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Hepatoprotective activity of *Phyllanthus emblica* Linn. extract against paracetamol-induced hepatic damage in rats

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Abstract

The efficacy of the medicinal plant *Phyllanthus emblica* Linn. to prevent paracetamol-induced hepatotoxicity in rats was examined by determining the concentrations of biochemical markers of liver enzymes Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP), and triglycerides, protein, cholesterol and phospholipids in serum. Treatment with aqueous extract of fruits of *Phyllanthus emblica* Linn. improved the levels of biochemical molecules to near normal levels suggesting the hepatoprotective effect of this medicinal plant.

Keywords: aqueous extract, hepatoprotective activity, *Phyllanthus emblica* Linn.

INTRODUCTION

Liver diseases remain to be serious health problems. Management of liver diseases is still a challenge to the modern medicine. 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 toxic substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign (xenobiotic) compounds culminating in liver dysfunction (Devarshi *et al*., 1986). Hepatic dysfunctions due to ingestion or inhalation of hepatotoxins (such as acetaminophen, cadmium chloride, ethanol, carbon tetrachloride and allyl alcohol) are increasing worldwide (Datta *et al*., 1998). Despite the tremendous strides in modern medicine, there is still a need for a drug that stimulates liver function or offers protection to the liver from damage or helps regeneration of hepatic cells (Rajesh and Latha, 2001). Poly herbal drugs are frequently considered to be less toxic and free from side effects than synthetic drugs. Medicinal plants such as *Andrographis paniculata, Eclipta alba, Phyllanthus amarus, Phyllanthus debilis* and *Boerhaavia diffusa* are well known for their hepatoprotective effects (Rana and Avadhoot, 1991; Saxena *et al*., 1993; Sane *et al*., 1995; Rawat *et al*., 1997). *Phyllanthus emblica* Linn. of the family Euphorbiaceae, is a perennial small deciduous tree. Its fruit is commonly used in the treatment of burning sensation, anorexia, constipation, urinary discharges, diarrhoea and dysentery (Chopra *et al*., 2002). The fermented liquor prepared from its fruit is used for treating jaundice and cough (Anonymous, 1959; Chopra *et al*., 2002). The fruit of this plant is used in the treatment of ulcer (Bhattacharya *et al*., 2000; Zhang *et al*., 2000). This paper examines the efficacy of aqueous extract of dried fruits of *P.emblica* as a hepatoprotective agent.

MATERIALS AND METHODS

Collection of plant materials

Fruits of *Phyllanthus emblica* were collected from Kumbakonam market, Thanjavur district, Tamil Nadu, India during 2004 and identified by the third author.

Taxonomic authenticity was confirmed by referring to herbarium specimen at Rapinat Herbarium, Tiruchirappalli and a specimen (STET-117) has been deposited in the Herbarium, Department of Botany & Microbiology, STET Women's College, Mannargudi.

Preparation of extract

Fruits were shade dried for a week, powdered mechanically, sieved (Sieve no. 10/44) and stored in airtight containers. About 250 g of the powdered material was boiled in 500 ml distilled water for 30 min, kept for three days with intermittent shaking, filtered and concentrated using rotary flash evaporator to get the completely dried aqueous extract. The yield was 20.2% w/w.

Drug formulation

Oral suspension containing 35 mg/ml of the aqueous fruit extract was prepared in 1% w/v gum tragacanth.

Animals

Adult male Wistar albino rats weighing 200 to 250 gm were procured from the Haliwell Research Centre, Thanjavur. The animals were housed in polypropylene cages and maintained at 27 ± 2°C, 60 ± 5% RH and at 12 h light/dark cycle. They were fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water *ad libitum* during the experiment.

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Evaluation of hepatoprotective activity

The animals were divided into four groups of six rats each. The animals in group-I served as control and received the vehicle [1 ml/kg /bw of 1% w/v gum tragacanth, po (per oral)] for 14 days. All the animals of group II to IV received 2 g/kg/bw, po paracetamol for 14 days. Aqueous extracts of 100 and 200 mg/kg/day, po of *P. emblica* were administered to the animals of groups III and IV, respectively, for 14 days.

