the liver tonic Liv-52. The related species *Ficus racemosa*, leaf extract contains steroids and triterpenoids (Mandal *et al.*, 1998) and has been shown to be a liver protectant also. However, a more detailed phytochemical study of *F. hispida* is to identify the active principle(s) and elucidate the mechanism of action.

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