

# The antiinflammatory activity and LD<sub>50</sub> of *Ocimum forskolei* Benth., family Lamiaceae

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## Abstract

The present study aimed to determine the lethal dose {LD<sub>50</sub>} using acute toxicity models and evaluate the anti-inflammatory activity of the total ethanolic extract (TEE) and different fractions of *Ocimum forskolei* Benth. aerial parts (leaves and stems) following the carrageenan-induced paw edema model in rats. The results obtained revealed that the LD<sub>50</sub> value of the TEE was 8 g/kg body weight, while the extract and different fractions showed a significant decrease in paw edema, especially the aqueous fraction treated group with a % inhibition of (40.77 %, P < 0.001) in comparison with indomethacin (55.55 %, P < 0.001) as a standard.

## Key words

*Ocimum forskolei*, Lamiaceae, Antiinflammatory, aqueous fraction

## 1. Introduction

Family *Lamiaceae* is a large dicotyledonous family comprising about 236 genera and up to 7200 species categorized into 7 subfamilies. Plants of the family are annual or perennial herbaceous plants or shrubs and subshrubs distributed in the temperate and warm regions of the world [1]. The genus *Ocimum*; commonly known as Tulsi, and often referred to as the king of herbs, is one of the largest genera of the family *Lamiaceae* currently comprising 160 species; and includes aromatic herbs and shrubs distributed in tropical and warm regions of Asia, Africa, Central and South America [2]. Species of the genus are widely used in traditional medicine to alleviate mental fatigue, rhinitis, cold spasm, in treatment of cough, respiratory disorders, poisoning and as insect repellents [3, 4]. Among the metabolic diversity of the genus *Ocimum*, flavonoids, terpenoids (mainly oxygenated monoterpenes and sesquiterpene hydrocarbons), sterols and phenolic compounds were found to prevail. Moreover, biological investigations have been carried out on these compounds and demonstrated their varied activities as cytotoxic, antioxidant, antimicrobial, and gastroprotective ones [5-7].

*Ocimum forskolei* Benth. (syn. *O. menthiifolium*, common name. Habak) ranges in East Africa from Egypt, south to Kenya, and the Southern Arabian Peninsula, including Oman, Yemen and UAE. It is traditionally used in Yemen as a cosmetic, antipyretic and in treating skin infections. In UAE, the crushed leaves of *O. forskolei* are used to treat headaches, colds and ear aches. It was used as a mosquito repellent in Eritrea and many studies highlighted its repellent activity [8] [9].

Surveying literature demonstrated the absence of any phytochemical or biological investigation of *O. forskolei*. which encouraged us to investigate some biological activities of that plant as acute toxicity testing to evaluate the LD<sub>50</sub> value and a detailed anti-inflammatory study of the TEE and other fractions. The results indicated the high safety and anti-inflammatory activity of that medicinal herb.

## 2. Materials and methods

### 2.1. Chemicals

Carboxymethylcellulose {CMC} was obtained from El-Nasr pharmaceutical and chemical co., Egypt (ADWIC). Carrageenan was obtained from Sigma-Aldrich (St. Louis, MO, USA). Indomethacin was obtained as (Liometacin®, El-Nile co., Egypt).

### 2.2. Plant materials

*Ocimum forskolei* Benth. aerial parts (stems and leaves) were collected in the period from July to September 2016, from the national garden of Jazan, KSA. Authentication of plant was established by Prof. Dr. Mahmoud Abdelhady Hassan, Professor of Horticulture, Faculty of Agriculture, Minia University. A voucher specimen (Mn-ph-Cog-38) was kept in the herbarium of Pharmacognosy Department, faculty of Pharmacy, Minia University, Minia, Egypt.

