

Subchronic Toxicity Studies of the Hydroalcoholic Extract of *Rungia pectinata* Leaves

S. R. Swain^{*}, B. N. Sinha¹ and P. N. Murthy²

^{*}Institute of Pharmacy & Technology, Salipur, Cuttack – 754 202, India

¹Birla Institute of Technology, Mesra, Ranchi – 835 215, India

²Royal College of Pharmacy and Health Sciences, Berhampur – 760 001, India

Summary

The acute and subchronic toxicity studies of *Rungia pectinata* (*R. pectinata*) leaves in albino mice and rats were investigated. Phytochemical analysis was also carried out. 1000, 2000 and 4000 mg/kg of the hydroalcoholic leaf extract was administered orally to the test groups while distilled water was given to the control group. The parameters measured include food and fluid intake, body weight, absolute and relative weight of various organs, 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 (LD₅₀) 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. A significant increase in fluid intake was seen without any significant change in their food intake and body weight in rats treated with hydroalcoholic extract of *R. pectinata* leaves. 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. *R. pectinata* hydroalcoholic leaf extract is non toxic in albino rats.

Key words *Rungia pectinata*, subchronic toxicity, liver function, packed cell volume, absolute weight, relative weight.

* For correspondence

Institute of Pharmacy & Technology, Salipur, Cuttack – 754 202, India

E-mail: sudhansu74us@yahoo.com

Introduction

Rungia pectinata (Acanthaceae) is a much branched, procumbent or erect herb found as a weed throughout the warmer parts of India^{1,2}. The juice of the leaves is considered cooling and aperient, and is given to children suffering from smallpox. Bruised leaves are applied to contusions to relieve pain and reduce swelling. In Bihar, the roots are used as a febrifuge by the tribal population^{3,4}. There are also reports that it is of use as diuretic and vermifuge⁵. Although *R. pectinata* is widely used in folklore medicine, there is no information about the toxicity even as the local population administers it. The present study was undertaken to investigate the acute and subchronic toxicity studies of the hydroalcoholic leaf extract of *R. pectinata* in rats.

Materials and Methods

The leave of *Rungia pectinata* was obtained from nearby areas of Salipur, Orissa 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 7.64 % w/w.

Phytochemical Screening:

Phytochemical screening gave positive test for phytosterols, terpenes, tannins, flavonoids and carbohydrates⁶.

Experimental Animals:

Wistar rats (140-190 g) of both sexes and Swiss albino mice weighing 20 to 25 g were used for the study. The animals were housed in cages under standard laboratory condition (12 hr light and 12 hr darkness). They had free access to standard diet and water.

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 mice⁷. 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.*⁸

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.⁹

Statistical Analyses

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

Results and Discussion

The acute and subchronic toxicity studies of the hydroalcoholic leaf extract of *R. pectinata* were carried out. Phytochemical tests indicate that the hydroalcoholic extract contains phytosterols, terpenes, tannins, flavonoids and carbohydrates. The LD₅₀ (*p.o.*) of hydroalcoholic leaf extract of *R. pectinata* 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 *R. pectinata* 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. The results showed significant increases ($p < 0.05$) in water intake among the test groups compared to the control throughout the exposure period may be due to diuretic effects of *R. pectinata*⁵. The effect of the extract on fluid intake was dose dependent (Table 1).

Table 1. Effect of *R. pectinata* Hydroalcoholic Extract on Weekly Food (g) and Fluid (ml) Intake in Rats (n = 6)

Treatment	Week		
	1	2	3
Control	299.16 ± 14.4 (822.33 ± 35.01)	307.16 ± 10.26 (816.83 ± 32.76)	311.83 ± 9.94 (832.50 ± 31.40)
1000 mg/kg	304.16 ± 11.03 (877.16 ± 46.70)	308.83 ± 8.68 (844.5 ± 31.61*)	311.33 ± 8.77 (1070.66 ± 49.83*)
2000 mg/kg	300.66 ± 11.21 (1257.16 ± 26.82*)	308.50 ± 8.80 (1034.83 ± 30.32*)	314.16 ± 8.11 (1170 ± 36.99*)
4000 mg/kg	302.50 ± 10.31 (1525.66 ± 46.90*)	307.50 ± 8.59 (1344.16 ± 35.14*)	311.5 ± 8.59 (1175.66 ± 42.69*)

Values in parenthesis indicate volume of fluid ingested.

