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# Acute Oral Toxicity Study of *Lepidagathis keralensis Madhus. & N.P.Singh*

**Research Article**

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## Abstract

Systematic documentation of local health tradition related knowledge is important for validating and preserving medicinal heritage of any place. *Lepidagathis keralensis* Madhus. & NP Singh. is a species belonging to Acanthaceae family It is a perennial, prostrate, woody herb tenaciously attached to hard laterite soil. It is used by traditional practitioners of North Malabar for various ailments. Safety is of primary importance as far as any drug is concerned. No scientific data is available on the safety profile of this drug. Acute Oral toxicity study is the foremost step among toxicity studies. Hence this study was taken up. Aim & objective: To assess the Acute Oral Toxicity of water extract of *Lepidagathis keralensis* Madhus. & NP Singh. Materials and methods: Aqueous extract of the drug was orally administered in Wistar albino rats at different dose levels and various parameters for signs and symptoms of gross behavioural and physical changes starting from 30 minutes to 48 hours at various intervals were observed. Results and observations: The drug did not produce any signs of toxicity and mortality upto the dose of 2000 mg/kg in rats. Hence this drug which is extensively in use among traditional practitioners can be further studied and brought forward to main stream.

**Keywords:** *Toxicity study, Rats, Lepidagathis keralensis, Aqueous extract, OECD 425 guideline.*

## Introduction

Herbs are used extensively in prevention and treatment of various diseases. It has attracted public attention widely over past 20 years. Investigation of acute toxicity is important as a public health concern because exposure to ill studied plant extracts may cause undesirable effects on consumers(1).

Acute toxicity is defined as adverse effects occurring within a short time of administration of a substance or multiple doses given within 24 hours. Acute oral toxicity tests are meant for obtaining knowledge on biological activity of a drug and to understand its mechanism of action. The data obtained can be utilised for hazard identification and risk management(2). Several synthetic drugs act on single molecular targets and provide symptomatic relief, but multi target responses of plant drugs are proven to be beneficial in chronic conditions(3). Plants are frequently used in medicine that more than 30% of entire plant species is utilised for medicinal purpose at one time or other(4). There is a general blind belief on natural products to be safe for consumption. Hence it is particularly essential to prove safety with proper toxicological studies(5) . Local health traditions form the integral component of medical heritage of any country. Systematic documentation of such knowledge is crucial to preserve the medical heritage of the country besides scientific validation of its attributes and principles(6). *Lepidagathis* Willd. Comprises about 100 species, distributed in the tropical and warm regions of the world. In India, the genus is represented by 23 species and 8 varieties, among them 15 species are endemic to the Eastern Ghats and Western Ghats of Southern India (7). Spines of this plant are used to prepare gruel for treating malnutrition, malabsorption and digestive disorders(8). It is also used as medicine by folk practitioners of Vannan community in malabar(9). Since the drug is used orally, it is essential to assess its toxicity, hence this study was conducted.

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## Materials and methods

Acute Oral Toxicity study was carried out using OECD 425 guideline(10).

1. Animals

Wistar albino Rats sourced from Animal house attached to SDM Research centre, SDM Ayurveda College Udyavara. A total of 5 healthy either sex of body weight 160-200g. Rats were selected according to AOT software. All the selected animals were kept under acclimatisation for 7 days before dosing. The animals were marked with saturated Picric acid solution

in water for proper Identification. The marking within the cages was as follows.

**Table 1: Animal numbering and identification marking**

| Animal number | Marking |
|---|---|
| 1 | Head |
| 2 | Neck |
| 3 | Middle of the back |
| 4 | Base of the tail |
| 5 | No mark |

The group number, animal number and sex of the animal were identified with the help of cage cards, as presented in following table.

**Table 2: Dose calculation based on body weight**

| Sl. no | Identification of animals | Desired dose (according to AOT) | Body weight (grams ) | Calculated dose (ml) |
|---|---|---|---|---|
| 1 | Head | 175mg/kg | 167 | 1.67 |
| 2 | Neck | 550mg/kg | 155 | 1.55 |
| 3 | Back | 2000mg/kg | 160 | 1.60 |
| 4 | Base of the tail | 2000mg/kg | 158 | 1.58 |
| 5 | No mark | 2000mg/kg | 172 | 1.72 |

Husbandry condition: Rats were housed in each cage of poly propylene with stainless steel top grill. The dry husk was used as bedding material and was changed every morning. The animals were exposed to 12 hours light and 12 hours dark cycle with relative humidity 50 to 70 % and the ambient temperature was 22 ± 03®c., rat pellet feed supplied by Sai Durga feed Bangalore, was provided throughout the study period except on previous night of dosing i.e. (overnight) fasting before dosing. The drinking water was given *ad libitum* in polypropylene bottles with stainless steel sipper tube.

