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Original Article

# EVALUATION OF ACUTE AND DERMAL TOXICITY OF ESSENTIAL OIL OF *ETLINGERA FENZLII* (KURZ) K. SCHUM: AN *IN VIVO* STUDY

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**ABSTRACT**

**Objective:** The present investigation describes the *in vivo* acute toxicity and dermal toxicity of essential oil of *Etlingera fenzlii* (*E. fenzlii*) in Wistar albino rats.

**Methods:** The essential oil was extracted by hydro-distillation using Clevenger apparatus. The acute toxicity study was conducted in Wistar albino rats at different doses (175,550 and 2000 mg/kg) while dermal toxicity study was carried out with simple ointment base and essential oil at a dose of 2000 mg/kg body weight. Animals were observed for 14 d and parameters like body weight, feed intake and water intake were studied.

**Results:** No mortality and no significant changes were observed in body weight and wellness parameters at 175,550 and 2000 mg/kg body weight doses. Further, the dermal absorption with essential oil also did not reveal any treatment-related changes in body weight, food consumption and water intake in any of the animals tested.

**Conclusion:** These findings suggest that the acute and dermal application of the essential oil of *E. fenzlii* in rats did not divulge any significant toxicity, and the oil extracts were found to be safe up to 2000 mg/kg *in vivo* concentration.

**Keywords:** *Etlingera fenzlii*, Acute toxicity, Dermal toxicity, Median Lethal Dose ($LD_{50}$)



## INTRODUCTION

*Etlingera fenzlii* (Kurz) K. Schum. of Zingiberaceae family, is an endemic species of the Andaman Nicobar Islands exclusively used by the Shompens as a bee repellent source for their honey collection [1]. Shompens are one of the primitive off shots of the Mongoloid race believed to have migrated from Malayan peninsula [2]. The favourite food of Shompens is the fruit of screw pines and honey from wild bees. They chew the plant parts and spit out the sap-filled in the mouth as a coarse spray on the bee hives which tranquilize the honey bees and protect them from bee stings. Roots and flower juice are also used to treat malarial fever, stomach disorders, and gastrointestinal disorders. The chemical characterization on essential oils of the species carried out proved that *E. fenzlii* has an effective repellent or tranquilizing property towards insects. The major constituents identified were long chain aliphatic compounds (n-dodecanol, n-undecanol, and n-tetradecanol etc.). Other major compounds are the oxygenated sesquiterpenoids humulene epoxide, caryophyllene oxide, p-cymene, geraniol, linalool and eugenol. It is documented that the volatile oils having all these constituents/ isolates have proved to be effective eco-friendly, possess varying degrees of insects and pest controlling properties [3]. The repellency properties of essential oils are well recognized for many years and used in some of the repellent products as active ingredients [4]. Recently commercial repellent products containing plant-based ingredients have gained increasing popularity among consumers, as these are commonly perceived as “safe” in comparison to long-established synthetic repellents although this is sometimes a misconception. To date, insufficient studies have followed standard WHO Pesticide Evaluation scheme guidelines for repellent testing. There is a need for further standardized studies in order to better evaluate repellent compounds and develop new products that offer high repellency as well as good consumer safety because natural insecticides are usually prescribed to be applied for a long period and hence may cause adverse effects including allergic contact dermatitis, photosensitization, neurotoxicity and carcinogenicity in human beings, thereby warranting evaluation of their efficacy and safety profile.

Because of the importance of dermal applications of *E. fenzlii*, the present study was undertaken to assess the toxicity profile of the essential oil of *E. fenzlii* in Albino rats as per OECD (Organization for Economic Cooperation and Development) guidelines [5].

## MATERIALS AND METHODS

### Plant material

Plants were collected from Mount Harriet hill ranges of South Andamans, Nancowary Island, Katchal Island and Great Nicobar Island. Living plant collections are introduced into the field gene bank of Jawaharlal Nehru Tropical Botanical Garden and Research Institute (JNTBGRI) campus and a voucher specimen (BT 025) was deposited in the herbarium of the Institute. Fresh leaves were collected, washed under running tap water and were used immediately to extract the essential oil.

