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Research Article

Acute Toxicity Evaluation of *Rauvolfia tetraphylla* Leaf Extract in Rat by Up and Down Method

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ABSTRACT
The objective of this study was to evaluate the acute toxicity of the methanolic leaf extract of *Rauvolfia tetraphylla* via the oral route in female Sprague Dawley rats. In this study, dosing was initiated, based on OECD guideline 425, using the default dose level of 175 mg/kg and approximately 3.2 multiplier dose progression. Subsequent animals were dosed at 550 and 2000 mg/kg. Rats were observed for 14 days. Rauvolfia tetraphylla leaf extract produces signs of toxicity at 2000 mg/kg but no lethality at any of the dose (175, 550 & 2000 mg/kg) tested. There is no effect on the body weight. Necropsy examination did not reveal any treatment related changes. The median lethal dose (LD50) of this plant extract could be considered higher than 2000 mg/kg and classified as toxicity "Category 5" according to Globally Harmonized system of Classification.

Keywords: *Rauvolfia tetraphylla*; Acute Toxicity; Leaf Extract

INTRODUCTION

Rauvolfia tetraphylla L. (Apocyanaceae), a small, much branched woody shrub, native to West-Indies, is found in plains of India especially in Tamil Nadu, Andhra Pradesh, Karnataka, Kerala, Bihar, Orissa, West Bengal, and Madhya Pradesh[1].The leaf extract of the plant is used for treatment of cholera, eye-disease and fever. It is also used as antihypertensive, as well as in intestinal disorders, diarrhea and dysentery (Anonymous, 1969).

Plant extracts have gained importance in the area of pest control, being considered environmentally safe, less hazardous to non-target biota, simple to use, inexpensive and can be applied effectively by using techniques more suitable for developing countries[2, 3] . Previously several plant extracts have been found effective as potentially acute or chronic insecticides, insect growth inhibitors or antifeedants against a variety of insect species[4, 5].

R.tetraphylla leaf extract has exhibited (pilot in-house studies) larvicidal and oviposition deterrent activity against the vector of lymphatic fileriasis, *Culex quinquefasciatus*. Owing the leaf extract as a potent biopesticide, the need to generate its safety data to assess risk to human population is imperative. In an attempt to generate safety data, methanolic leaf extract of *R. tetraphylla* was tested in Ames test and was found to be non-mutagenic with and without metabolic activation[6].

The objective of this study was to evaluate the acute toxicity of the methanolic leaf extract of *R. tetraphylla* via the oral route in female Sprague Dawley rats.

MATERIALS AND METHODS

Plant Collection and Extraction: *R. tetraphylla* plant leaves were collected from University of Pune campus. Identification & confirmation was performed by Botanical Survey of India, Western Regional Office, Pune.

Leaves were washed with water; shed dried and powdered using a mechanical grinder. Powdered leaves were extracted with methanol solvent in Soxhlet apparatus. Extract was then concentrated under reduced pressure 22-26 mm of Hg at 45 ^{0}C using rotary evaporator, and the residue was stored at 4 to 8 ^{0}C till further used for the experiment[7].

Animals and Husbandry: Female Lrpp: Sprague Dawley rats, approximately 8–10 weeks of age were used for acute toxicity study. These animals were raised in the research animal facility of Lupin Research Park, Pune (India). Animals were fed standard laboratory animal diet (Altromin 1324P, Spezialfutter GmbH & Co. KG, Germany) and given drinking water filtered through water filtration system *ad libitum*. The animal rooms were maintained at 19-26 ^{0}C temperature and 30-70% relative humidity with a 12 h light–dark cycle. The study was conducted in accordance with OECD guideline No.425, "Acute Oral Toxicity-Up and Down Procedure" [8]. Authorization for the use of

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Table 1: Individual animal dosing and mortality details

Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Females	Outcome
175	17.5	10	1	Survived
550	55	10	1	Survived
2000	200	10	1	Survived
2000	200	10	1	Survived
2000	200	10	1	Survived

