RESEARCH ARTICLE

# Acute Oral Toxicity Studies on Ethanol Extract of Aerial Parts of *Blumea laevis* (EABL) in Rats

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## Abstract

Acute toxicity of ethanol extract of aerial parts of *Blumea laevis* (EABL) plant material was studied *in-vivo* in six female Sprague-Dawley rats as per OECD guidelines 425. Results revealed that there was no significant difference in feed intake in both the treatment groups (*i.e.,* 1750 & 5000 mg/kg b.wt.) even though water intake was significantly higher ($p<0.01$) in animals receiving daily or 48 h interval per orally at 5000 mg/kg of EABL as compared to 1750 mg/kg body weight. There was an increase in body weight in animals receiving EABL @ 5000 mg/kg as compared to treatment at 1750 mg/kg body weight. Furthermore, the EABL did not produce toxic effects on signs, general behaviour, mortality and gross appearance of internal organs of rats during the 14-days observation period. Hence the $LD_{50}$ of EABL was found to be more than 5000 mg/kg body weight. In conclusion, this pilot study demonstrated that EABL did not cause acute oral toxicity in rats at given dose rate and dose 5000 mg/kg was found to be safer. However, additional studies in sub-acute and chronic toxicity evaluation are needed to further determine the long-term safety of this plant extract.

**Key words:** Acute toxicity, *Blumea laevis,* Ethanol extract, Sprague-Dawley rats.

*Ind J Vet Sci and Biotech* (2023): 10.48165/ijvsbt.19.3.12

## Introduction

Plants and animals are related to each other and evolved together. Plants are essential to the survival of animals as they provide food, shelter, and oxygen. Animals, in turn, help plants by pollinating them, dispersing their seeds, and preventing overgrowth of certain species. The relationship is always asymmetric with animals benefited and plants harmed. This made the plants to evolve with multiple defense mechanisms to combat both biotic and abiotic factors (Mazid *et al.,* 2011) with high range of secondary metabolites that accounts for the major contribution for specific taste, odor and color which attracts the herbivores. A large number of toxic plants may be ingested accidentally or willfully during grazing which leads to extreme toxicity. Recently, many veterinarians working in Malappuram district of Kerala have reported heavy mortality in goats, ascribing the cause as ingestion of *Blumea laevis* locally called "Kaattappa".

*Blumea* is an erect glabrous shrub in Asteraceae family. This plant is commonly found in tropical and sub-tropical zones of Asia, especially the Indian Subcontinent and Southeast Asia. A very few species are found in Australia and still few are in Africa (Fadrique *et al.,* 2018). *Blumea balsamifera* is one plant species of *Blumea* genus found to be popular as veterinary medicines (Shan *et al.,* 2020). Plants constitute numerous phytochemical constituents which contribute to the medicinal properties and toxic effects. *Blumea laevis* possess medicinal properties such as diuretic, carminative and anthelmintic. Since toxicity of B. laevis has not been intensively evaluated, and hence this study was undertaken to contribute to the scientific data on safety of the 95% ethanol extract of aerial parts of *B. laevis* (EABL) in experimental animal model study.

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**How to cite this article:** Kumar, Y.R., Nair, D.N., Anoopraj, R., Prasanna, K. S., Palekkodan, H., George, A.J., & Rani, S. (2023). Acute Oral Toxicity studies on Ethanol Extract of Aerial Parts of Blumea laevis (EABL) in Rats. Ind J Vet Sci and Biotech. 19(3), 53-57.

**Source of support:** Nil

**Conflict of interest**: None

**Submitted:** 05/12/2022 **Accepted:** 02/02/2023 **Published:** 10/05/2023

## Materials and Methods

This study was performed as per the Organization for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals No.425 (OECD, 2001) and World Health Organization (WHO) guideline (2000) (WHO, 2000). The study was approved by Institutional Animal Ethics Committee of Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Pookode. Kerala (India)

### Procurement and Preparation of Plant Material

Aerial parts of *B. laevis* plant in its flowering stage (Fig. 1) were collected in the month of December 2016 from Malappuram district of Kerala. The plant was identified and authenticated by plant taxonomist and plant specimen was assigned with accession no. 88480. Herbarium and passport data sheets of plant were prepared accordingly and voucher specimen was deposited in the Department of Botany, University of Calicut. The plant materials were cleaned, shade dried and ground to coarse powder using blender. Powdered samples were collected and stored in a closed container until required for extraction.