### 2.3. Preparation of the TEE and fractions

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The air dried, powdered leaves and stems (5 kg) of *O. forskolei* were extracted by maceration with 95 % ethanol at room temperature. The TEE was then concentrated under reduced pressure to a syrupy consistency (300 g) which was further subjected to preliminary phytochemical screening [10]. TEE was then suspended in a small amount of distilled water to yield an aqueous solution which was successively partitioned with petroleum ether (pet. ether), dichloromethane (DCM), and ethyl acetate (EtOAc) using a separating funnel. The organic phase in each step was separately evaporated under reduced pressure to afford the corresponding fractions I (120 g), II (35 g) and III (32 g), respectively. The remaining mother liquor was then concentrated to give the aqueous fraction IV.

#### 2.4. Experimental animals

Wister male albino rats of (200 ± 50 g) were obtained from Animal House, pharmacology department, faculty of Medicine, Assuit University. All animal rules were conducted in stratification with the internationally accepted principles for using and caring of laboratory animals as found in the European Community Guidelines and Institutional Ethical Committee. Animals were housed under standardized environmental conditions in the pre-clinical Animal House in plastic cages. Temperature was adjusted at 25 ± 2 °C and relative humidity was 55 - 70 %. A 12:12 hs light/dark cycle was kept constant throughout the experiment. They were fed a standard diet, water provided *ad libitum* and they were acclimatized for one week before the experiment. All conditions were well kept to minimize animal suffering.

#### LD<sub>50</sub> determination

The acute toxicity of the TEE of *O. forskolei* Benth. aerial parts was determined by measuring the lethal dose for 50 % of the animals tested (LD<sub>50</sub> method) [11]. Rats were divided into six groups, each containing five animals. The TEE was administered orally at different dose levels (0.5, 1, 2, 4 and 8 g/kg, p.o.) and suspended in 0.5% CMC. The control group received an equivalent dose (0.5% CMC solution; 10 ml/kg, p.o.) of the total extract vehicle. The test and control groups were observed for 48 hs under normal environmental conditions, and all groups were allowed free access to food and water.

#### Anti-inflammatory activity

The TEE and other different fractions were evaluated for anti-inflammatory activity following Whistar male albino rats (200 ± 50 g) were grouped into seven groups (five animals each). The tested fractions at the specified dose were suspended in 0.5 % CMC. The negative control group was given the vehicle (0.5 % CMC solution), the positive control one was given indomethacin at a dose of 25 mg/kg and the other five groups received the tested fractions at a dose of 300 mg/kg suspended in 0.5 % CMC. All the doses were given orally 30 m before sub-plantar injection of 0.1 ml freshly prepared 1 % Carrageenan in the right hind paw of each rat. A plethysmometer was used to

measure the paw thickness after 30 m, 1, 2, 3, 4 and 5 hs. Percentage of variation of edema and percentage of inhibition were calculated as follows:

$$\% \text{ Variation (edema)} = \frac{\text{Right paw thickness} - \text{Left paw thickness}}{\text{Right paw thickness}} \times 100$$

$$\% \text{ Inhibition} = \frac{(V_0 - V_t)}{V_0} \times 100$$

V<sub>0</sub> = The average paw thickness of the control group, and

V<sub>t</sub> = The average paw thickness of the treated group.

#### Statistical analysis

Analysis of data was done by the one-way analysis of variance (ANOVA) using SPSS 20 version software. Results were expressed as "mean increase in paw volume ± SD". Analysis of variance (one way ANOVA) followed by Tukey's test for control, standard and test group comparisons were used for statistical assessment. P values < 0.05 were considered as significant and P < 0.001 were considered as highly significant.

### 3. Results and discussion

#### LD<sub>50</sub> determination

The acute toxicity (LD<sub>50</sub>) result was 8 g/kg bw during 24 h of observation of the animals after the TEE oral administration. Therefore, *O. forskolei* is considered a safe herbal drug with a wide safety margin.