Table 2: Individual animal clinical observations

Observation	An. No.	Dose (mg/kg)	Hours Post-Dose on day-1							Day Post- Dose											
			0.5	1	2	3	4	5	6	2	3	4	5	6	7	8	9	10	11	12	13	14
	1	175	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
No	2	550	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Abnormality	3	2000	-	-	-	-	-	-	-	-	-	P	P	P	P	P	P	P	P	P	P	P
Detected	4	2000	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	5	2000	-	-	-	-	-	-	-	P	P	P	P	P	P	P	P	P	P	P	P	P
Lethargy	3	2000	P	P	P	P	P	P	P	P	-	-	-	-	-	-	-	-	-	-	-	-
	5	2000	P	P	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hypoactivity	3	2000	P	P	P	P	P	P	P	P	-	-	-	-	-	-	-	-	-	-	-	-
Abdominal	3	2000	-	P	P	P	P	P	P	P	P	-	-	-	-	-	-	-	-	-	-	-
breathing	5	2000	-	P	P	P	P	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prostration	3	2000	-	P	P	P	P	P	P	-	-	-	-	-	-	-	-	-	-	-	-	-
Cold Extremities	3	2000	-	P	P	P	P	P	P	-	-	-	-	-	-	-	-	-	-	-	-	-
Intermittent shivering	3	2000	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-

P: Present; -: Not seen at that interval

laboratory animals was obtained from the Institutional Animal Ethics Committee.

Acute Toxicity Study: On receipt, the animals were acclimated to the laboratory conditions for at least five days prior to first day of dosing. As there were no predefined dose groups, no randomization procedure was used and animals were randomly selected for dosing. However, at the commencement of dosing, weight of animal was within ± 20% of the mean initial weight of previously dosed animals.

Test item formulation was prepared daily. Weighed quantity of plant extract was triturated with small quantity of 0.5% carboxymethylcellulose with pestle and mortar. The final volume of formulation was made by adding required quantity of 0.5% carboxymethylcellulose. The formulation was stirred for 15 minutes using stir bar and magnetic stirrer. The prepared dose formulation was administered through oral gavage at the dose volume of 10 mL/kg.

The dose levels were selected based on OECD Guideline 425. Dosing was initiated using the default dose level of 175 mg/kg and approximately 3.2 multiplier dose progression.

First animal selected for dosing was fasted overnight. Animal was weighed prior to dosing and test item was administered orally at the dose of 175 mg/kg as a single dose using a syringe attached to gavage cannula. Animal was returned to feed 3-4h after dosing. Subsequent animal (second animal) was dosed at 48h interval (or as necessary) with higher or lower doses depending on survival of the previous animal.

Animals were examined twice daily for mortality. On day of dosing, animals were observed frequently (30 min, 1h, 2h, 3h, 4h, 5h and 6h post dose) for clinical signs and at least once daily afterwards for a period of 14 days. Body weights were recorded once prior to dosing, day 7, day 14 and just prior to necropsy. Animals survived till the end of observation period were euthanized by carbon dioxide and were subjected to necropsy examination.

RESULTS

Based on OECD guideline 425, first animal was dosed at 175 mg/kg. Based on its survival, second animal was dosed at 550 mg/kg dose level which also survived. Hence, third animal was dosed at 2000 mg/kg. Survival of an animal at 2000 mg/kg leads to dosing of two more animals at 2000 mg/kg. Two out of three animals treated at 2000 mg/kg exhibited signs of toxicity, however no lethality was observed. The present study revealed that the said extracts did not produce any mortality at any of the dose level tested i.e. 175, 550 and 2000 mg/kg, throughout the study period of 14 days (Table-1). No mortality in all the three animals tested at 2000 mg/kg dose level resulted in meeting likelihood ratio stopping rule as per OECD guideline No. 425 and no further doses were tested.