The animals of all the groups were sacrified by diethyl ether anaesthesia on the 14th day. The blood sample of each animal was collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37° C. The clear serum was centrifuged at 3000 rpm for 10 min and the supernatant was subjected to biochemical investigations.

Assay of serum enzyme and lipid levels

Activities of serum enzyme such as serum glutamate pyruvate transaminase (SGPT) or alanine amino transaminate (ALT) (EC. No. 2.6.1.2), serum glutamate oxaloacetate transaminase (SGOT) or asparate transaminase (AST) (EC.No.2.6.1.1), alkaline phosphatase (ALP) (EC.No. 3.1.3.1), acid phosphatase (ACP) (EC.No. 3.1.3.2) and serum bilirubin were assayed as described by Yoshiyuk *et al.* (1992). The total cholesterol was estimated by the method of Abell *et al.* (1952), phospholipids by the method described by Varley (1988), triglycerides by the method of Van Hendle and Zilversmith (1957), and protein content of serum by the method of Gornal *et al.* (1949).

Histopathology

The liver samples were excised from the experimental animals of each group and washed with normal saline. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. They were then processed for paraffin embedding. The sections were taken at 5 µm thickness using microtome, processed in alcohol–xylene series and stained with alum-haematoxylin and eosin (Galigher and Koyloff, 1971). The sections were examined microscopically for the evaluation of histopathological changes.

Estimation of liver lipid peroxides

Malondialdehyde (the product of lipid peroxidation) in the liver homogenate was measured as described by Buege and Steven (1978). Protein in the liver homogenate was measured by the method of Lowry *et al.* (1951).

Determination of bile flow rate

Overnight fasted animals were anaesthetized by intraperitoneal injection of pentabarbitone (30 mg/kg). The common bile duct was surgically exposed by middle line caprotomy and cannulated with polyethylene tubing (No. 48). A heating lamp was used to maintain the body temperature of rats. Bile collected in first 10 min was discarded and then it was collected in pre weighed, graduated tubes. The drug was given introduodenally at indicated doses and bile collection was made at 1 h proceeding treatment and another collection was made at 4 h after drug treatment.

Calculation of hepato-protection (%)

Percentage of protection for each biochemical parameter was calculated as follows assuming that there was no protection (100% damage) in paracetamol control group.

$$\text{Protection \%} = 100 - \frac{100}{\text{PC} - \text{NC}} \times \text{Drug and PC–NC}$$

PC–Paracetamol Control; NC–Normal Control.

RESULTS AND DISCUSSION

Paracetamol induced hepatotoxicity

Administration of paracetamol (2 g/kg/bw, po) resulted in a marked increase in SGPT, SGOT, ALP and bilirubin levels after 48 h intoxication and decrease in ACP levels, when compared with the control (Table 1).

The water extract of the dried powder of *P. emblica* + paracetamol (100 mg/kg/day, po) provided excellent hepatoprotection as judged from the levels of SGPT, SGOT, ALP, ACP and bilirubin (Table 1).

Paracetamol treatment resulted in an increase in the lipid peroxide levels in liver. Administration of water extract of the fruits of *P. emblica* prevented the accumulation of lipid peroxides. At a lower dose (100 mg/kg/day, po) there was a marginal effect in the lipid peroxide levels whereas at a higher dose (200 mg/kg/day, po) the drug effectively prevented paracetamol-induced elevation of lipid peroxides in liver (Table 1).

Histological changes

The histoarchitecture of paracetamol treated rat liver sections showed cloudy swelling and fatty degeneration of hapatocytes. Necrosis of cells was also seen. Such degenerative changes were not observed in the rats treated with of the aqueous extract (200 mg/kg/day, po) of the fruits of *P. emblica* thus confirming its hepatoprotective effect. Hepatoprotective effects of *P. emblica* Linn. extract on ethanol induced hepatic injury in rats was reported earlier by Pramyothin *et al.* (2006) also.