### Essential oil isolation

Fresh leaves (400 g) were hydro distilled for 3 h using a Clevenger–type apparatus to obtain the essential oil. The oils were collected and dried over anhydrous sodium sulphate ($NA_2SO_4$) and stored at 10 °C until analysed.

### Experimental animals

Healthy young adult nulliparous and nonpregnant Wistar albino female rats, weighing 95-105 g at the start of the experiment, were procured from College of Veterinary and Animal Science, Mannuthy, Thrissur District. The present study protocol has been reviewed and approved by Institutional Animal Ethics Committee (IAEC) of CARe KERALAM Ltd (CKL/TOX/IAEC/44-15). Animals were housed under standard laboratory conditions, air-conditioned environment with adequate fresh air supply through IVC system (Air changes 15 per hour), room temperature 21.0 to 24.0 °C and relative humidity 57-65 %.

The temperature and relative humidity were recorded daily. A single animal was housed in a standard poly sulphonate cage (Size: L 300 x B 170 x H 140 mm) with stainless steel top grill mesh having

facilities for holding pelleted food and drinking water in water bottle fitted with stainless steel sipper tube. Sterilized paddy husk was provided as bedding material. The animals were acclimatized for a minimum period of five days to laboratory conditions and were observed for clinical signs daily. Veterinary examination of all the animals was recorded on the day of receipt and on 5th day of acclimatization. The animals were fed *ad libitum* throughout the acclimatization and study period. Amrut lab rodent feed was provided. Water was provided *ad libitum* throughout the acclimatization and study period. Deep bore-well water passed through activated charcoal filter and exposed to ultraviolet rays in Aquaguard water filter cum purifier (Manufactured by Eureka Forbes Ltd., Mumbai) was provided in plastic water bottles with stainless steel sipper tubes.

**Oral acute toxicity study**

The overnight fasted Swiss albino rats (95-105 g) were weighed and administered the essential oil of *E. fenzlii* as a single dose (175,550, 2000 mg/kg) by oral route and animals were observed for toxic symptoms for first four hours. Dosing was initiated at 175 mg/kg. Dosing was sequential, allowing at least 48 h before dosing the next animal. There was no mortality at starting dose of 175 mg/kg, hence further animals were treated at the next higher doses with the single animal at 550 mg/kg and three animals at 2000 mg/kg as per the OECD guideline procedure [5].

A number of survivors were noted after 24 h and the observation made daily for a period of 14 d. throughout the acute toxicity study, individual animal body weight, food and water intake were recorded daily during the study period. The toxicological effect was assessed on the basis of mortality, which was expressed as Median Lethal Dose ($LD_{50}$).

**Dermal toxicity**

The acute dermal toxicity with the single highest test dose was conducted as per the OECD guideline [6]. Wistar Albino rats were selected and acclimatised to laboratory conditions for at least five days prior to the experiment. The fur was removed from the dorsal area of the trunk of all animals by clipping or shaving, and care was taken to avoid abrading the skin and only animals with intact skin were used for the present study. At least 10 % of the body surface area of each animal was cleared, and the control, and test substances were applied uniformly over this area. Animals were divided into two groups. A simple ointment base and test item essential oil of *E. fenzlii* 2000 mg/kg body weight were applied dermally over an area with a porous gauze dressing and non-irritating tape in group I and II respectively throughout a 24-hour exposure period.

All the animals were observed for clinical signs of toxicity and mortality at 30-40 min, 1 hr, 2 hr, 3 hr and 4 hr following dosing and thereafter once daily during the 14 d observation period.

**Statistical analysis**

The results are expressed as mean±SD. Data were analysed by one-way analysis of variance (ANOVA). The one-way ANOVA was done for expressing experimental significance as accepted at a level $p<0.05$.

