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Ficus racemosa Affords Antihepatotoxic Activity Against Paracetamol-Induced Acute Liver Damage in Rats

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Abstract – The effect of oral treatment with *Ficus racemosa* leaf extract (400 mg/kg for seven days) were studied on hepatic damage induced by paracetamol (750 mg/kg, i.p.) in rats. Biochemical parameter like SGOT, SGPT, serum bilirubin and alkaline phosphatase were estimated to assess liver function. These biochemical observations were supplemented by histopathological examination of liver sections. The activity of extract was also comparable to *Neutrosec* a known hepatoprotective formulation.

Key words – Paracetamol, hepatotoxicity, biochemical changes, histopathology, liver, *Ficus racemosa*, hepatoprotective effect.

Introduction

Ayurvedic and other traditional medical practitioners from different countries have claimed for centuries that extracts of plants can be effectively used for the alleviation of different types of liver disease (Attygalle, 1952; Kirtikar and Basu, 1956; Jayaweera, 1981; Ding, 1987), but most claims are anecdotal and very few have received adequate medical or scientific evaluation until the 1970s. Investigations carried out since then have provided experimental evidence which confirms that many of these plants do indeed have hepatoprotective properties (Bertelli, 1975; Handa *et al.*, 1986; Yan *et al.*, 1987; Wagner, 1989). Recent progress in the study of such plants has resulted in the isolation of about 170 different phytoconstituents from plants belonging to about 55 plant families which exhibit hepatoprotective activity (Sharma *et al.*, 1991). Many of these individual plants have also been used in the preparation of commercial herbal formulations sold in different countries for treatment of liver diseases; in India alone there are 40 patent polyherbal formulations representing a variety of combinations of about 93 Indian herbs from 44 plant families (Handa *et al.*, 1986; Sharma *et al.*, 1991). Hepatitis from the different viral causes is a major public health problem throughout the world and causes considerable morbidity and mortality in the population both from acute infection as well as from the consequences of chronic viral infection such as chronic active or persistent hepatitis, cirrhosis and primary liver cancer.

Plants traditionally used in the alleviation of liver dysfunction might therefore provide a useful source of new hepatoprotective compounds for development as pharmaceutical entities or as simple dietary adjuncts to

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existing therapies (Ira Thabrew and Hughes, 1996).

Plants are known to play a vital role in the management of various liver disorders. Ayurveda, the ancient science of Indian traditional system of medicine identified liver disorder quite early and recommends a number of herbal remedies, one such plant, commonly known as "Jagya-dumur" (Bengali), "Gular" (Hindi) and "Udumbara" (Sanskrit).

The leaf of *Ficus racemosa* Linn. Syn. *F. glomerata* Roxb. (Moraceae) is considerable useful in dysentery, diarrhoea, diabetes and billious affection (Chopra *et al.*, 1958; Kirtikar and Basu, 1975; Nadkarni *et al.*, 1976). The hypoglycaemic (Mandal *et al.*, 1997a) and antidiarrhoeal activity (Mandal *et al.*, 1997b) of *F. racemosa* leaves has been reported. *Neutrosec* (Tablet India Ltd., Madras, India), is used as a standard liver tonic (Maity *et al.*, 1997), each 15 ml containing methionine USP 100 mg, choline dihydrogen citrate NF XIII 100 mg, vitamin B-complex and vitamin E acetate. Presently it has come to our notice that the rural tribal people of Khatra region of Bankura district, West Bengal, India use this plant to get cure of jaundice.

In the light of above information the present study was undertaken to evaluate the antihepatotoxic activity of the leaves extract of this plant and is being reported in the present communication.

Experimental

Plant material – The leaves of *Ficus racemosa* Linn. (Moraceae) were collected from Hetyasole, Khatra region of Bankura district of West Bengal (India) in August 1995. A voucher specimen was deposited in the Central National Herbarium, Botanical Survey of India, Shibpur, Howrah (CNH/7-3/(20) Tech.II/95/239) and was authenticated by Dr. Malick. The leaves were collected and dried under shade and pulverised in a mechanical grinder and stored in closed container for further use.