2. Preparation of Test formulation for administration:

*Lepidagathis keralensis* Madhus.& NP Singh. whole plant. Water was used as vehicle. The test drug powder was made in to fine suspension in vehicle with suitable concentration. All the animals were dosed constant dose volume (1 ml/ 100g body weight) 175mg/kg, 550mg/kg, 2000mg/kg. Schedule: Single dose per animal. The test formulation was administered through oral route at different dose levels to respective animals through oral feeding needle. Dose fixation was done according to the AOT Software. Dose : 175mg/ kg, 550mg/kg, 2000mg/kg test substance.

## Observations

### Examination of Physical and Behavioural changes

The animal was observed continuously for 4 hours after the dosing. The careful cage side observation was done without disturbing the animal attention and at the end of the every hour the animal was individually exposed to open arena for recording the behavioural changes like increased or decreased motor activity , convulsions, Straub's reaction, muscle spasm, catatonia, spasticity, Ophisthotonos, hyperesthesia, muscle relaxation, anaesthesia, arching and rolling, lacrimation, salivation, diarrhoea, writhing, mode of respiration, changes in skin colour etc. exitus, CNS depression – hypo activity, passivity, relaxation, ataxia, narcosis, etc.

### Mortality

All the animals were observed at ½, 1, 2, 3, 4, 24 h, 48h after dosing and there after daily once for mortality during the entire period of the study (i.e.14 days).

**Table 3: Signs and symptoms during Gross Behavioural Study in group 1 to 5**

Group 1:175mg/kg ( Dose: 1.67 ml) & Group 2:550 mg/kg ( Dose: 1.55 ml),Group 3:2000 mg/kg (Dose: 1.60 ml), Group 4:2000mg/kg (Dose:1.58 ml), Group 5 2000 mg/kg (Dose:1.72ml),
Animal: Wistar albino rats:
Route: Oral
Drug: *Lepidagathis keralensis* Madhus. &N.P.Singh

| Signs and Symptoms | Basal | 30min | 1h | 2h | 3h | 4h | 24h | 48h |
|---|---|---|---|---|---|---|---|---|
| General impression | N | N | N | N | N | G-1N, G-2 Active | N | N |
| Increased motor activity | - | - | - | G1-,G2-,G3-,G4+ | G1-,G2-,G3+,G4+ | G1-,G2-, G3+,G4+ | - | - |
| Convulsion: Tonic | - | - | - | - | - | - | - | - |
| Clonic | - | - | - | - | - | - | - | - |
| Straub's reaction | - | - | G1-,G2+,G3+, G4-,G5+ | G1-,G2-,G3-,G4+, G5+ | G1-,G2-,G3+,G4+, G5+ | G1-,G2-,G3-, G4+, G5+ | - | - |
| Muscle spasm | - | - | - | - | - | - | - | - |
| Catatonia | - | - | - | - | - | - | - | - |
| Opisthotonos | - | - | - | - | - | - | - | - |
| Hyperaesthesia | - | - | - | - | - | - | - | - |
| Decreased motor activity | - | - | - | - | - | - | - | - |
| Muscle relaxation | - | - | - | - | - | - | - | - |
| Anaesthesia | - | - | - | - | - | - | - | - |
| Arching and rolling | - | - | - | - | - | - | - | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Lacrimation | | - | - | - | - | - | - | - | - |
| Diarrhoea | | - | - | - | - | - | - | - | - |
| Writhing | | - | - | - | - | - | - | - | - |
| Salivation | Viscid | - | - | - | - | - | - | - | - |
| | Watery | - | - | - | - | - | - | - | - |
| Respiration | Stimulation | - | - | - | - | - | - | - | - |
| | Depression | - | - | - | - | - | - | - | - |
| | Failure | - | - | - | - | - | - | - | - |
| Skin colour | Blanching | - | - | - | - | - | - | - | - |
| | Cyanosis | - | - | - | G1-,G2-,G3-,G4+, G5+ | G1- G2+,G3+,G4+, G5+ | G1- G2+, G3+, G4+, G5+ | - | - |
| | Vaso-dilatation | - | - | - | - | - | - | - | - |
| Grip strength | | N | N | N | N | N | N | N | N |
| Visual placing response | | N | N | N | N | N | N | N | N |
| Tail pinch response | | N | N | N | G1N, G2N, G3N, G4N, G5+ | G1N, G2N, G3+, G4+, G5+ | G1-, G2+, G3+,G4+, G5+ | N | N |
| Auditory response | | N | N | N | N | N | N | N | N |
| mucus membrane | | N | N | N | N | N | N | N | N |
| Piloerection | | - | - | - | - | - | - | - | - |

