TOXICOLOGICAL STUDIES OF THE HYDROALCOHOLIC EXTRACT OF *PTEROSPERMUM ACERIFOLIUM* FLOWERS

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Summary

The objective of the present study was to evaluate acute and subchronic toxicity studies of *Pterospermum acerifolium (P. acerifolium)* flowers in albino mice and rats. Phytochemical investigation was also carried out to know type of phytoconstituents present in the flowers of *P. acerifolium*. Hydroalcoholic flower extracts at a dose of 1000, 2000 and 4000 mg/kg was administered orally to the test groups while distilled water was given to the control. The parameters measured include food and fluid intake, body weight, absolute and relative weight of various organs [Lung, Liver, Pancreas, Kidney, Heart and Spleen], haematological parameters [total white blood cell (WBC) and packed cell volume (PCV)], and tests for liver function: Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase and total bilirubin. The lethal dose (LD_{50}) was found to be greater than 4000 mg/kg (*p.o.*) in both mice and rats. Rats treated with the extract had no progressive increase in body weight. No significant increase in fluid and food intake was observed in rats treated with hydroalcoholic extract of *P. acerifolium* flowers. There were no significant changes in both the absolute and relative organ weights between the control and the test groups. The liver enzymes and haematological parameters were statistically equal in all the groups. *P. acerifolium* hydroalcoholic flower extract is found to be non-toxic in albino rats.

Key words *P. acerifolium*, subchronic toxicity, liver function, packed cell volume, absolute weight, relative weight.

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Introduction

The family Sterculiace consists of about 68 genera and 150 species, mostly herbs or shrubs. *P. acerifolium* (karnikara tree) is an angiosperm indigenous to Southeast Asia, from India to Burma. Pterospermum acerifolium is an angiosperm that is traditionally included in the Sterculiaceae family; however, it is grouped in the expanded Malvaceae family as well. An evergreen tree, up to 24.0 m. height and *c.* 2.5 m. in girth with a clean bole up to 12m., found in the sub-Himalayan tract and outer valleys from Yamuna eastwards to West Bengal, and in Assam and Manipur, up to an altitude of *c.* 1,200 m., extending southwards into Ramanagar hills of Bihar and in western Ghats of Konkan and North Kanara; it is also common in the Andamans. Bark greyish brown; leaves variable in size and shape, 25-35 cm.× 15-20 cm., entire or variously lobed, oblong, cordate or sometimes peltate; flowers large, 12-35 cm. in diam., axillary, solitary or in pairs, white fragrant; capsules oblong, 5-angled, dark brown, woody; seeds winged, brown.

Pterospermum acerifolium Linn. has a wide application in traditional system of Indian medicine for example, in ayurvedic anticancer treatment flowers are mixed with sugars and applied locally [1]. Flowers and bark, charred and mixed with kamala applied for the treatment of smallpox. Flowers made into paste with rice water used as application for hemicranias [2]. Stem bark of the plant was found to have antimicrobial activity [3]. Isolation of boscialin glucosides from leaves of *P. acerifolium* have been reported [4]. Hepatoprotective effect of ethanolic extract of leaves of *P. acerifolium* was also reported [5]. Chronic effects of *P. acerifolium* on glycemic and lipidemic status of type 2 model diabetic rats was found beneficial [6]. The barks are reported to be used as anti-ulcer [7] anti-inflammatory [8] analgesic [8] and anti-oxidant activity [9]. Flavonoids like keampferol, keampferide, luteolin, steroids and triterpenoids like sitosterol, taraxerol, friedelin, sugars, and fatty acids are present in the plant [10, 11]. The present study was undertaken to investigate the acute and subchronic toxicity studies of the hydroalcoholic flower extract of *P. acerifolium* in rats.

Materials and Methods

P. acerifolium was obtained from Salipur, Orissa, India and identified at Botanical Survey of India, Howrah. Their voucher specimen was deposited in the herbarium. All other chemicals and reagents used were of analytical grade. The experiment protocols were approved by the Institutional Animal Ethics Committee prior to the conduct of the animal experiments (1053/ac/07/CPCSEA).

Preparation of Extracts

Air-dried, powdered plant material was soxhlet extracted for 75 h in a mixture of ethanol and water (50:50). The hydroalcoholic extract was concentrated and dried using a rotary flash evaporator to give solid residue. The yield was 9.32 % w/w.

Phytochemical Screening

Phytochemical screening gave positive test for phytosterols, alkaloids, flavonoids and carbohydrates [10].