Bile flow

The drug treatment resulted in stimulation of bile output in anaesthetized normal rats. Among the two concentrations of the drugs tested, 200 mg/kg/day, po;

Table 1. Effect of different concentrations of aqueous extract of dried fruit of *P. emblica* on the activities of enzymes and the concentration of total protein, cholesterol, triglycerides, phospholipids and lipid peroxide in serum of rats. Values are mean ± S.D. of six rats in each group.

Parameters	Groups			
	Control (Group I)	Paracetamol (2 g/kg/bw,po) (Group II)	Paracetamol (2 g/kg/bw,po) + *P.emblica* dried fruit extracts (100 mg/kg/day,po) (Group III)	Paracetamol (2 g/kg/bw,po) + *P.emblica* dried fruit extracts (200 mg/kg/day,po) (Group IV)
SGPT (IU/l)	59 ± 8.0	170 ± 18.2	60 ± 7.8	62 ± 3.4
SGOT (IU/l)	97 ± 9.4	210 ± 19.1	99 ± 13.1	95 ± 10.2
ALP (K.A unit)	47 ± 8.2	114 ± 14.2	48 ± 8.6	42 ± 8.1
ACP (K.A unit)	1.14 ± 0.038	0.95 ± 0.03	1.02 ± 0.04	1.09 ± 0.03
Bilirubin (mg/100 ml)	0.39 ± 0.40	2.37 ± 0.40	0.58 ± 0.10	0.52 ± 0.11
Total protein (mg/100 ml)	5.16 ±0.13	4.02 ± 0.14	4.68 ± 0.24	4.84 ± 0.21
Cholesterol (mg/100 ml)	50 ± 1.26	66.6 ± 2.3	53.3 ± 2.7	51.3 ± 2.1
Triglycerides (mg/100 ml)	5.7 ± 0.14	8.7 ± 0.3	5.9 ± 0.3	6.0 ± 0.4
Phospholipids (mg/100 ml)	150 ± 3.77	187.5 ± 6.58	150 ± 7.67	160 ± 5.4
Lipid peroxides (µ mole of MDA / mg protein)	1.10 ± 0.21	2.08 ± 0.35	1.81 ± 0.34	1.23 ± 0.22

SGPT: Serum Glutamate Pyruvate Transaminase; SGOT: Serum Glutamate Oxaloacetate Transaminase; ALP: Alkaline Phosphatase; ACP: Acid Phosphatase

Table 2. Choleretic effect of aqueous extracts of dried fruit of *P.emblica* in anaesthetized rats. Values are mean ± S.D. of six rats in each group

Bile flow (ml/100 g)	Control (Group I)	Paracetamol (2g/kg/b.w./po) (Group II)	Paracetamol (2g/kg/b.w./po) + *P.emblica* dried fruit extracts (100 mg/kg/day,po) (Group III)	Paracetamol (2g/kg/b.w./po) + *P.emblica* dried fruit extracts (200 mg/kg/day,po) (Group IV)
For 1 hr preceeding drug treatment (A)	0.28 ± 0.027	0.12 ± 0.012	0.26 ± 0.021	0.27 ± 0.023
For 4 hr following drug treatment (B)	0.74 ± 0.057	0.10 ± 0.082	1.08 ± 0.093	1.05 ± 0.075
B/A	2.64 ± 0.26	2.01 ± 0.031	4.14 ± 0.32	3.85 ± 0.25

was found to produce more than 50% increase in bile flow (Table 2).

Paracetamol is a well-known antipyretic and analgesic drug, which produces hepatic necrosis in high doses (Crippin, 1993; Boutaud *et al.*, 2002). Most of the hepatotoxic chemicals including paracetamol and alcohol damage liver mainly by inducing, directly or indirectly, lipid peroxidation (Zimmerman and Maddrey, 1995). Since, the plant drug treatment reduced the levels of lipid peroxides in liver, the antioxidant property of this could, possibly, be one of the mechanisms of its action.

The active principles involved in the hepatoprotective mechanisms of this herbal drug is not known. So further experimental evaluation and clinical trial of this drug are needed.

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