#### 4. Results and discussion

The TEE and the other tested fractions of *O. forskolei* showed a potent anti-inflammatory activity using the carrageenan-induced paw edema model in rats. Animals from the -ve control group expressed a progressive increase in the paw volume, while those from the +ve control one as well as the test groups showed progressive decrease in the paw volume throughout the time of the experiment. The aqueous fraction exhibited the most potent anti-inflammatory potential with a significant decrease in the paw volume throughout time intervals of 2, 3, 4 and 5 hs with the highest value at the 4h interval (percentage of inhibition being 40.77 % which is considered highly significant, P < 0.001). The mean paw volume of rats from the -ve control group reached its peak after 5 hs from beginning the experiment. Groups treated with TEE, pet. ether, DCM and EtOAc fractions displayed values of 35.22 %, 33.33 %, 33.33 % and 31.44 % inhibition of paw edema at 4 hs, respectively with values ranged from significant to highly significant. The results also showed that The DCM fraction had the shortest onset of action with 15.82 % inhibition at 30 m, and 18.36 % at 1 hr interval indicating high absorption. Moreover, the EtOAc fraction showed % inhibition of 13.64 % and 14.32 %, at the same time interval with better values than that of indomethacin treated group although a slow onset of action of 2.18 m and 6.12 m, respectively, was observed. In general, all the tested fractions as well as the TEE exhibited significant to highly significant values that extended over the whole period of the experiment as shown in (Table 1 & 2) and (Figure 1 & 2)

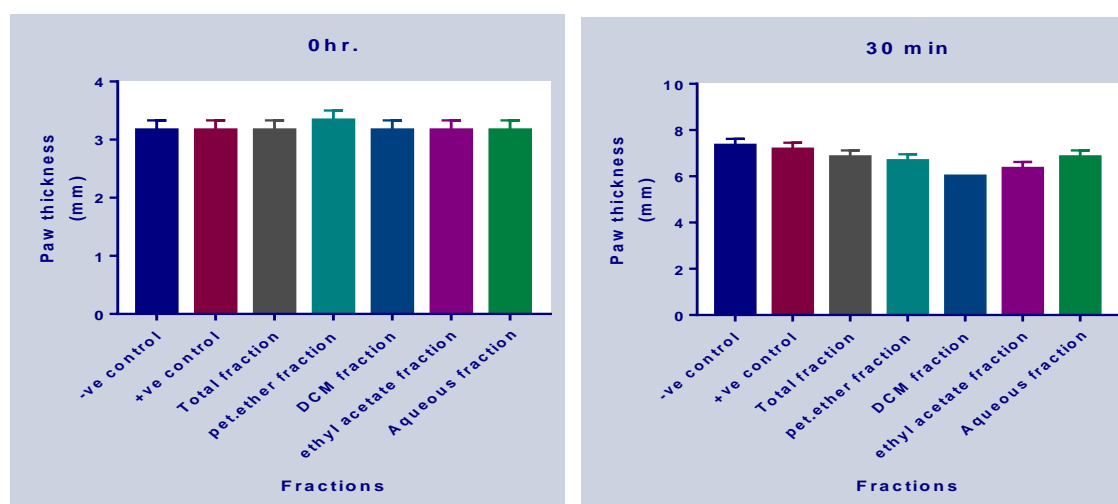
**Table 1:** Effect of the TEE and different fractions of *O. forskolei* on the carrageenan-induced paw edema thickness in rats.

Group	Dose mg/ kg	Thickness of the paw (mm) (mean +- SD)						
		Pre-treatment 0h	30 m	1h	2h	3h	4h	5h
-ve control	-	3.17±0.17	7.33 ±0.17	8.17 ±0.17	8.67±0.17	8.83± 0.17	9± 0.29	9.67± 0.17
Indomethacin (+ve control)	25	3.17±0.17	7.17 ±0.17	7.67 ±0.17	5.17± 0.17***	4.17±0.17****	4± 0****	3.67±0.17****
TEE	300	3.17±0.17	6.83 ±0.17	7.33±0.17*	7.17± 0.17**	6.67±0.17***	5.83± 0.17***	7.17± 0.17***
Pet. ether fr.	300	3.33±0.17	6.67±0.17*	7.33± 0.33	6.5± 0.29**	6.33±0.17***	6±0.29**	6.5 ±0.29***
DCM fr.	300	3.17±0.17	6.17±0.17**	6.67±0.33*	5.83± 0.17***	5.5±0.29***	6±0.29**	6.67± 0.17***
EtOAc fr.	300	3.17±0.17	6.33 ±0.16*	7.0**	6.5± 0.29**	6.17±0.17***	6.17±0.17**	6.67± 0.44**
Aqueous fr.	300	3.17±0.17	6.83 ±0.17	7.5± 0.29	5.83± 0.17***	5.5±0.29***	5.33±0.17***	6 ±0.29***

Each value represents the mean ± SD (N = 5). Statistical analysis by One-way ANOVA followed by the student's T-test (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 ).

