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## The Anti-inflammatory Activity of the *Foeniculum vulgare* L. Essential Oil and Investigation of its Median Lethal Dose in Rats and Mice

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**Abstract:** The aim of this study was to gas chromatographic analysis of extract of *Foeniculum vulgare* L. essential oil and to investigate its median lethal dose ( $LD_{50}$ ) and possible anti-inflammatory effects. The composition of the essential oil was as follows: 74.8% (E)-anethole, 11.1% limonene, 4.7% methyl chavicol, 2.5% fenchone and 1.3%  $\alpha$ -pinene. The  $LD_{50}$  dose was found to be  $1.038 \text{ mL kg}^{-1}$ . The essential oil of *Foeniculum vulgare* L. was investigated the model of carrageenan induced rat paw edema and it had an anti-inflammatory effect matching to that of etodolac at 0.050 and  $0.200 \text{ mL kg}^{-1}$  doses.

**Key words:** *Foeniculum vulgare* L., fennel, anti-inflammatory activity, median lethal dose

### INTRODUCTION

Fennel (*Foeniculum vulgare* Miller, family Umbelliferae) is an annual, biennial or perennial aromatic herb, depending on the variety, which has been known since antiquity in Europe and Asia Minor<sup>[1]</sup>. The dried, aromatic fruits are widely employed in culinary preparations for flavoring bread and pastry, in candies and in alcoholic liqueurs of French type, as well as in cosmetic and medicinal preparations<sup>[2]</sup>. Volatile components of subsequent fennel seed extracts is analysed by chromatographic methods. Trans-anethole, fenchone, methylchavicol, limonene,  $\alpha$ -pinene, camphene,  $\beta$ -pinene,  $\beta$ -myrcene,  $\alpha$ -phellandrene, 3-carene, camphor and cisanethole<sup>[3]</sup>. Major components of oil samples are  $\alpha$ -pinene,  $\beta$ -pinene, limonene, fenchone, methychavicol and trans-anethole detected. Özbek *et al.*<sup>[4-6]</sup> also suggested that *Foeniculum vulgare* Essential Oil (FEO) has hypoglycemic activity in mice, potent hepatoprotective action in rats and analgesic activity in mice. Fennel and its herbal drug preparations are used for dyspeptic complaints, spasmodic gastric-intestinal complaints, bloating and flatulence<sup>[7-9]</sup>. Extracts of the fennel seeds are used in Turkish traditional medicine as an anti-inflammatory agent<sup>[10]</sup>. In this study we investigated whether, FEO had an anti-inflammatory effect as supposed by traditional medicine.

### MATERIALS AND METHODS

The fennel seeds used were purchased from a local market from Van in Turkey. Voucher specimens were kept in Herbarium of Yuzuncu Yıl University, Faculty of Art and Science (VANF Nr. 691). The dried fruits were finally

grounded in an electrical grinder and boiled in Clevenger device (Ildam, Turkey). Collected essential oil was kept in tubes and the yield was determined as 1%. The analysis of FEO was done by Gas Chromatography device in University of Anotalia, The Research Center of Medical and Aromatic Plant and Drug, Eskişehir.

Male, Sprague-Dawley rats and Swiss albino mice were used in these experiments. The animals were housed at room temperature ( $20\pm2^\circ\text{C}$ ) in standard cages with standard pellet food and water *ad libitum*, in rooms lit in a rhythm of 12 h light, 12 h dark and kept under controlled environment following the standard operating procedures of the animal house with approval of animal ethics committee.

Lambda-carrageenan Type IV and indomethacin were obtained Sigma (Steinheim, Germany), Etodolac was obtained FAKO İlaçları A.Ş. (İstanbul, Turkey).

**Acute toxicity:** Male and female Swiss albino mice were randomly assigned to seven groups with 8 animals in each group. First group was treated with normal saline and considered as control and the other six groups were treated with FEO extract given intraperitoneally (i.p) in increasing dosages of 0.20, 0.40, 0.80, 1.60, 3.20 and  $6.40 \text{ mL kg}^{-1}$  body weight. The mortality in each cage was assessed 72 h after administration of FEO. The percentage mortalities were converted to probits. Regression lines were fitted by the method of least squares and confidence limits for the  $LD_1$ ,  $LD_{10}$ ,  $LD_{50}$ ,  $LD_{90}$  and  $LD_{99}$  values were calculated by the method of Litchfield and Wilcoxon<sup>[11,12]</sup>.

**Anti-inflammatory activity:** The method of Winter *et al.*<sup>[13]</sup> with slight modification was used. Seventy rats of either sex were divided into seven groups of ten animals each.

The rats were fasted for 12 h and deprived of water only during the experiment. Deprivation of water was to ensure uniform hydration and to minimize variability in edematous response. Inflammation of the hind paw was induced by injecting 0.05 mL fresh lambda carrageenan (phlogistic agent) into the subplantar surface of the right hind paw. The control Group I was given normal saline and the control group II was given ethyl alcohol. The third group (reference Group-I) received indomethacin (3 mg kg<sup>-1</sup>, i.p)<sup>[14]</sup> and the fourth group (reference Group-II) received etodolac (50 mg kg<sup>-1</sup>, i.p)<sup>[15]</sup> while the remaining three groups received the extract at doses of 0.050, 0.100 and 0.200 mL kg<sup>-1</sup>, i.p. These doses of the extract utilised in the current study has been chosen accordingly LD<sub>1</sub> value (LD<sub>1</sub> = 0.449 mL kg<sup>-1</sup>).

