**International Journal of Research in Pharmacy and Pharmaceutical Sciences**
**ISSN: 2455-698X; Impact Factor: RJIF 5.22**
**www.pharmacyjournal.in**
**Volume 2; Issue 1; January 2017; Page No. 01-03**

# Acute toxicity studies of petroleum ether, methanol and aqueous extracts of *Leptadenia reticulata*

**[1] Dr. C Girish, [*2] Y Narsimha Reddy**

[1] Division of pharmacy, Department of Biochemistry, Sri Venkateshwara University, Tirupati, Andhra Pradesh, India
[2] Prof., Department of pharmacology, University College of pharmaceutical sciences, Kakatiya University, Warangal, Andhra Pradesh, India

**Abstract**
The purpose of the study was to test the acute oral toxicity of the different extracts of the plant *Leptadenia reticulata*. Acute toxicity of petroleum ether, methanol and aqueous extracts of *Leptadenia reticulata* was evaluated in Swiss mice. The acute toxicity studies were carried out based on OECD guidelines 423. The animals were orally administered with a single dose of 100, 250, 500, 750, 1000, 2000mg/kg body weight of each extract. Signs of toxicity and mortality were noted after 1, 4 and 24h of administration of the extract for 14 days. The highest dose administered (2000mg/kg body weight) did not produce mortality or changes in general behaviour of the test animals. These results indicate the safety of the oral administration of petroleum ether, methanol and aqueous extracts of *Leptadenia reticulata*.

**Keywords:** acute toxicity studies, anaphylaxis, *leptadenia reticulata*, methanolic extract

## Introduction
Ayurveda, an ancient system of Indian medicine, has recommended a number of drugs for the treatment of various diseases, like anaphylaxis, bronchial asthma and allergic disorders [1]. Allergy is one of the common diseases that affect mankind with diverse manifestations and is responsible for significant morbidity and mortality [2]. Anaphylaxis is triggered by different substances like foods (nuts, fish, wheat etc), medications (Penicillin), venom from insects, latex from natural rubber, allergy shots and extreme temperature also act as stimuli for anaphylaxis [3]. The available treatment options for upper and lower respiratory tract allergic diseases have major limitations owing to low efficacy, associated adverse events and compliance issues [4].
*Leptadenia reticulata* has been used in the Ayurvedic system of Indian medicine for the treatment of bronchial asthma, eczema, insect bites etc [5]. Plants or drugs must be ensured to be safe before they could be used as medicines. By conducting toxicity tests in appropriate animal models, acute toxicity studies, we ensuring the safety of drugs.
So, in the present study, the petroleum ether, methanol and aqueous extracts of *Leptadenia reticulata* were analysed for their acute toxicity profile with reference to behavioural aspects, in Swiss Albino mice. The limit test dose of 2000mg/kg body weight was used following OECD guidelines [6, 7].

## Materials and methods
### Plant material collection
The plant material of *Leptadenia reticulata (Retz.)* was collected from Tirumala hills after taxonomic verification and were identified and authenticated in Department of Botany, S.V. University, Tirupathi. The plant materials were coarsely powdered using a rotary grinder and stored in airtight plastic containers. This powder was used for preparation of extracts.

### Preparation of extracts
The freshly collected plant material was washed, dried at room temperature for 15-20 days under shade and was treated with a rotary grinder for size reduction. The fine powder was collected and was used for preparation of extracts. Dried plant material (100 g) was extracted with Soxhlet apparatus using 400 mL petroleum ether for about 48 h. After defatting, the marc was dried in hot air oven at 50°C, packed in soxhlet apparatus and further extracted with 400 mL of 95% Methanol until it does not show the presence of any residue on evaporation. The aqueous extract was prepared by cold maceration with 3% methanol-water for 7 days with occasional shaking. The solvents were removed from the extracts under reduced pressure by using rotary vacuum evaporator.

### Experimental animals
Acute oral toxicity test was performed as per Organization for Economic Co-operation and Development (OECD) guidelines 423 [8]. Experiments were performed using healthy young adult Swiss albino mice weighing 25-35 g [9].