**Table 2:** Effect of the TEE and different fractions of *O. forskolei* on the percentage of inhibition of edema in rats.

Group	Dose mg/kg	% of inhibition of edema					
		30 m	1h	2h	3h	4h	5h
Control	-	-	-	-	-	-	-
Indomethacin	25	2.18	6.12	40.37	52.77	55.55	62.05
TEE	300	6.82	10.28	17.3	24.46	35.22	25.85
Pet.ether fr.	300	9	10.28	25.03	28.31	33.33	32.78
DCM fr .	300	15.82	18.36	32.76	37.71	33.33	31.02
EtOAC fr.	300	13.64	14.32	25.03	30.12	31.44	31.02
Aqueous fr.	300	6.82	8.2	32.76	37.71	40.77	37.95

**Figure 1:** Effect of the TEE and different fractions of *O. forskolei* on the percentage of inhibition of edema in rats

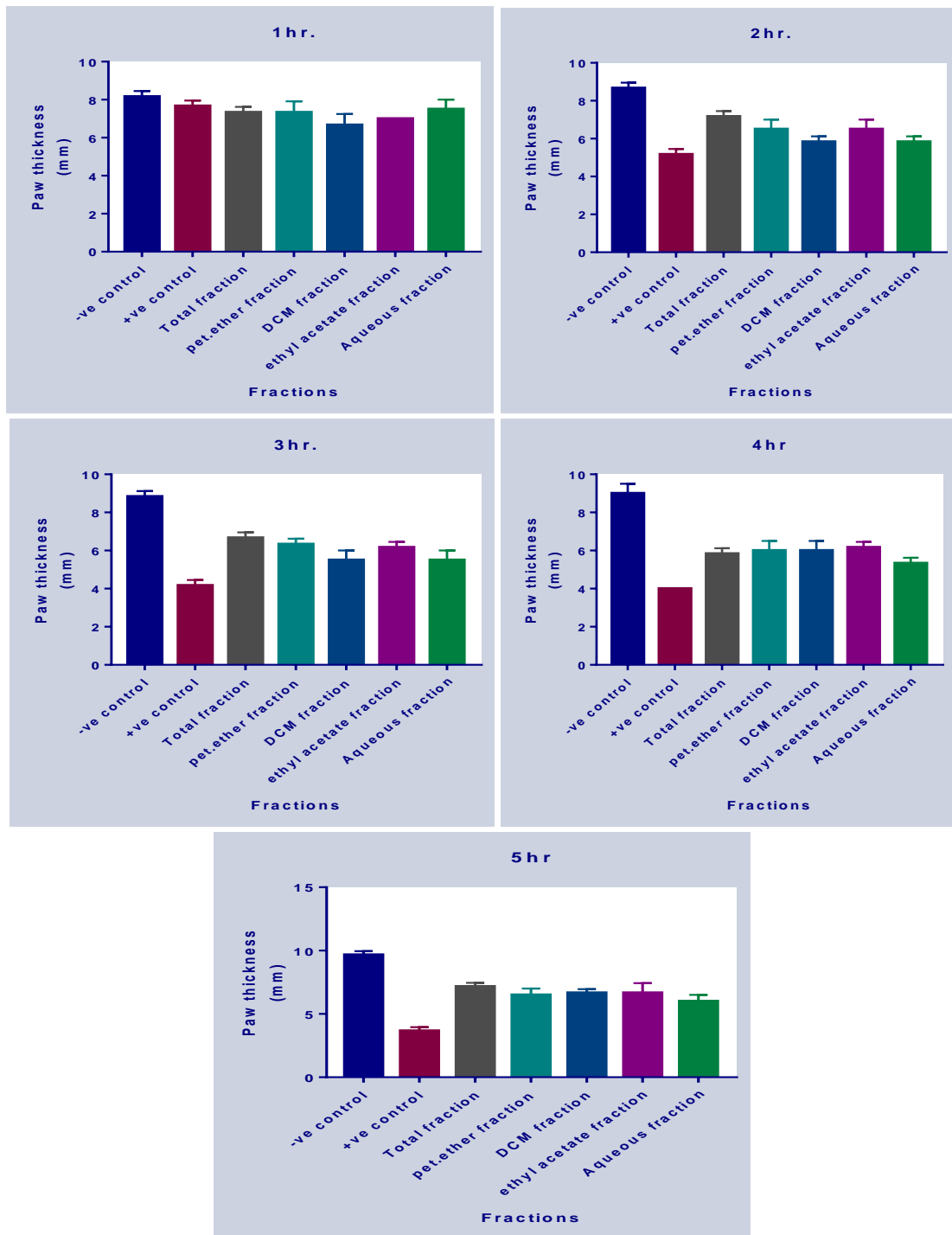


Figure 1: Effect of the TEE and different fractions of *O. forskolei* on the percentage of inhibition of edema in rats (cont.)

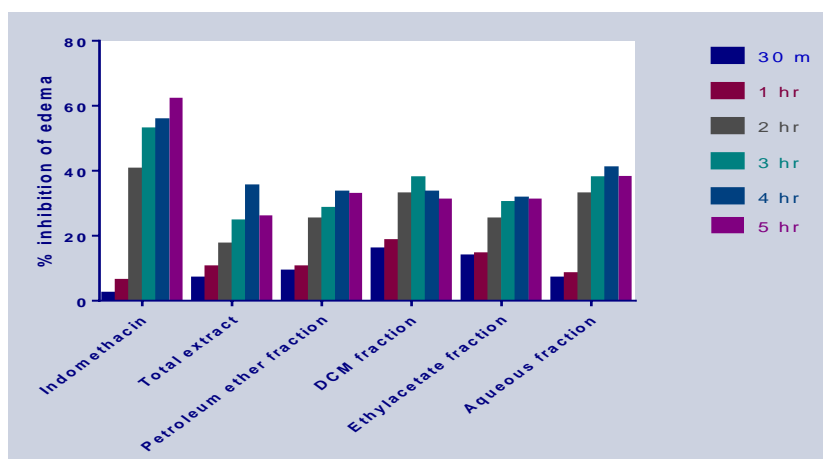


Figure 2: Effect of the TEE and different fractions of *O. forskolei* on the percentage of inhibition of edema in rats. J. Adv. Biomed. & Pharm. Sci.

## 5. Conclusion

Investigating the anti-inflammatory potential of TEE and different fractions of *O. forskolei* Benth. aerial parts revealed that the aqueous fraction was the most potent one followed by the TEE (at 4 hs interval) which could be related to the abundance of flavonoids and phenolic compounds in the extract. Flavonoids and phenolic acids extracted from various species of *Ocimum* have been reported to exhibit significant antiinflammatory activity in various studies and using different models [12, 13]. The strong activity of both pet. ether and DCM fractions could be attributed to the presence of terpenoids and sterols which also demonstrated different levels of antiinflammatory activity [14].

Moreover, the TEE showed a high safety margin with LD<sub>50</sub> (8 g / kg bw). Therefore, this plant could be considered as a source for discovering new bioactive natural products, although the exact bioactive metabolites responsible for the activity still remain speculative. Further investigations of *O. forskolei* using a wider range of doses and covering longer periods of observation are still needed before reaching a clear cut conclusion. Future studies are needed to refine the extraction procedure of the plant that can lead to improved pharmaceutical products. Owing to its easy availability, efficacy, safety and potency it can be used as an adjuvant with the-available anti-inflammatory agents, leading to reducing the dose needed for treatment and therefore minimizing its harmful side effects.

## Conflict of interest

The authors declare that they have no conflict of interest.

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