The measurement of foot volume was accomplished by displacement technique using the plethysmometer (Ugo Basile 7140 plethysmometer, Italy), immediately before and 3 h after the injection. The inhibition percentage of the inflammatory reaction was determined for each animal by comparison with controls and calculated by the formula<sup>[16]</sup>:

$$I\% = [(1 - (dt/dc)) \times 100]$$

where, dt is the difference in paw volume in the drug-treated group and dc the difference in paw volume in the control group.

Results of the paw edema were reported as mean±SEM. The total variation was analysed by performing one-way Analysis Of Variance (ANOVA). Tukey's HSD test (Tukey's honestly significant difference test) was used to determining significance. Probability levels of less than 0.05 were considered significant. Medium effective dose (ED<sub>50</sub>) value was calculated by non-linear regression analysis.

## RESULTS AND DISCUSSION

According to the results of GC analysis, FEO consists of 74.8% (E)-anethole, 11.1% limonene, 4.7% methyl chavicol, 2.5% fenchone, 1.3%  $\alpha$ -pinene and (Z)- $\beta$ -ocimen. LD<sub>1</sub>, LD<sub>10</sub>, LD<sub>50</sub>, LD<sub>90</sub> and LD<sub>99</sub> of FEO was determined as 0.449, 0.654, 1.038, 1.648 and 2.402 mL kg<sup>-1</sup>, respectively.

Table 1 shows the results on antiedema effect of FEO on carrageenan paw oedema in rats. Essential oil of FEO extract showed significant anti-inflammatory effect in 0.05 and 0.200 mL kg<sup>-1</sup> doses studied which peaked at dose of 0.200 mL kg<sup>-1</sup> (56.78% inhibition) with a lesser degree of inhibition at 0.050 mL kg<sup>-1</sup> (48.89%) and 0.100 mL kg<sup>-1</sup> (26.83%). Compared to the controls,

Table 1: Anti-inflammatory effect of *Foeniculum vulgare* essential oil

Groups	Dose	Paw edema (%mL)	Inhibition (%)
Control-I (ISS)	0.1 mL	1.043±0.084	-
Control-II (ethyl alcohol)	0.1 mL	0.988±0.075	-
Indomethacin	3.0 mg kg <sup>-1</sup>	0.042±0.015 <sup>b</sup>	95.70
Etdolac	50.0 mg kg <sup>-1</sup>	0.572±0.033 <sup>acd</sup>	43.42
FEO-I	0.050 mL kg <sup>-1</sup>	0.531±0.100 <sup>acd</sup>	48.89
FEO-II	0.100 mL kg <sup>-1</sup>	0.760±0.104 <sup>d</sup>	26.83
FEO-III	0.200 mL kg <sup>-1</sup>	0.449±0.111 <sup>abe</sup>	56.78
F-value		28.949	
p-value		0.000	

Data presented as mean±SEM

FEO-1: *Foeniculum vulgare* essential oil (0.050 mL kg<sup>-1</sup>)

FEO-II: *Foeniculum vulgare* essential oil (0.100 mL kg<sup>-1</sup>)

FEO-III: *Foeniculum vulgare* essential oil (0.200 mL kg<sup>-1</sup>)

Post-hoc Tukey's HSD test:

a: p<0.001 compared to control-I (SF) group

b: p<0.001 compared to control-II (ethyl alcohol) group

c: p<0.01 compared to control-II (ethyl alcohol) group

d: p<0.001 compared to indomethacin group

e: p<0.01 compared to indomethacin group

greatest anti-inflammatory activity was observed in first reference group receiving indomethacin with a 95.7% regression of the inflammation. FEO has significantly lower anti-inflammatory effect compared to indomethacin at all doses. When compared to etodolac the extract had statistically similar effect at 0.050 and 0.200 mL kg<sup>-1</sup>. The median effective dose (ED<sub>50</sub>) of FEO essential oil was determined as 0.228 mL kg<sup>-1</sup>.

The current study clearly demonstrated anti-inflammatory effect of FEO in vivo, which equals to that of etodolac at 0.050 and 0.200 mL kg<sup>-1</sup> doses. Souza *et al.*<sup>[17]</sup> reported that essential oil of *Conyza bonariensis* was able to inhibit the LPS (Lipopolysaccharide) induced inflammation on the mice and the main monoterpene constituents of the essential oil is limonene. In an experimental model of carrageenan induced rat paw edema essential oil of *Bupleurum fruticosum* was found that it has anti-inflammatory effect and the anti-inflammatory activity could be attributed to the two major components,  $\alpha$ -pinene and  $\beta$ -caryophyllene<sup>[18]</sup>. In addition Lorente *et al.*<sup>[19]</sup> showed that essential oil of *Bupleurum fruticosum* has anti-inflammatory effect and the anti-inflammatory activity shown by the essential oil could be attributed in part to the two major components,  $\alpha$ -pinene and  $\beta$ -pinene. Limonene and  $\alpha$ -pinene are among the major components of the FEO (Table 1). Besides  $\beta$ -pinene is a minor component of the FEO<sup>[3]</sup>. When we consider the former studies<sup>[17-19]</sup> we can say that anti-inflammatory effect of FEO was thought to be due to limonene,  $\alpha$ -pinene and  $\beta$ -pinene. However, all other constituents of FEO may contribute to that activity. These are mixed together in the essential oil extract so they may have some enhancing or interfering effects on one another. Therefore, in the next step, each constituent

molecule of FEO should be tested for its anti-inflammatory activity and undergone all necessary stages of modern drug development process.

In conclusion we can say the current study showed that FEO has an anti-inflammatory effect on the rats.

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