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Hepatoprotective activity of *Artocarpus heterophyllus* leaf extracts against paracetamol-induced liver damage in rats

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ABSTRACT

In the present study, the defatted successive ethyl acetate and methanol extracts from *Artocarpus heterophyllus* leaf were evaluated for their protective effects on paracetamol-induced liver damage in Wistar albino rats. Serum biochemical parameters viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), total protein, bilirubin, cholesterol, triglycerides were evaluated. All biochemical findings indicated that both the test extracts exerted significant hepatoprotective efficacy against paracetamol-induced hepatic damage in rats. The methanol extract was found to be more effective than the ethyl acetate extract. Therefore, from the present study it can be concluded that *A. heterophyllus* leaf had remarkable hepatoprotective activity in rats.

Introduction

India is endowed with a rich wealth of medicinal plants. These plants have made a good contribution to the development of ancient Indian Materia Medica. India is one of the 12 mega diversity centers of the world and the richest country in plant wealth as well as in medicinal plants heritage. Human beings have been utilizing plants for their basic preventive and creative health care since time immemorial. A recent estimate suggests that over 9,000 plants have been known to medicinal applications in various cultures and countries, and this is without having conducted compressive research amongst several indigenous and other communities. These are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used. The major merits of traditional or herbal medicine seem to be their perceived efficacy, low incidences of serious adverse effects and comparatively low cost.

Artocarpus heterophyllus Lam. (Moraceae), commonly known as Jackfruit in English, *Kathal* in Bengali is a large evergreen tree native to South-East Asia. The jackfruit tree is a widely cultivated and popular food item in tropical regions of India, Bangladesh, Nepal, Sri Lanka, Cambodia, Vietnam, Thailand, Malaysia, Indonesia, and the Philippines. Jackfruit is also found across Africa (e.g., in Cameroon, Uganda, Tanzania, Madagascar, and Mauritius), as well as throughout Brazil and in Caribbean nations such as Jamaica. Jackfruit is the national fruit of Bangladesh. Its leaves are widely used as cattle feed. The present study was aimed to investigate the possible hepatoprotective effects of defatted successive solvent extracts from *A. heterophyllus* leaf against paracetamol-induced liver damage in Wistar albino rats.

Materials and methods

Plant material

The mature leaves of *Artocarpus heterophyllus* Lam. (Moraceae) were collected during October-November 2013 from Dumdum, Kolkata, West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen (CNH/12/2014/Tech.II/069) was maintained in our research laboratory for future reference. The leaves were shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an airtight container for use in the study.

Drugs and chemicals

Paracetamol and silymarin from SISCO Research Laboratory, Mumbai, India; potassium dichromate and glacial acetic acid from Ranbaxy, Mumbai. All the other reagents used were of analytical reagent grade obtained commercially.

Preparation of extracts

The powdered plant material was extracted successively with benzene (for de-fatting), ethyl acetate and methanol for 72 h in the cone shaped percolator. The solvents were distilled off in reduced pressure and resulting semisolid mass was vacuum dried to yield the dry benzene, ethyl acetate and methanol extracts. Preliminary phytochemical studies revealed the presence of triterpenoids and steroids in the benzene extract; triterpenoids, steroids, saponins, glycosides and carbohydrates in ethyl acetate extract; saponins, glycosides, tannins and phenolic compounds in methanol extract [1].

Experimental animals

Adult male Wistar albino rats weighing 170-200 g were used for the present investigation. They were housed in a clean polypropylene cage and maintained under standard laboratory conditions (temperature 25 ± 2ºC with dark/light cycle 12/12 h). They were fed on standard pelleted diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week prior to the commencement of experiment. All experimental procedures described were reviewed and approved by the Institutional Animal Ethics Committee.

Treatment protocol

The rats were divided into five groups (n = 6). A single dose of 650 mg/kg paracetamol in 2 % methyl cellulose was administered orally to each animal in group II, III, IV and V.

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Table 1. Effect of the extracts on body weight, liver weight and kidney weight of normal and paracetamol-treated rats

Group	Dose	Initial body wt (g)	Final body wt (g)	Final liver wt (g)	Final kidney wt (g)
I (Normal saline)	5 ml/kg	168.76±7.8	174.54±5.2	6.55±2.9	1.46±1.3
II (PCM)	650 mg/kg	171.68±7.2	164.54±4.5*	3.25±2.3*	0.94±1.1*
IV (PCM + Ethyl acetate extract)	200 mg/kg	163.18±5.3	156.78±1.8**	5.28±3.4**	1.27±1.8**
V (PCM + Methanol extract)	200 mg/kg	175.15±3.6	165.67±4.3**	6.11±3.3**	1.25±1.8**
VI (PCM + Silymarin)	25 mg/kg	177.53±4.5	168.76±2.3**	6.26±3.6**	1.29±1.6**

Values are expressed as mean ± SEM ($n = 6$); *$p < 0.001$ compared with normal control and ** $p < 0.001$ compared with Paracetamol control group. PCM: Paracetamol.

