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Evaluation of Median Lethal Dose and Analgesic Activity of *Foeniculum vulgare* Miller Essential Oil

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Abstract: Gas-chromatographic analysis, analgesic effect and median lethal dose (LD_{50}) of *Foeniculum vulgare* Essential Oil (FEO) extract were investigated in mice. In all the experiments mice were tested twice, 30 min before drug administration in the baseline latency determinated and 30, 90 and 150 min after drug administration by tail-flick device. Aspirin (150 mg kg^{-1}, peroral) and Morphine hydroclorure (10 mg kg^{-1} subcutan) were used as reference drugs. Only isotonic saline solution (0.2 mL, intraperitoneal) was given to the control group. 0.25 and 0.50 mL kg^{-1} FEO extract were given intra peritoneally to FEO groups. LD_{50} of the FEO was determined as 1.038 mL kg^{-1}. At the 150th min of the study it is determined that all of the study groups (except Morphine group) had significantly analgesic effect when compared with control group and there was no difference between Aspirin and FEO groups. It was concluded that FEO had statistically significant and same analgesic effect with Aspirin showed at the 150th min of the study.

Key words: Fennel essential oil, analgesic activity, median lethal dose, mice

INTRODUCTION

Fennel (*Foeniculum vulgare* Miller, family of Umbelliferae) a perennial aromatic herb has volatile components. Fennel seed extracts by chromatographic analysis contain trans-anethole, fenchone, methyl chavicol, limonene, α-pinene, camphene, β-pinene, β-myrcene, α-phellandrene, 3-carene, camphor and cisanethole (Baytop, 1999; Akgül, 1993). Fennel and its herbal drug preparations are used for dyspeptic complaints catarrh of the upper respiratory tract, as useful pediatric colic. *Foeniculum vulgare* Essential Oil (FEO) has a potent hepatoprotective action against CCl_4 induced acute liver injury and antiinflammatory effect in rats (Ozbek *et al*., 2003; Ozbek, 2005). In this study analysis of FEO by Gas-chromatography (GC), median lethal dose (LD_{50}) and analgesic effect has investigated.

MATERIALS AND METHODS

This study was conducted in 2003. The fennel seeds used were purchased from a local market from Van in Turkey. Voucher specimens were kept in Herbarium of Yüzüncü Yıl University, Faculty of Art and Science (VANF Nr. 691). The plant sample were kept at room temperature until they were finely ground. 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 GC device in University of Anotalia, The Research Center of Medical and Aromatic Plant and Drug, Eskişehir.

Male and female Swiss albino mice (21-26 g) were used in these experiments. The animals were housed at room temperature (22±2°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.

Mice were randomly assigned to 7 groups (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) with 8 animals in each group. One group was (T_1) treated with Isotonic Saline Solution (ISS) (0.2 mL) and considered as control and the other six groups were intra peritoneally treated with the FEO in increasing dosages of 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 mL kg^{-1} body weight. The mortality in each group was assessed 24 h, 48 h and 72 h after administration of the FEO. The percentage mortalities were converted to probits and plotted against the log_{10} of the dose of the extract (Litchfield and Wilcoxon, 1949; Kouadio *et al*., 2000).

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Analgesic response was assessed with a tail-flick apparatus (LSI Letica LE 7106, Spain) using a method initially described by D'Amour and Smith (1941).

The animals were gently immobilized by using a glove and the radiant heat was focused on a blackened spot 1-2 cm from the tip of the tail. Beam intensity was adjusted to give a tail flick latency of 5-8 sec in control animals. Measuring was terminated if the latency exceeded the end of time (15 sec) to avoid tissue damage. In all the experiments mice were tested twice, 30 min before drug adminstration in the baseline latency determinated and 30, 90 and 150 min after drug administration. Aspirin (150 mg kg^{-1}, per oral) and Morphine hidroclorure (10 mg kg^{-1}, subcutan) were used as reference standard (Matsumoto *et al.*, 2003; 2004). Only Isotonic Saline Solution (ISS) (0.2 mL, intraperitoneal) was given to the control group. 0.25 mL kg^{-1} and 0.50 mL kg^{-1} FEO were given intra peritoneally to FEO groups.

The data derived from groups for statistic analysis were standardized by using the formula by Tanker *et al.* (2001).

% analgesic activity = 100 x ($Measurement_n$ – $Measurement_{initial}$)/$Measurement_{initial}$

$Measurement_n$: Tail-flick results at 30th, 90th and 150th min.

$Measurement_{initial}$: Tail-flick results before drug administration.

All data were represented as mean±standard error mean (SEM) or as percentages. Analysis of variance (ANOVA) used for the statistical analysis of data. LSD (least significant difference) test were used for determining significance. Results with $p<0.05$ were considered as statistically significant.

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% α-pinene.

LD_1, LD_{10} LD_{50} and LD_{90} of FEO was determined as 0.449, 0.654, 1.038, 1.648 and 2.402 mL kg^{-1}, respectively.

Analgesic activity results are shown in Table 1 according to that, it was observed that at 30th and 90th min of the study there was not a significant difference between groups as analgesic effect. At the 150th min of the study, it was observed that when compared with the control group (ISS) all of the other groups have significantly analgesic activity.

Table 1: Results of FEO, Aspirin, Morphine and control groups on tail-flick test

	Analgesic activity (%)		
Groups	30th min	90th min	150th min
*ISS (control)	2.03±01.45	3.26±04.26	1.21±4.98
Aspirin (150 mg kg^{-1})	22.74±07.24	23.55±08.87	$37.29±6.91^{a}$
Morphine (10 mg kg^{-1})	$46.31±10.99^{ab}$	$69.38±12.28^{ab}$	$0.16±6.61^{b}$
FEO (0.25 mL kg^{-1})	$14.26±09.65^{c}$	$23.41±08.84^{c}$	$40.01±8.95^{ac}$
FEO (0.50 mL kg^{-1})	$0.94±04.43^{c}$	$13.75±07.08^{c}$	$30.91±9.63^{ac}$
F-value	5.792	8.680	5.506
p-value	0.001	0.000	0.000

*Isotonic saline solution, Post-hoc LSD (least significant difference) test: a: $p<0.5$ compared to control-I (ISS) group, b: $p<0.05$ compared to aspirin group, c: $p<0.05$ compared to morphine group

Morphine group showed analgesic effect at the 30th and 60th min. However at the 150th min Morphine group did not show an analgesic effect. According to the measurements in the 150th min there was not a significant difference between Aspirin group and FEO 0.25 mL kg^{-1}, FEO 0.50 mL kg^{-1} groups, so these two study groups showed same analgesic effect with Aspirin group. The analgesic effect of FEO and Aspirin began later than Morphine. FEO consists of (E)-anethole, limonene, methyl chavicol, fenchone and α-pinene. One of the components of FEO may be responsible for the analgesic effect.

There is no study related to analgesic activities of (E)-anethole, limonene, methyl chavicol, fenchone and α-pinene. However, it has been reported that *Dracocephalum kotschyi* had analgesic activity and this effect could be due to its limonene and terpineol ingredients (Golshani *et al.*, 2004).

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