### Preparation of Ethanol Crude Extract

The powdered sample (100 g) was extracted by continuous hot extraction using the Soxhlet apparatus with 95% ethanol. The extract was then concentrated and reduced under pressure through Rotary vacuum evaporator (M/s. Buchi, Switzerland) at temperature ranging from 40-50°C. The concentrated extract was air dried at room temperature for about 24 h and placed in suitable containers and preserved in refrigerator for further studies.

### Phytochemical Screening of Aerial Parts

Ethanol extract of aerial parts of *Blumea laevis* was subjected to various phytochemical screening by various qualitative tests for detection of bioactive molecule, which included alkaloids, carbohydrates, fat tests, flavonoids, proteins, amino acids, phenolic compounds, tannins and steroids as per Harborne *et al*. (1978) and Raaman (2006).

### Experimental Animals

A total of six adult nulliparous and non-pregnant Sprague-Dawley female rats 6 to 8 weeks old, weighing between 130 and 200 g, obtained from the Small Animal Breeding Station of College of Veterinary and Animal Sciences, Mannuthy, Kerala were used for the study. The rats were kept in polypropylene cages in the animal house with an ambient temperature of 25°C, 12 h light and 12 h dark periodicity. The rats were fed with standard diet and water *ad libitum* and allowed to acclimatize for seven days before the procedure. They were randomly assigned to the cages and the individual animal was further marked with felt tip marker pen. Veterinary examination was done before allocation of animals to groups and after the completion of acclimatization period.

### Acute Toxicity Study

The experiment was performed as per OECD guidelines 425 for acute oral toxicity study. Since there was no information available on the toxicity of *B. laevis* the primary test was conducted to find out $LD_{50}$ of the extract. A software programme for conducting acute oral toxicity study named AOT *StatPgm* (Version: 1.0, 2001) was employed to select the sequential doses with the dose progression factor 0.5 on sigma log dose scale. 5000 mg/kg body weight per oral was selected as limit dose in order to avoid any likely miss of toxicity of extract above 2000 mg/kg.

Prior dosing each animal was fasted overnight and weighed prior to extract administration. The animal was orally given a single dose of the ethanol extract with a volume of 2 mL/100 g body weight. Since no estimate of the extract's lethality was available, the dosing was initiated at 175 mg/kg body weight. The animals were observed for appearance of signs of toxicity at every 30 min for 48 h after dosing. Since there was no mortality in any of the progression doses, *viz*., 550, 1750 mg/kg, the dosing was continued till 5000 mg/kg body weight at 48 h interval. Finally, three animals each were dosed at the dose level of 1750 mg/kg (T1) and 5000 mg/kg (T2) body weight at 48 h interval. The dosing was stopped

**Fig 1:** *Blumea laevis* plant in its flowering stage

after 14 days since there was no mortality in the upper limit dose (*i.e.*, 5000 mg/kg).

The visual observations included changes in the skin and fur, eyes and mucous membranes, and behavioural pattern. Attention was given for observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, coma and mortality. Body weights of animals were recorded on 7$^{th}$ and 14$^{th}$ day of the study. The number of survivors was noted after 24 h, and they were then maintained for a further 14 days. On the 15$^{th}$ day after administration, all surviving rats were weighed and sacrificed. The animals that died during the experiment were necropsied.

### Pathological Examination

All the animals were randomly sacrificed after 14 days of observation and systematic necropsy was conducted. Organs such as liver, kidneys, stomach, heart, brain, spleen, and different segments of intestine were observed for any gross pathology, and specimens were collected in 10% neutral buffered formalin (NBF) for histopathology. 5 µm thick sections were obtained from paraffin embedded tissues and were stained by Hematoxylin and Eosin (H & E) and examined for histopathological changes, if any (Bancroft and Gamble, 2008).

### Statistical Analysis

The data on different parameters obtained from distinct groups were subjected to one-way analysis of variance (ANOVA) and paired t-test, followed by Duncan's multiple range test for significance at $p<0.05$ using software SPSS version 16.0.