**RESULTS**

**Oral acute toxicity study**

No mortality was recorded during the treatment period in either the control or treated groups given 2000 mg/kg of essential oil of *E. fenzlii* orally in the 14-day study period. The animals did not show any changes in general behaviour or other physiological activities. There were no significant differences between the control and treated groups in the body and organ weights. Thus, the $LD_{50}$ values of the essential oil of were found to be greater than 2000 mg/kg per orally. There were no clinical signs of toxicity and mortalities at any of the doses tested. There were no treatment-related changes in body weight, percent body weight, food intake and water intake during the study period at the dose of 2000 mg/kg [table 1, 2, 3].

**Table 1: Mean body weight (g) of rats after 14 d treatment with essential oil of *E. fenzlii***

| Groups | Change in body weight (g) | | |
|---|---|---|---|
| | Day 0 | Day 7 | Day 14 |
| 175 mg/kg | 119±7.8 | 150±8.9* | 140±8.3 |
| 550 mg/kg | 127±11.9 | 156±5.6* | 144±8.3 |
| 2000 mg/kg | 111±10 | 130±18 | 130±14 |

n=3; Values are expressed as mean±SD. Data were analyzed by one-way ANOVA. *P<0.05 when compared to Day 0

**Table 2: Mean food intake (g) of rats after 14 d treatment with essential oil of *E. fenzlii***

| Groups | Change in food intake (g) | | |
|---|---|---|---|
| | Day 0 | Day 7 | Day 14 |
| 175 mg/kg | 13±3.7 | 14.3±1.7* | 13.1±2.7* |
| 550 mg/kg | 17±6.2 | 18.6±3.5* | 17.1±4.9* |
| 2000 mg/kg | 13±1.0 | 12.1±1.5* | 12. 7±1.2 |

n=3; Values are expressed as mean±SD. Data were analyzed by one-way ANOVA. *P<0.05 when compared to Day 0

**Table 3: Mean water intake (ml) of rats after 14 d treatment with essential oil of *E. fenzlii***

| Groups | Change in water intake (ml) | | |
|---|---|---|---|
| | Day 0 | Day 7 | Day 14 |
| 175 mg/kg | 15±5.2 | 15±3.0 | 14±2.6* |
| 550 mg/kg | 18±2.6 | 13±1.7* | 13±2.2 |
| 2000 mg/kg | 19±4.3 | 16±2.6* | 12±2.3 |

n=3; Values are expressed as mean±SD. Data were analyzed by one-way ANOVA. *P<0.05 when compared to Day 0

**Dermal toxicity**

In dermal toxicity study, at a concentration of 2000 mg/kg did not produce any burning sensation or rashes on the skin of experimental animals. Further, no mortality was seen throughout the observation period either in the control or in any of the treated groups. Therefore, the essential oil of *E. fenzlii* was found to be safe up to a concentration of 2000 mg/kg and their acute dermal $LD_{50}$ was found to be greater than 2000 mg/kg. Treatment with test item essential oil of *E. fenzlii* up to 2000 mg/kg dose level was well tolerated.

There were no clinical signs of toxicity and mortalities noticed in any of the doses tested. There were no treatment-related changes in body weight, food intake and water intake [Table 4, 5 & 6].

**Table 4: Effect of essential oil of *E. fenzlii* treatment on body weight gain (g) in rats during acute dermal toxicity study**

| Groups | Change in body weight (g) | | |
|---|---|---|---|
| | Day 0 | Day 7 | Day 14 |
| Simple ointment base | 154±6.6 | 163±10.8* | 176±13.9* |
| Essential oil (2000 mg/kg) | 157±9.8 | 169±12.4* | 182±11.7* |

n=3; Values are expressed as mean±SD. Data were analyzed by one-way ANOVA. *P<0.05 when compared to Day 0

**Table 5: Effect of essential oil of *E. fenzlii* treatment on food intake (g) in rats during acute dermal toxicity study**

| Groups | Change in food intake (g) | | |
|---|---|---|---|
| | Day 0 | Day 7 | Day 14 |
| Simple ointment base | 12±0.41 | 9.33±3.27 | 12.33±3.27 |
| Essential oil (2000 mg/kg) | 11±1.22 | 13±1.63* | 12±0.41* |

n=3; Values are expressed as mean±SD. Data were analyzed by one-way ANOVA. *P<0.05 when compared to Day 0