Each value is mean of ± S.E.M (n = 6); *Denotes significant difference when compared to control values at p <0.05 (Student's t-test).

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 rats (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 nephrotoxicity¹⁰. The hydroalcoholic leaf extract of *R. pectinata* 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 Aqueous Leaf Extract of *R. pectinata* on the Relative (%) and Absolute (g) Weights of Organs (n = 6)

Organ	Treatment			
	Control	1000 mg/kg	2000 mg/kg	4000 mg/kg
Lung	0.85 ± 0.07 (1.72 ± 0.13)	0.76 ± 0.05 (1.62 ± 0.18)	0.80 ± 0.06 (1.59 ± 0.13)	0.91 ± 0.07 (1.60 ± 0.18)
Liver	3.71 ± 0.41 (7.19 ± 0.43)	4.08 ± 0.27 (7.65 ± 0.82)	4.28 ± 0.45 (7.92 ± 0.44)	4.45 ± 0.44 (7.28 ± 0.34)
Pancreas	0.27 ± 0.04 (0.52 ± 0.04)	0.32 ± 0.04 (0.60 ± 0.07)	0.25 ± 0.03 (0.46 ± 0.04)	0.27 ± 0.03 (0.49 ± 0.05)
Kidney	0.78 ± 0.04 (1.46 ± 0.03)	0.77 ± 0.04 (1.52 ± 0.07)	0.75 ± 0.05 (1.50 ± 0.07)	0.83 ± 0.06 (1.48 ± 0.02)
Heart	0.37 ± 0.03 (0.74 ± 0.04)	0.37 ± 0.03 (0.79 ± 0.06)	0.38 ± 0.04 (0.78 ± 0.06)	0.37 ± 0.04 (0.70 ± 0.07)
Spleen	0.42 ± 0.05 (0.82 ± 0.05)	0.48 ± 0.06 (0.91 ± 0.06)	0.46 ± 0.06 (0.83 ± 0.04)	0.47 ± 0.07 (0.86 ± 0.06)

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

The effect of hydroalcoholic extract of *R. pectinata* 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 haemolysis¹¹. The hydroalcoholic leaf extract of *R. pectinata* 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 Aqueous Leaf Extract of *R. pectinata* on the Liver Function of Rats ($n = 6$)

Treatment	Analyte			
	SGOT (iu/l)	SGPT (iu/l)	ALP (iu/l)	Total Bilirubin (mg/dl)
Control	55.53 ± 2.68	22.56 ± 1.09	180.26 ± 8.31	0.08 ± 0.008
1000 mg/kg	56.66 ± 2.12	22.63 ± 1.05	179.24 ± 7.23	0.07 ± 0.01
2000 mg/kg	54.83 ± 3.01	23.04 ± 0.96	180.38 ± 5.88	0.89 ± 0.006
4000 mg/kg	58.35 ± 1.76	23.06 ± 0.99	179.76 ± 5.07	0.084 ± 0.008

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 anemia¹². 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 *R. pectinata* extract. The above results suggest the nontoxicity of hydroalcoholic extracts of *R. pectinata* in rats.

Table 4. Dose Effect Relationship of Aqueous Leaf Extract of *R. pectinata* on the Haematological Parameters of Rats ($n = 6$)

Treatment	PCV (%)	WBC (cells/mm ³)
Control	59.15 ± 3.02	6053.66 ± 145.66
1000 mg/kg	59.96 ± 3.09	6121.5 ± 164.08
2000 mg/kg	59.26 ± 3.01	6208.33 ± 170.61
4000 mg/kg	59.54 ± 2.70	6252.83 ± 216.83

Values are expressed as mean ± S.D.

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