## Results and Discussion

The percentage extractive yield of aerial parts of *Blumea laevis* was found to be 8.051%. The preliminary phytochemical analysis from extract of *Blumea laevis* was first of its kind and showed the presence of secondary phytochemical constituents such as Saponins (Foam test), Terpenoids (Salkowskis test), Flavonoids (Alkaline reagent test), Phytosterols (Leibermann Burchards test) and Carbohydrates (Molisch test). Phytochemical analysis has aided in rapid and accurate methods of screening plants for determining the particular bioactive compounds

### Acute Oral Toxicity Study

In the acute oral toxicity study as per the OECD Test Guideline 425, the animals fed with EABL at the initial dose rate of 175 mg/kg body weight did not show any mortality or signs of toxicity during the initial 48 h interval and 14 days of observational period. On administration of subsequent doses of 550, 1750, and 5000 mg/kg body weight as per the instructions of AOT StatPgm (Version: 1.0, 2001), the similar findings were observed. No changes were observed in the skin and fur, eyes, mucous membranes, behavioural pattern and other wellness parameters even after administration of the highest dose level of 5000 mg/kg body weight.

### Feed and Water Intake

The effects of EABL on mean feed and water intake of the experimental animals during the 14 days of observation period at 1750 mg/kg (T1) and 5000 mg/kg (T2) dose levels are shown in Table 1. There was no significant difference in feed intake between treatment groups ($p>0.05$) even though water intake was significantly higher ($p<0.01$) in animals receiving 5000 mg/kg of EABL (T2) as compared to 1750 mg/kg body weight (T1).

### Body Weight

The animals administered with single dose of EABL at dose level of 1750 mg/kg and 5000 mg/kg body weight showed increase in the body weight during the observation in all the experimental rats indicating that EABL is practically non-toxic and safe even at the highest dose of 5000 mg/kg after acute oral administration (Table 2). This could be due to the organoleptic hotness or bitterness induced by sesquiterpene lactones that escalated the consumption of water (Chadwick *et al.,* 2013).

**Table 1:** Effect of acute oral administration of EABL on feed and water Intake (n=3 each)

| Parameter | Treatment 1 (1750 mg/kg) | Treatment 2 (5000 mg/kg) | t-value | p-value |
|---|---|---|---|---|
| Average feed intake (g/day) | 18.45±0.16 | 18.47±0.36 | 0.53$^{ns}$ | 0.958 |
| Average water intake (mL/day) | 31.64±1.38 | 41.64±1.64 | 4.64** | <0.001 |

ns: non-significant, ** significant at $p<0.01$ level.

**Table 2:** Effect of acute oral administration of EABL on body weight (n=3 each)

| | Body weight (g) | | |
|---|---|---|---|
| Dose Group | Day 0 | Day 7 | Day 14 |
| 1750 mg/kg b. wt. (T1) | 180±0.18$^{ns}$ | 185±0.17$^{ns}$ | 196±0.27$^{ns}$ |
| 5000 mg/kg b. wt. (T2) | 181±0.14$^{ns}$ | 187±1.03$^{ns}$ | 198±0.30$^{ns}$ |

ns: non-significant, ** significant at $p<0.01$ level.