Experimental Animals

Inbreed Albino Wistar rats (12weeks old) of either sex weighing between 150-180 g and Swiss albino mice (both sex) weighing 20 to 25 g was used for the study. They were housed in polyacrylic cages and fed with standard rodent pellet diet and given water *ad libitum*. The animals were maintained in cages under standard laboratory condition (12 hr light and 12 hr darkness) at $24^0 \pm 2^0$C.

Acute toxicity studies

The acute toxicity of the extract was determined by the method of Lorke using the oral route on both Wister rats and Swiss albino mice [12]. The animals were divided into 9 groups of six animals each. The control group received 2 ml/kg distilled water orally. The other groups received the extracts at dose levels of 100, 200, 400, 800, 1000, 2000, 3000 and 4000mg/kg in distilled water as suspension through oral route. After administration of dose the animals were observed continuously for first 4 h for behavioral changes and for mortality, if any, at the end of 24, 48 and 72 h respectively.

Subchronic Toxicity Study

A total of twenty four mature Wister rats were used in this study. They were divided into four groups of six rats each. Three of the groups received 1000, 2000 and 4000 mg/ kg body weight of the hydroalcoholic extract (*p.o.*), respectively, while the control group received distilled water only. Food and water intake were monitored daily. After 30 days of exposure, blood was collected from the animals, by cardiac puncture, for haematological and biochemical analysis. Thereafter, the animals were sacrificed and the following organs isolated and weighed: kidney, liver, heart, lungs, spleen and pancreas. Relative weight of the respective organs was calculated from each organ's wet weight and the animal's body weight.

Effect of Extract on Liver Function

About 5 ml of whole blood collected into a plain tube was centrifuged at 3500 rpm for 5 min. using table centrifuge (Remi, India) and the serum separated and analyzed for the liver enzymes. Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT) were assayed using the methods of Reitman and Frankel, alkaline phosphatase (ALP) was analysed by the method of King and Armstrong, while total bilirubin level was determined by the method of Malloy and Evelyn. All assay methods employed were as reported by Varley *et al* [13].

Haematological Assay

EDTA-anticoagulated tubes were used to collect whole blood for these investigations. Packed cell volume (PCV) was determined by the microhaematocrit method, while total WBC was determined by visual method [14].

Statistical Analysis

Data were analyzed using Student's *t*-test.

Results and Discussion

The acute and subchronic toxicity studies of the hydroalcoholic flower extract of *P. acerifolium* were carried out. Phytochemical tests indicate that the hydroalcoholic extract contains phytosterols, alkaloids, flavonoids and carbohydrates. The LD_{50} (*p.o.*) of flower extract of *P. acerifolium* was found to be greater than 4000 mg/kg indicative of the safety of these extracts in both mice and rats. Table 1 shows the effect of various doses of *P. acerifolium* hydroalcoholic extract on weekly food and fluid intake. The extract did not increase the food intake of the animal compared to control at $p < 0.05$ throughout the three weeks of exposure. There is no significant change in water intake among the test groups compared to the control throughout the exposure period.

Table 1. Effect of *P. acerifolium* hydroalcoholic extract on weekly food (g) and fluid (ml) intake in rats (n = 6)

	Week		
Treatment	**1**	**2**	**3**
Control	309.5 ± 8.22 (814.5 ± 5.33)	308.83 ± 3.74 (819.83 ± 2.73)	304.17 ± 3.77 (816.67 ± 2.25)
1000 mg/kg	306.17 ± 7.37 (816 ± 6.17)	304.5 ± 5.25 (815 ± 7.03)	303 ± 4.219 (809.83 ± 7.53)
2000 mg/kg	297.83 (812.17 ± 5.18)	297.17 ± 4.25 (810.67 ± 3.2)	299.5 ± 2.57 (804.5 ± 6.09)
4000 mg/kg	308 ± 5.34 (817.5 ± 5.38)	302.83 ± 3.5 (813 ± 5.89)	305.67 ± 2.46 (811.5 ± 4.9)

Values in parenthesis indicate volume of fluid ingested. Each value is mean of ± S.E.M (n = 6)

Rats treated with the various doses of the extract (1000, 2000 and 4000 mg/kg) had no significant change in body weight. No statistically significant differences existed in the absolute and relative weights of all the isolated organs between the treated and the control groups (Table 2). Kluwe documented that the absolute organ weight has been observed to be a relative sensitive indicator of nephrotoxicity for known nephrotoxicants. An increase in kidney weight (either absolute or relative) indicates nephrotoxicity [15]. The hydroalcoholic flower extract of *P. acerifolium* did not induce any toxic effect on the kidneys and the other organs going by this

indicator, since the absolute and relative weights of the organs were not significantly different from control values.