Abbreviations- N – Normal, G- Group

**Table 4: Gross behaviour - CNS and ANS -in Rat:1, Rat-2, Rat-3, Rat-4, Rat-5 one each from groups 1 to 5**

Drug: : *Lepidagathis keralensis* Madhus. &N.P.Singh
Group1, (Rat-1): 175mg/kg, Group 2, (Rat-2): 550mg/kg, Group 3, (Rat-3):2000 mg/kg, Group 4, (Rat-4):2000 mg/kg, Group 5, (Rat-5):2000 mg/kg
Route: Oral
Animal: Wistar albino rats
Study: OECD- 425

| **Time interval** | | **B** | **1h** | **2h** | **3h** | **4h** | **24h** | **48h** |
|---|---|---|---|---|---|---|---|---|
| **Rat No. 1** | | | | | | | | |
| **Time after drug administration** | | | | | | | | |
| **Exit us** | | - | - | - | - | - | - | - |
| **CNS Depression** | **Hypo activity** | - | - | - | - | - | R1-, R2-, R3-,R4-,R5+ | - |
| | **Passivity** | - | - | - | - | - | - | - |
| | **Relaxation** | - | - | - | - | - | - | - |
| | **Ataxia** | - | - | - | - | - | - | - |
| | **Narcosis** | - | - | - | - | - | - | - |
| **ANS** | **Ptosis** | - | - | - | - | - | - | - |
| | **Exophthalmoses** | - | - | - | - | - | - | - |
| **CNS Stimulation** | **Hyperactivity** | - | - | R1-, R2-, R3-, R4+ | R1-, R2-, R3+, R4+, G5+ | R1-, R2-, R3-, R4+, G5+ | - | - |
| | **Irritability** | - | - | R1-, R2-, R3-, R4+ | R1-, R2, R3+, R4+ | R1-, R2+, R3+, R4+, G5+ | - | - |
| | **Stereotypy** | - | - | - | - | - | - | - |
| | **Tremors** | - | - | - | - | - | - | - |
| | **Convulsion** | - | - | - | - | - | - | - |
| | **Straub tail** | - | R1-, R2+, R3+, R4-, R5+ | R1-, R2-, R3-, R4+, R5+ | R1-, R2-, R3+, R4+, R5+ | R1-, R2-, R3-, R4+, R5+ | - | - |
| **Analgesia** | | - | - | - | - | - | - | - |
| **Others** | | N | N | N | N | N | N | N |

Abbreviations: G- Group, N – Normal, R-Rat

# Results

**Physical and behavioural examination**

There were no physical and behavioural changes-(except mild increase in motor activity, cyanosis, Straub tail, irritability and rearing seen in 4 rats in the group 550mg/kg and 2000mg/kg) in all the treated animals on day one at ½, 1,2,3,4 hours intervals after dosing and there after once daily for 14 consecutive days. Thus the data obtained from the study on single dose administration of *Lepidagathis keralensis* Madhus.& NP Singh. oral administration up to 14 days of observation period does not result in any physical and behavioural changes.

**Mortality**

All the animals belonging to the treated group survived throughout the 14 days observation period after dosing.

- AOT 425 stat pgm (Version: 1.0) Test Results
- Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program
- Test/Substance: *Lepidagathis keralensis* Madhus.& NP Singh.
- Test type: Main Test
- Limit dose (mg/kg): 2000
- Assumed LD50 (mg/kg): Default
- Assumed sigma (mg/kg): 0.5
- Recommended dose progression: 2000, 550, 175, 55, 17.5, 5.5, 1.75
- Since signs and symptoms of toxicity did not occur at 175, further lower doses were not examined. then 55, 17.5, 5.5, 1.75 dose levels are to be done.

**Data**

| Test Seq. | Animal ID | Dose Mg/kg | Short-term Result | Long-term Result |
|---|---|---|---|---|
| 1 | 1 | 175 | O | O |
| 2 | 2 | 550 | O | O |
| 3 | 3 | 2000 | O | O |
| 4 | 4 | 2000 | O | O |
| 5 | 5 | 2000 | O | O |

(X = Died, O = Survived)

Dose Recommendation: The main test is complete. Stopping criteria met: 3 at Limit Dose.