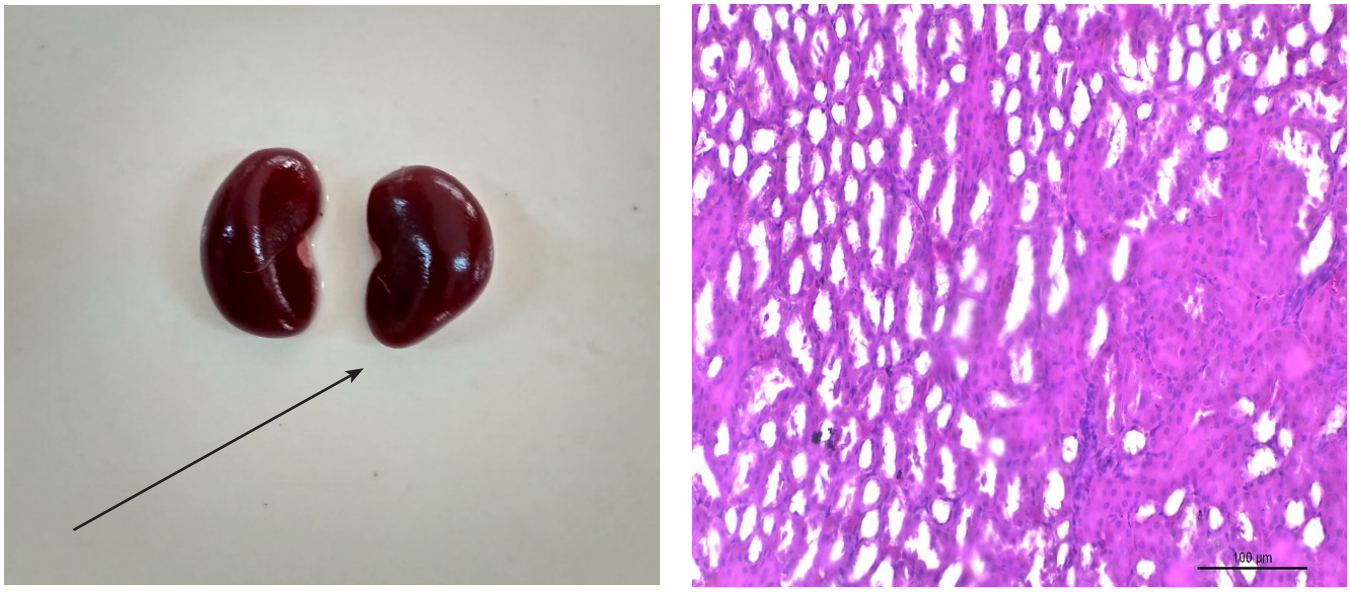


**Fig 2:** Gross normal kidneys (a) and kidney section showing focal tubular epithelial hyperplasia on histopathology (b) of rats dosed at 5000 mg/kg b.wt. (Group II)

### Wellness Parameters

The animals were observed for assessing wellness parameters at different time intervals, *viz.,* during the first 30 min, 4 h, 24 h, 48 h and daily for 3-14 consecutive days. It showed no signs of toxicity or mortality in the observation period. Physiological parameters such as skin & fur, alertness, grooming behaviour, sleep, gait, gripping, pinna reflex, corneal reflex, pupils, salivation, lacrimation, urination and defecation were found to be normal in rats at all intervals in all dose levels without tremors.

### Pathology

The gross pathological examination did not reveal any major abnormalities for EABL at both the dose levels, *i.e.*, 1750 mg/kg and 5000 mg/kg body weight. During the 14 days observation period the rats receiving two dose levels also did not show any clinical signs of toxicity or mortality. Gross examination revealed apparently normal visceral organs. On microscopic examination one among the three animals receiving EABL at the dose rate of 5000 mg/kg body weight showed focal tubular epithelial hyperplasia in the kidney (Fig. 2). The toxicity, if any, could be attributed to the presence of phytoconstituents of the plant under investigation since literature evidenced that many of the phytoconstituents like steroids, alkaloids, triterpenes and glycosides in plant have been associated with toxicity (Hoffmann *et al.,* 2003). Since all the animals survived with normal body weight gain and without exhibiting any clinical signs and mortality, it was inferred that the $LD_{50}$ of EABL was found to be more than 5000 mg/kg body weight. *Blumea* genus belongs to the Asteraceae family, in which many other species were reported to be toxic. Similar findings were also reported earlier on acute oral toxicity study of ethanol extract of *Cosmos caudautus*, a member of the Asteraceae family (Amna *et al.,* 2013).

## Conclusions

Based on the findings of the present study, it was demonstrated that EABL was found to be safe up to 5000 mg/kg body weight at a single dose oral administration to female Sprague-Dawley rats and the $LD_{50}$ was more than 5000 mg/kg body weight. To the best of our knowledge this is the first pilot study, which demonstrated that EABL did not cause toxicity in oral acute dosage in rats and the highest dose of 5000 mg/kg b. wt. was found to be safer. However, additional studies in sub-acute and chronic toxicity evaluation are needed to further determine the long-term safety of this plant extract.

## Acknowledgements

Authors would like to thank the Kerala Veterinary and Animal Sciences University for providing the research grants and rendering facilities to carry out this work. First author makes a special note of thanks to his teachers and co-authors for their constant support and expert advice.

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