Table 2. Effect of the extracts on serum biochemical parameters of normal and paracetamol-treated rats

Group	Dose	SGOT(IU/L)	SGPT(IU/L)	SALP(IU/L)	Total Bilirubin (mg/dL)	Total Protein (mg/dL)	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)
I(Normal saline)	5 ml/kg	19.15±1.05	21.73±1.66	77.17±2.71	1.05±0.10	7.96±0.18	121.57±2.09	97.97±6.76
II (PCM)	650 mg/kg	36.13±1.31*	38.85±1.14*	134.81±3.88*	2.78±0.25*	4.07±0.27*	162.21±1.88*	171.51±7.67*
IV (PCM + Ethyl acetate extract)	200 mg/kg	21.25±1.48**	25.33±0.27**	87.78±4.31**	1.19±0.15**	6.81±0.29**	125.15±3.33**	118.95±6.63**
V (PCM + Methanol extract)	200 mg/kg	19.18±0.97**	22.71±0.82**	81.57±1.33**	1.18±0.27**	7.93±0.17**	124.94±1.85**	108.43±7.62**
VI (PCM + Silymarin)	25 mg/kg	19.38±0.68**	21.91±0.72**	79.33±1.43**	1.17±0.19**	7.93±0.13**	123.31±1.65**	105.15±7.33**

Values are expressed as mean ± SEM ($n = 6$); *$p < 0.001$ compared with normal control and ** $p < 0.001$ compared with Paracetamol control group. PCM: Paracetamol.

Group I served as normal (vehicle) control and group II served as paracetamol control and both received normal saline (5 ml/kg b.w., p.o.) daily for 14 days. After administration of paracetamol suspension, the ethyl acatate and methanol extracts were administered orally (p.o.) at the dose of 200 mg/kg body weight (b.w.) to groups III and IV respectively, daily for 14 days. Group V received reference drug silymarin (25 mg/kg b.w; p.o.) daily for 14 days. After 24 h of last dose, blood was collected from overnight fasted rats of each group by cardiac puncture for estimation of serum biochemical parameters [2].

Body weight, liver and kidney weights

The body weight of rats of each group were measured just before and 14 days after treatment. Liver and kidney weights of all rats were measured after post treatment sacrifice.

Serum biochemical parameters

The collected blood was used for the estimation of serum biochemical parameters viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), total bilirubin, total cholesterol and triglycerides contents were estimated by using commercially available reagent kits (Span Diagnostic Ltd., Surat, India). Serum total protein was estimated according to the reported method [3].

Statistical analysis

All results were expressed as the mean ± standard error of mean (SEM). The results were analyzed for statistical significance by one-way ANOVA followed by Dunnett's *post hoc* test of significance. $P < 0.001$ was considered as statistically significant.

Results

Body weight, liver and kidney weights

The body weight, liver and kidney weights of rats from paracetamol control group (after 14 days) were significantly ($p < 0.001$) decreased when compared with normal control group. Both the extracts at 200 mg/kg b.w. significantly ($p < 0.001$) maintained the body weight, liver and kidney weights towards normal as compared to paracetamol control (Table 1).

Serum biochemical parameters

Serum biochemical parameters like SGOT, SGPT, SALP, bilirubin, total cholesterol and triglycerides in the paracetamol control group were significantly ($p < 0.001$) elevated as compared to the normal saline group. Treatment with the test extracts at the dose of 200 mg/kg significantly ($p < 0.001$) reduced their levels towards the normal values. The total protein content was found to be significantly decreased in the paracetamol control group as compared with the normal saline group ($p < 0.001$). Administration of both the extracts in paracetamol-intoxicated rats significantly ($p < 0.001$) increased the total protein content as compared with the paracetamol control (Table 2).

Discussion

Paracetamol is a widely used as an antipyretic and analgesic drug which is safe in therapeutic doses but can cause fatal hepatic damage in human and animals at higher toxic doses [4]. Bio-activation of paracetamol by hepatic cytochrome P-450 enzymes leads to formation of a highly reactive and toxic metabolite N-acetyl-*p*-benzoquinone imine (NAPQI). NAPQI is normally detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid which is excreted in urine. Toxic overdose of paracetamol depletes hepatic reduced glutathione (GSH) content so that free NAPQI binds covalently to cellular macromolecules causing acute hepatocellular necrosis. The NAPQI then causes acylation or oxidation of cytosolic and membrane proteins and generation of reactive oxygen species (ROS). This leads to further oxidation of protein thiols, lipid peroxidation and DNA fragmentation [5].

It has been well established that elevated levels of SGOT, SGPT and SALP are indicative of cellular leakage and loss of functional integrity of the hepatic cell membranes implying hepatocellular damage. Serum total protein and bilirubin levels on the other hand are related to the function of the hepatic cells revealing the functional status of the hepatic cells [2]. Elevated serum cholesterol and triglyceride levels in paracetamol challenged rats indicated impaired fat metabolism due to hepatic

damage. Both the extracts decreased the elevated serum enzyme activities, bilirubin and lipid contents with elevation of total protein content in the paracetamol treated rats which are comparable to the normal control group. It appears that the extracts preserved the structural integrity of the hepatocellular membrane which is evident from the significant reduction in paracetamol-induced rise in serum marker enzymes in rats. The methanol extract was found to be more active. The extracts also ameliorated the harmful effect paracetamol on body weight loss, liver and kidney weights of rats.

The methanol extract was found to me more potent than the ethyl acetate extract. Preliminary phytochemical studies revealed the presence of tannins and phenolic compounds only in methanol extract. Polyphenols are well known natural products having several important biological activities [6]. Higher activity in methanol extract may be due to presence of tannins and phenolic compounds which are found absent in the ethyl acetate extract.

From the present investigation, it can be concluded that both the defatted ethyl acetate and methanol extracts from *Artocarpus heterophyllus* leaf offered potential hepatoprotection against paracetamol-induced hepatic damage, normalizing altered serum biochemical parameters in Wistar albino rats. The methanol extract was found to be more active than ethyl acetate extract. Further studies are necessary to confirm the bioactive principles responsible for the observed effects.

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