Table 3: Incidence summary of clinical observations

Observations	Dose					
	175 mg/kg (n=1)	550 mg/kg (n=1)	2000 mg/kg (n=3)			
	Day 1-14	Day 1-14	Day 1	Day 2	Day 3	Day 4-14
Lethargy	0/1	0/1	2/3	1/3	0/3	0/3
Hypoactivity	0/1	0/1	1/3	1/3	0/3	0/3
Abdominal Respiration	0/1	0/1	2/3	1/3	1/3	0/3
Prostration	0/1	0/1	1/3	0/3	0/3	0/3
Cold Extremities	0/1	0/1	1/3	0/3	0/3	0/3
Intermittent Shivering	0/1	0/1	0/3	1/3	0/3	0/3

Table 4: Individual animal body weights (g) and body weight change (%)

Animal No.	Dose Level (mg/kg)	Body Weight (g)			Body Weight Change (%)	
		Day 1	Day 7	Day 14	Day 1-7	Day 7-14
1	175	175.6	200.1	220.1	13.9	9.9
2	550	208.1	225.3	237.5	8.2	5.4
3	2000	197.7	220	232.7	11.2	5.7
4	2000	190.1	214.1	219.2	12.6	2.3
5	2000	220.5	242.9	247.3	10.1	1.8

Animals treated at 2000 mg/kg revealed clinical observations (Table-2 & 3) such as lethargy (2/3), hypoactivity (1/3), abdominal breathing (2/3), prostration (1/3), cold extremities (1/3) and intermittent shivering (1/3). However, no signs of toxicity were observed in the animals treated at 175 and 550 mg/kg.

No treatment related reduction in body weights was observed. Instead normal weight gain was noted in all treated animals during study period (Table-4). Gross pathological examination did not show any lesions or abnormal changes.

DISCUSSION

Plant extracts are good source of biologically active substances but knowing the side effects before its application is essential to know the safety of the extract. In the current study the safety of methanolic extract of *R. tetraphylla* leaves was evaluated by performing acute oral toxicity tests in rat. In acute oral toxicity study the median lethal dose (LD50) of *R. tetraphylla* leaf extract was found to be >2000-5000 mg/kg b.wt and could be classified as "Category 5" according to Globally Harmonized Classification System[9].

Similarly, ethyl acetate extract, aqueous extract and methanol fractionate of aqueous extract of waterhyacinth used in the treatment of wound healing was found to be non-lethal in acute toxicity study in Swiss Albino mice[10]. Acute toxicity assessment of aqueous extract of Phragmanthera capitata (AEPC) by Up and Down method revealed its safety up to a concentration of 3000 mg/kg in mice. Ethanolic extract of Zingiber zerumbet rhizomes tested by oral route in Wistar rat did not cause lethality up to 15g/kg dose[11].

Animals were observed frequently on day one of dosing and once daily thereafter, for a total of 14 days. Clinical signs viz. lethargy, hypoactivity, abdominal respiration, prostration, cold body extremities and intermittent shivering, observed in two of three animals treated at 2000 mg/kg were considered due to toxicity of plant extract. However, clinical signs were observed for a short duration and recovered during observation period of 14 days.

Body weight is an important factor to monitor the health of an animal. Loss in body weight is frequently the first indicator of the onset of an adverse effect. A dose, which causes 10% or more reduction in the body weight, is considered to be a toxic dose. In the present study, treated animals did not show any significant decrease in body weights. Instead, all the animals showed increase in body weight gain during study period.

To study the systemic effect of the plant extract, animals were subjected to necropsy examination. Animals were observed externally for body surfaces and orifices and internally for different organ/tissues and body cavities. In the present study, gross pathological examination did not show any lesions or abnormal changes.

CONCLUSION

Under the test conditions of this study, the acute oral LD50 of *R. tetraphylla* leaf extract was determined to be greater than 2000 mg/kg in the female rat.

This study is a preliminary investigation in the toxicity evaluation of methanolic leaf extract of *R. tetraphylla* offering an outset to continue the research regarding the effect of this extract in repeat dose toxicity studies.

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