**Summary of Long-term results:**

| Dose | O | X | Total |
|---|---|---|---|
| 175 | 1 | 0 | 1 |
| 550 | 1 | 0 | 1 |
| 2000 | 3 | 0 | 3 |
| All Doses | 5 | 0 | 5 |

Statistical Estimate based on long term outcomes: The LD50 is greater than 2000 mg/kg.

# Discussion

Apart from routine findings of Acute oral toxicity study observations like Straubs reaction, mild Increase in motor activity, hyperactivity, Irritability and Straub tail shows mild Central Nervous System stimulant activity. Hence further studies are suggested on it. Even though mild irritability and cyanosis were observed at 550 mg /kg and 2000 mg/kg dose levels, the drug did not cause any mortality.

# Conclusion

*Lepidagathis keralensis* Madhus.& NP Singh. did not produce any mortality up to the dose of 2000mg/kg per oral which is equivalent to 22.4g total dose for a human being weighing 70 kg man. At the dose level studied the drug also did not produce any observable toxic effect except for mild increase in motor activity, cyanosis, Straub tail, irritability and rearing in animal receiving the dose550mg/kg and 2000mg/kg and thus it could be concluded that the test drug is without any toxic potential even at the dose of 2000mg/kg in animals equivalent to 22.4g for human being. This data can be useful for further clinical research on this drug.

# References

1. Subramanion L, Jothy,Zuraini Zakaria, Yeng Chan,Yee Ling Lau, Lachimanan Yoga Latha & Sreenivasan Sasidharan, Acute Oral Toxicity of Methanolic Seed Extract of Cassia fistula in Mice,Molecules,2011,16,5268-5282;doi: 10.3390/ molecules 16065268
2. .Walum E. Acute oral toxicity. Environ Health Perspect. 1998 Apr;106 Suppl 2(Suppl 2):497-503. doi: 10.1289/ehp.98106497. PMID: 9599698; PMCID: PMC1533392.
3. Patel AG, Nariya MB, De S. Acute toxicity and repeated dose 28-day oral toxicity study of metriviv syrup in female rats. Ayu. 2018 Apr-Jun;39(2):107-112. doi: 10.4103/ayu.AYU_18_18. PMID: 30783366; PMCID: PMC6369600.
4. www.nhp.gov.in/introduction- and- importance -of-medicinal -plants-and-herbs_mtl developed and hosted by the centre for health informatics(CHI), set up at National Institute of Health & Family welfare(NIHFW), by the ministry of Health and Family Welfare, Government of India, accessed on16/08/2021
5. JOUR Hsieh, Ming-Fa Pariyani, Raghunath Safinar Ismail, Intan Azam, Amalina Ahmad Abas, Faridah Shaari, Khozirah Sulaiman, Mohd RoslanPY 2015,DA - 2015/12/27,TI - Phytochemical Screening and Acute Oral Toxicity Study of Java Tea Leaf Extracts,SP - 742420,VL - 2015
6. Narayanan Srikanth, Thugutia Maheswar, Sobaran singh, Methodical documentation ofLocal Health Traditions and Folklore Claims: scope, Relevance and Suggested format, Journal of Drug Research in Ayurvedic Sciences, April- June 2017;2(2): 149.155
7. Raja. P, Dhatchanamoorthy N, Sooiraj S & Jansirani P, New distribution record of two endemic plant species, Euphorbia kadapensis sarojin. & R.R.V.Raju (Euphorbiaceae) and Lepidagathis keralensis Madhus.&N.P. Singh(Acanthaceae) for Karnataka, India,Journal of threatened Taxa, 26 October 2020/volume 12/No.14/Pages17045-17048,

DOI: 10.11609/jot.4978.12.4, www.threatened taxa,org

8. Leena Palakkal, N.H. Zeinul Hukuman, Jisha Mullappally, department of Post graduate studies and Research in Chemistry, sir Syed College, Taliparamba, kannur, kerala, India, Journal of Applied Pharmaceutical Science,Volume7(06), pp:182-189, June,2017 available online at http:// www.japsonline.com,DOI:10.7324/ JAPS.2017.70626
9. Envis centre, Ministry of Environment& Forest, Government of India, Sacred groves of North Kerala, printed date: Monday, 16/08/2021
10. OECD, Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure 2022 doi:https://doi.org/ 10.1787/9789264071049-enhttps://www.oecd-ilibrary.org/content/publication/9789264071049-en.

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