**Table 6: Effect of essential oil of *E. fenzlii* treatment on water intake (ml) in rats during acute dermal toxicity study**

| Groups | Change in water intake (ml) | | |
|---|---|---|---|
| | Day 0 | Day 7 | Day 14 |
| Simple ointment base | 14±1.20 | 14±1.29 | 14±2.23 |
| Essential oil (2000 mg/kg) | 18.29±2.1 | 18.29±2.69* | 14.86±2.06 |

n=3; Values are expressed as mean±SD. Data were analyzed by one-way ANOVA. *P<0.05 when compared to Day 0

## DISCUSSION

The safe dose range of drug can be determined by the $LD_{50}$ values through acute toxicity studies [7]. The result obtained in the acute oral toxicity studies reveals that the oral administration of essential oil of *E. fenzlii* did not cause any behavioural and toxicological effects during the 14-days study period with a limited test dose of 2000 mg/kg. As no mortality is reported in any of the treated groups, it is safe to state that oral $LD_{50}$ value of the essential oil exceeds 2000 mg/kg. In general, the changes in body and organ weights are probably due to the toxic effects of the xenobiotic [8]. Furthermore, the changes in the organ to weight ratio or the relative weight could also be due to organ injury as a result of exposure to the toxic material [9]. The $LD_{50}$ value is a statistical estimate of the acute lethality of a chemical administered under specific circumstances; it provides a measure of relative toxicities of the chemical under similar or identical conditions. Thus, the major application of the $LD_{50}$ is comparative, allowing for semi-quantitative toxic evaluation of compounds.

However, when the body weight was analysed no differences were observed between control and experimental groups. A similar situation was shown by essential oil of *Cymbapogan citrates* did not present on extensive toxic effect in rodents, since the $LD_{50}$ in mice was around 3-5 g/kg [10]. In oral acute toxicity study, as high dose of *Ocimum sanctum* essential oil at 2000 mg/kg did not show any observable toxic effects in mice in terms of any deaths or abnormal symptoms which points to its being nontoxic and safe in mice [11] and natural biopesticides against stored food grain pests like dust from roots of *Securidaca longipedunculata* (polygonaceae) and leaves from *Chamaecrista nigricans* (Leguminoseae) also revealed negligible toxicity in tested animals [12]. Determination of food intake and water consumption of rat is also important in the study of the safety of a product with a therapeutic purpose, as proper intake of nutrients is essential to the physiological status of the animal and to the accomplishment of the proper response to the drugs tested [13]. The absence of any significant differences in the body weight provides support for the safety of essential oil of *E. fenzlii*. Also, there were no significant changes in animal behaviour and food consumptions in essential oil-treated group at any dosage. These observations indicate no effects of the essential oil on the general well-being of the animals.

The dermal application was carried out to check the toxicity of the *E. fenzlii* essential oil; pathological analysis did not reveal any toxic lesions in the natural architecture of the skin. This indicates the dermal safety of the essential oil sample. Since the dermal dose of essential oil (200 mg/kg) did not induce any biochemical, haematological, anatomical and histopathological signs of toxicity, it can be taken as the no-observed adverse effect level (NOAEL) for rats under experimental conditions used. This conclusion correlates well with findings from dermal toxicity analysis of essential oil of *Eucalyptus globules* did not indicate any significant sign of allergies like edema or erythema and thus, it was considered to be safe for dermal application [14].

## CONCLUSION

The acute and dermal toxicity treatment with various concentrations of essential oil of *E. fenzlii* demonstrates no systemic rashes allergic contact dermatitis, photosensitization, neurotoxicity and other treatment-related adverse effects, no mortality, and no significant weight loss was attributable to the application of essential oil in any of the animals treated. The animals exhibited signs of good health and well-being throughout the study and the application of essential oil is safe up to 2000 mg/kg *in vivo* concentration. Therefore, the results indicate the potential application of volatile oil isolates of *E. fenzlii* as effective plant-based repellent towards insects.

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## CONFLICT OF INTERESTS

Declared none

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