Table 2. Effect of various doses of *P. acerifolium* flower hydroalcoholic extract on the relative (%) and absolute (g) weights of organs (n = 6)

	Treatment			
Organ	**Control**	**1000 mg/kg**	**2000 mg/kg**	**4000 mg/kg**
Lung	0.86 ± 0.04	0.82 ± 0.05	0.91 ± 0.03	0.86 ± 0.06
	(1.72 ± 0.04)	(1.65 ± 0.05)	(1.57 ± 0.08)	(1.64 ± 0.06)
Liver	3.75 ± 0.32	3.88 ± 0.26	4.14 ± 0.31	3.95 ± 0.17
	(7.04 ± 0.28)	(6.54 ± 0.42)	(7.19 ± 0.42)	(7.43 ± 0.45)
Pancreas	0.19 ± 0.03	0.21 ± 0.04	0.25 ± 0.03	0.23 ± 0.04
	(0.24 ± 0.04)	(0.19 ± 0.02)	(0.25 ± 0.05)	(0.25 ± 0.05)
Kidney	0.65 ± 0.05	0.62 ± 0.06	0.58 ± 0.05	0.64 ± 0.04
	(1.52 ± 0.17)	(1.39 ± 0.09)	(1.33 ± 0.11)	(1.37 ± 0.11)
Heart	0.28 ± 0.04	0.23 ± 0.03	0.33 ± 0.04	0.36 ± 0.04
	(0.78 ± 0.03)	(0.82 ± 0.06)	(0.86 ± 0.06)	(0.83 ± 0.08)
Spleen	0.45 ± 0.08	0.54 ± 0.05	0.58 ± 0.04	0.56 ± 0.05
	(0.95 ± 0.1)	(1.04 ± 0.11)	(1.12 ± 0.14)	(0.98 ± 0.11)

Values in parenthesis indicate absolute weight. Values are expressed as mean ± S.D.

The effect of hydroalcoholic extract of *P.acerifolium* on liver enzymes and bilirubin is shown on Table 3. The levels of bilirubin and the liver enzymes: SGOT, SGPT and alkaline phosphatase were not significantly affected by the extract. Certain drugs and other substances are

known to affect and influence circulating bilirubin levels and elevation in bilirubin levels suggests increase in haemolysis [16]. The hydroalcoholic flower extract of *P.acerifolium* however, did not alter significantly, the bilirubin levels of the exposed rats, as well as other liver enzymes compared to the control.

Table 3. Dose effect relationship of hydroalcoholic flower extract of *P. acerifolium* on the liver function of rats (n = 6)

	Analyte			
Treatment	**SGOT (iu/l)**	**SGPT (iu/l)**	**ALP (iu/l) Total Bilirubin (mg/dl)**	**Total Bilirubin (mg/dl)**
Control	57.35 ± 3.18	26.91 ± 2.36	181.93 ± 8.88	0.08 ±0.005
1000 mg/kg	56.43 ± 2.55	25.55 ± 2.7	75.41 ± 6.02	0.07 ± 0.005
2000 mg/kg	55.52 ± 1.65	23.93 ± 1.85	176.73 ± 7.62	0.08 ± 0.05
4000 mg/kg	58.47 ± 1.84	26.09 ± 3.06	183.44 ± 8.89	0.09 ± 0.005

Values are expressed as mean ± S.D. SGOT = serum glutamic oxaloacetic transaminase,

SGPT = serum glutamic pyruvic transaminase, ALP = alkaline phosphatase

According to Onyenyili and co-workers, anemia following administration of an agent can be as a result of lysis of blood cells and/or inhibition of blood cell synthesis by the active constituents of the extract, and decrease in hematological parameters in experimental animals has been associated with anemia [17]. There was no significant change in haematological parameters in the extract-treated animals compared to the control (Table 4), which indicates that there is no lysis of blood cells and/or inhibition in blood cells synthesis by the active constituents of *P. acerifolium e*xtract. The above results suggest the nontoxicity of hydroalcoholic extracts of *P. acerifolium* in rats.

Table 4. Dose effect relationship of *P. acerifolium* hydroalcoholic extract (flower) on the hematological parameters of rats ($n = 6$)

Treatment	PCV (%)	WBC (cells/mm^3)
Control	60.29 ± 2.35	6266.52 ± 79.99
1000mg/kg	60.95 ± 2.81	6172.8 ± 90.99
2000mg/kg	60.06 ± 2.55	6082.9 ± 138.85
4000mg/kg	60.66 ± 1.94	6194.57 ± 74.06

Values are expressed as mean ± S.D.

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