### Housing and Diet
The animals were housed in polypropylene cages (55 x 32.7 x 19 cm) in a standard condition of temperature (22 ± $2^0$C) relative humidity (60 ± 5%). Lighting was controlled to supply 12 h of light and 12 h of dark for each 24-h period. The animals were fed with standard laboratory animal food pellets with water ad libitum.

### Grouping of animals
The animals were randomly divided into three batches. Each batch contains seven groups and each group containing four mice. Group 1 (Control Group), Group 2: Receives 100 mg/kg, Group 3: Receives 250 mg/kg, Group 4: Receives

500 mg/kg, Group 5: Receives 750 mg/kg, Group 6: Receives 1000 mg/kg, Group 7: Receives 2000 mg/kg of a specific extract of *leptadenia reticulata*.

**Mode of administration**

The test substance was administered orally in a single dose using specially designed mice oral needle. Animals were fasted 3 h prior to dosing (only food was withheld for 3 h but not water).

**Administration Dose**

Following the period of fasting, animals were weighed and test substance was administered orally at a dose of 100, 250, 500, 750, 1000 and 2000 mg/kg. After the administration of test substance, food for the mice was withheld for 2 h.

**Test substance administration volume**

The administration volume was 1ml/kg body weight of the animal. Based on the body weight of the animal on the day of treatment, the quantity of the test substance was calculated.

**Observation period**

Animals were observed individually after atleast once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. All the mice were observed at least twice daily with the purpose of recording any symptoms of ill-health or behavioural changes and for mortality if any.

**Acute toxicity studies**

Direct observation parameters include Alertness. Writhing, Torch response, Corneal refiux, Tremors, Righting reflux, Gripping strength, Pinna refiux, Skin colour, Urination, Pupils diameter, Subcutaneous swellings, and Abdominal distensions. The time of death, if any, was recorded. After administration of the test substance, food was withheld for further 1-2 h. The number of survivors was noted after 24 h and then they were observed for further 14 days and Percentage of Mortality was calculated.

**Statistical Analysis**

Data are presented as a mean ± SEM (Standard Error of the Mean). Comparisons were made between the treated groups by the use of single way ANalysis Of VAriance (ANOVA). $P< 0.05$ was considered as the level statistical significance.

**Results**

The present study conducted as per the OECD guidelines 423 revealed that the said extracts did not produce any mortality throughout the study period of 14 days even when the limit dose was maintained at 2000mg/kg body weight. The oral LD50 was indeterminable being in excess of 2000mg/kg body weight. So, testing the extracts at a higher dose may not be necessary and the extracts were practically non-toxic.

Tables 1, 2, 3 indicates the parameters observed before and after the administration of the Petroleum ether, Methanolic and Aqueous extracts of *Leptadenia reticulata* respectively. The parameters observed were normal even at the highest dosage of 2000mg/kg body weight of the test animal. This clearly indicated that the above extracts of *Leptadenia reticulata* do not produce oral toxicity. The medium lethal dose (LD50) of the extracts is higher than 2000 mg/kg body weight and hence, in a single dose administration, the plant extracts had no adverse effect. Table 4 indicates the percentage of Mortality after 14 days of treatment with Petroleum ether, Methanolic and Aqueous extracts of *Leptadenia reticulata*.

**Table 1:** Effect of petroleum ether extract of *Leptadenia reticulata* on acute oral toxicity test in mice

| S. No | Response | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 |
|---|---|---|---|---|---|---|---|---|
| 1 | Alertness | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 2 | Writhing | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| 3 | Torch response | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 4 | Corneal reflux | Present | Present | Present | Present | Present | Present | Present |
| 5 | Tremors | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| 6 | Righting reflux | Present | Present | Present | Present | Present | Present | Present |
| 7 | Gripping strength | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 8 | Pinna reflux | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 9 | Skin colour | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 10 | Urination | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 11 | Pupils diameter | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 12 | Subcutaneous swellings | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| 13 | Abdominal distensions | Absent | Absent | Absent | Absent | Absent | Absent | Absent |

