



Hepatoprotective activity of *Trapa natans* fruit peel 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 *Trapa natans* fruit peel 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 *T. natans* fruit peel had remarkable hepatoprotective activity in rats.

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Introduction

Trapa natans L. (Lythraceae), commonly known as Water Chestnut in English, *Paniphāl* in Bengali is an annual aquatic floating herb occurring in ponds and lakes throughout the Indian subcontinent [1]. It is commercially cultivated across different parts of India for its consumable seasonal fruits. Traditionally the plant has been used in India for several important medicinal purposes. It has been used as nutritive, appetizer, astringent, diuretic, aphrodisiac, cooling, anti-diarrhoeal and tonic; it is also useful in lumbago, sore throat, bilious affections, bronchitis, fatigues and inflammation. Its fruits are also used in making liniments for the cure of rheumatism, sores and sunburn. Its stem is used in the form of juice in eye disorders [1-3]. The dried kernels of its fruits are recommended for use in bleeding disorders, threatened abortion, dysuria, polyuria and oedema [4]. Previous researchers have reported analgesic and psychopharmacological activities of its roots [5, 6], antibacterial, antifungal and anti-diabetic activity of its fruit peel [7-9]. Despite several important traditional medicinal usages the reports on the experimental pharmacological studies on this plant are comparatively scanty. Present study was therefore aimed to investigate the possible hepatoprotective effects of defatted successive solvent extracts from *T. natans* fruit peel against paracetamol-induced liver damage in Wistar albino rats.

Materials and methods

Plant material

The mature fruits of *Trapa natans* L. (Lythraceae) were collected during October-November 2012 from Kalyani, Nadia, 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/7/2013/Tech.II/965) was maintained in our research laboratory for future reference. The peels from the fruits were removed by hand and the peels 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 petroleum ether (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 petroleum ether, ethyl acetate and methanol extracts (yields 0.19, 1.10 and 8.01% w/w respectively). Preliminary phytochemical studies revealed the presence of triterpenoids and steroids in petroleum ether extract; triterpenoids, steroids, saponins, glycosides and carbohydrates in ethyl acetate extract; triterpenoids, steroids, alkaloids, saponins, glycosides, tannins and phenolic compounds in methanol extract [10].

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 $25 \pm 2^\circ\text{C}$ 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 experiment. All 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. 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 acetate and methanol extracts were administered orally (p.o.) at the dose of 100 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 [11].

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 [12].

Statistical analysis

All results were expressed as the mean \pm 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 100 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 100 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 [13]. Bioactivation of paracetamol by hepatic cytochrome P-450 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 [14].

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 [11]. 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 be 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 [15]. Higher activity in methanol extract may be due to presence of tannins and phenolic compounds which are absent in the ethyl acetate extract.

From the present investigation, it can be concluded that both the defatted ethyl acetate and methanol extracts from *Trapa natans* fruit peel 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.

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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*
III (PCM + Ethyl acetate extract)	100 mg/kg	162.15±5.6	155.83±1.8**	5.88±3.8**	1.29±1.5**
IV (PCM + Methanol extract)	100 mg/kg	176.14±3.7	166.66±3.3**	6.31±3.2**	1.27±1.7**
V (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*
III (PCM + Ethyl acetate extract)	200 mg/kg	20.27±1.58**	24.32±0.29**	88.58±4.21**	1.18±0.16**	6.83±0.27**	127.18±3.53**	119.95±6.93**
IV (PCM + Methanol extract)	200 mg/kg	19.88±0.98**	23.81±0.72**	80.67±1.53**	1.19±0.29**	7.96±0.15**	125.94±1.95**	109.45±7.63**
V (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.

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