Preparation of extract – The powdered leaves were extracted with petroleum ether (B.P. 60-80°C) in soxhlet extractor. On evaporation of petroleum ether from the petroleum ether extract *in vacuo*, a greenish residue was obtained [yield 6.43% (w/w) with respect to the dry starting material] and was stored in a desiccator. For pharmacological experiments 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 for steroid (Liebermann, 1885) and a positive Noller test for triterpenoid (Noller *et al.*, 1942), which were confirmed by thin layer chromatography with the solvent system hexane : ethyle acetate (1:1) over silica gel G (Stahl, 1969). Further separation of the specific phytochemical is under process.

Test animals – Albino Wister rats of either sex weighing 200-250 g supplied by M/s B. N. Ghosh & Co., Calcutta, India, were placed in cages with wire-net floors in a controlled room temperature 22±2°C, relative humidity 60-70% 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 by using six rats in each group.

Chemical used – Paracetamol used for this study was obtained from Alpha Remedies Ltd., Nagpur, India. Serum glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), bilirubin and alkaline phosphatase (ALP) were determined by using kits of Span Diagnostic Ltd., Surat, India. All other reagents were of analytical grade.

Paracetamol induced hepatotoxicity – Paracetamol intoxication in rats is an experimental model widely used to study

necrosis and steatosis of liver (Jollow *et al.*, 1973, Dwivedi *et al.*, 1991). Animal were randomized into four groups of six rats each. Group I was given normal saline 1.0 ml/kg (p.o.). Groups III and IV were given 400 mg/kg (p.o.) extract and *Neutrosec* 5 ml/kg respectively for a period of seven days. On seventh day paracetamol suspension (750 mg/kg, p.o.) was given to groups II, III and IV (Hiroshi *et al.*, 1987).

Assay of serum biochemical indices – Thirty-six hours after the last administration of paracetamol rats of each group were anesthetized with diethyl ether and blood samples were collected directly from the heart. It was centrifuged at 2000×g for 10 min in a refrigerated centrifuge at 4℃ to separate the serum. Serum activities of GOT and GPT were determined by the method of Reitman and Frankel (1957), ALP by Kind and King (1971) and bilirubin by Malloy and Evelyn (1937).

. **Histopathological study** – Each rat was laparotomized to obtain the liver immediately after collecting blood under anaesthesia. Small fragments of the liver were fixed in 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 haematoxylin-eosin 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 the student's *t*-test (Woodson, 1987).

Results and discussion

Paracetamol (acetaminophen), a widely used antipyretic-analgesic drug produces acute liver damage if accidental overdosage (which may occur in alcoholics and the elderly) are consumed. 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. Results in Table 1 depict a substantial increase in serum GOT, GPT, ALP and bilirubin activities 36 hours after acute paracetamol regimen relative to vehicle control group. The beneficial effects of *F. racemosa* extract at 400 mg/kg with regard to serum enzymes as well as to level of bilirubin were found statistically significant. The activity of this plant extract was also compared with a standard hepatoprotective formulation *Neutrosec*. Data presented in Table 1 show the response

Table 1. Effect of *F. racemosa* leaf extract on Serum biochemical parameters during paracetamol induced acute liver damage in rats (n=6)

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)	47.3±1.2	135.2±1.0[a]	54.4±1.2[b]	51.5±1.0[a]
SGPT (IU/L)	33.4±1.1	107.3±1.4[a]	41.2±1.0[a]	39.7±1.1[b]
Alkaline Phosphatase (U/L)	55.6±1.2	140.1±1.0[a]	61.3±1.3[b]	59.2±1.0[c]
Bilirubin (g/L)	1.6±0.01	6.3±0.04[a]	2.0±0.03[a]	1.9±0.2[a]

Experimental groups were compared with control
[a]$p<0.001$, [b]$p<0.01$, [c]$p<0.05$.