**Table 2:** Effect of methanolic extract of *Leptadenia reticulata* on acute oral toxicity test in mice

| S. No | Response | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 |
|---|---|---|---|---|---|---|---|---|
| 1 | Alertness | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 2 | Writhing | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| 3 | Torch response | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 4 | Corneal reflux | Present | Present | Present | Present | Present | Present | Present |
| 5 | Tremors | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| 6 | Righting reflux | Present | Present | Present | Present | Present | Present | Present |
| 7 | Gripping strength | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 8 | Pinna reflux | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 9 | Skin colour | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 10 | Urination | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 11 | Pupils diameter | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 12 | Subcutaneous swellings | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| 13 | Abdominal distensions | Absent | Absent | Absent | Absent | Absent | Absent | Absent |

**Table 3:** Effect of Aqueous extract of *Leptadenia reticulata* on acute oral toxicity test in mice.

| S. No | Response | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 |
|---|---|---|---|---|---|---|---|---|
| 1 | Alertness | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 2 | Writhing | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| 3 | Torch response | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 4 | Corneal reflux | Present | Present | Present | Present | Present | Present | Present |
| 5 | Tremors | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| 6 | Righting reflux | Present | Present | Present | Present | Present | Present | Present |
| 7 | Gripping strength | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 8 | Pinna reflux | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 9 | Skin colour | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 10 | Urination | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 11 | Pupils diameter | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 12 | Subcutaneous swellings | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| 13 | Abdominal distensions | Absent | Absent | Absent | Absent | Absent | Absent | Absent |

**Table 4:** % of mortality of mice after 14 days of treatment with different extract of *leptadenia reticulata.*

| Groups | No. of mice | Dose administered | Petroleum ether extract | | Methanol extract | | Aqueous extract | |
|---|---|---|---|---|---|---|---|---|
| | | | No. of mice died | % of mice died | No. of mice died | % of mice died | No. of mice died | % of mice died |
| 1 | 6 | Control | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 6 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 6 | 250 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 6 | 500 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 6 | 750 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 6 | 1000 | 1 | 16 | 0 | 0 | 0 | 0 |
| 7 | 6 | 2000 | 2 | 33 | 1 | 16 | 1 | 16 |

## Discussion and conclusion

The non-toxic nature of petroleum ether, methanol and aqueous extracts of *Leptadenia reticulata* is evident by the absence of mortality of the test animals at oral treatment of 2000mg/ kg body weight. The normal behaviour of the test animals during a period of 14 days suggests the non-toxic nature of the foresaid extracts. Hence *Leptadenia reticulata* could be safe up to the dose of 2000 mg/kg body weight of the animal. Further studies are warranted for determining chronic toxic symptoms.

## References


1. Charaka Samhita, Sri Gulabkunverba Ayurvedic Society, Jamnagar, Ayurvedic Mudranalaya, Jamnagar, 1949; 4:1953-2032.
2. Ring J, Kramer U, Shafer T, Beherendt H. Why are allergies increasing? Curr Opinions Immunol. 2001; 13:701-8.
3. Kim Kim EK, Li GZ, Chai OH, Song CH et al. Inhibitory effect of *Arctium lappa Linne* on compound 48/80-induced mast cell activation and vascular permeability. *Korean J. Phys. Anthropol.* 2004; 17:55-66.
4. Salib RJ, Drake-Lee A, Howarth PH. Allergic rhinitis: past, present and the future. Clin Otolaryngol. 2003; 28:291-303.
5. Anjaria JV, Varia MR, Janakiraman K, Gulati OD. Studies on *Leptadenia reticulata*: Lactogenic effects on rats. Ind. J. Exp. Biol. 1975; 13:448-449.
6. Lipnick RL, Cotruvo JA, Hill RN. Comparison of the Up-and-Down, Conventional LD50 and Fixed Dose Acute Toxicity Procedures. Fd Chem Toxicol. 1995; 33:223-231.
7. Kulkarni SK, Handbook of Experimental Pharmacology. 2nd Ed. Vallabh Prakashan Publication, New Delhi, India. 1993, 168.
8. OECD Guidelines for the Testing of Chemicals (No. 423) Acute Oral Toxicity-Acute Toxic Class Method Adopted on 17 December, 2011.
9. Halim SZ, Abdullah NR, Afzan A, Abdul Rashid BA, Jantan I, Ismail Z. Study of acute toxicity of *Carica papaya* leaf extract in Sprague Dawley rats. J Medicinal Plants Res. 2011; 5:1867-1872.