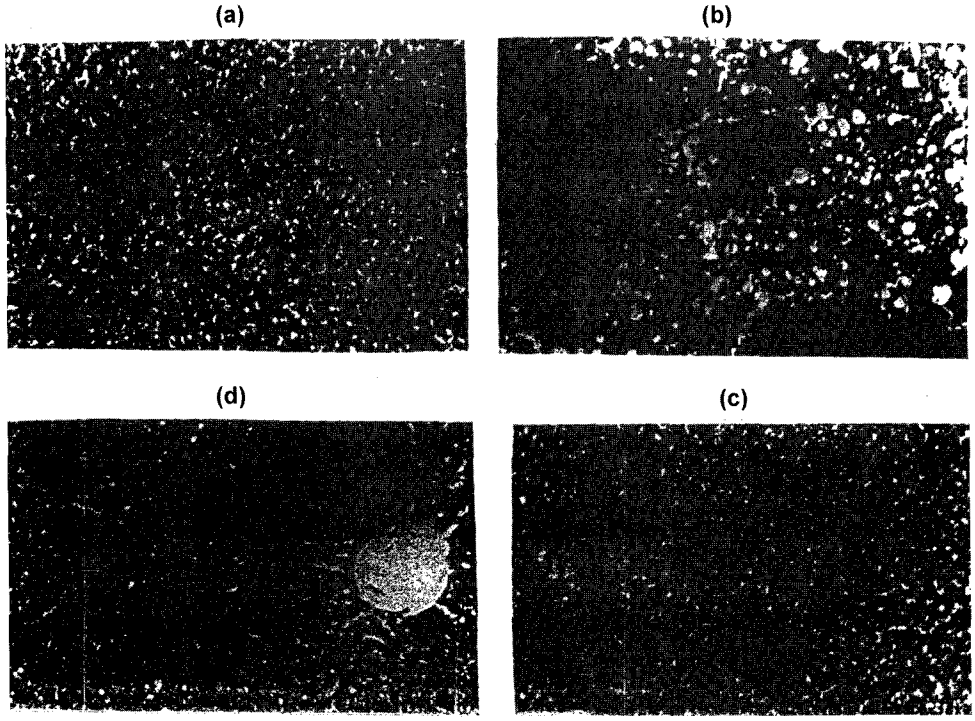

Fig. 1. Liver section taken from rats: (a) vehicle control group; (b) paracetamol (750 mg/kg); (c) paracetamol+*F. racemosa* leaf extract (400 mg/kg); (d) paracetamol+liver tonic (*Neutrosec*), (HE stain, 65x).

to the extract and *Neutrosec* is comparable in all parameter tested. However, *Neutrosec* provided better hepatoprotective in terms of the inhibition of elevated activities of GOT, GPT, ALP and bilirubin induced by paracetamol and almost similar response with regard to some parameters compared with the dose of the extract used in this study i.e. 400 mg/kg.

The histological changes associated with the hepatoprotective activity basically support the estimation of the serum enzymes and bilirubin. Administration of paracetamol to an animal leads to centrilobular necrosis in the liver including infiltration of lymphocyte, Kupffer cell, fatty change and ballooning degeneration. Necrosis, however, was recognised more particularly in the paracetamol intoxicated group than in the drug treated group. The inflammation of hepatocyte and ballooning degeneration was less severe after administration of extract and *Neutrosec* (Fig. 1). SGOT, SGPT, ALP and serum bilirubin are the most sensitive tests employed in the diagnosis of hepatic disease (Harper, 1961). Large doses of paracetamol is known to cause hepatotoxicity in man and laboratory animals (Yoshiyuki *et al.*, 1992) and cause significant rise in levels of GOT, GPT, ALP and bilirubin in blood serum of treated animals. Overdose of paracetamol produce hepatotoxicity by altering in liver microsomal membrance in experimental animal (Hiroshi *et al.*, 1987).

Elevated levels of serum enzymes are indicative of cellular leackage and loss of functional integrity of the cell membrane in liver (Drotmann and Lowhorn, 1978). In our study with paracetamol induced acute hepatotoxic model, pretreatment with *F. racemosa* extract and *Neutrosec* treatment group offered hepatoprotection as evidenced by the inhibition of the rise in SGOT, SGPT,

ALP and bilirubin levels. The absence of necrotic lesions in liver sample from extract treated group, suggested that its hepatoprotective action may be due to its membrane stabilizing effect on hepatic cells and comparable to that of *Neutrosec*. However, further studies are in progress to isolate the active principle (s) present in the extract and allied approaches are also in process in our laboratory.

So it can be concluded that on preliminary secreening the extract of *F. racemosa* produced significant antihepatotoxic and thus established the claim of using the plant as an antiheptatotoxic in folklore medicine (Chopra *et al.*, 1958; Kirtikar and Basu, 1975; Nadkarni *et al.*, 1976).

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