ANTINOCICEPTIVE ACTIVITY OF ALPHA-PINENE AND FENCHONE

Aydin Him[1*], Hanefi Ozbek[2], Idris Turel[3], Ahmet Cihat Oner[3]

[1] Yüzüncü Yil University, Medical School, Department of Physiology, Van-Turkey
[2] Yüzüncü Yil University, Medical School, Department of Pharmacology, Van-Turkey
[3] Yüzüncü Yil University, Veterinary School, Department of Pharmacology, Van-Turkey

Summary

We aimed to investigate antinociceptive activities of some components of *Foeniculum vulgare* Mill., commonly known as fennel. In this study, alpha-pinene, limonene, fenchone, trans-anethol and alpha-copaene were investigated for analgesic effects in mice using tail-flick tests which is commonly employed as a pain model. The drugs were injected intraperitoneally in doses of 0.05, 0.1 and 0.2 ml.kg^{-1}. Alpha-pinene and fenchone caused significant reduction in the nociceptive threshold in the tail-flick test. The other compounds tested did not show significant analgesic effects. The motor coordination of mice treated with alpha-pinene or fenchone, evaluated by using the "rotarod" test, was not impaired. The results obtained in the present study indicate that alpha pinene and fenchone, major constituents of *Foeniculum vulgare* essential oil, have antinociceptive activity in tail-flick test in mice.
Key Words: *Foeniculum vulgare*, alpha-pinene, fenchone, antinociception, tail-flick test, rotarod test.

*Corresponding author: ahim@yyu.edu.tr, Fax: +90 432 2167519

Introduction

Foeniculum vulgare Mill., (commonly known as fennel) a member of *Umbelliferae*, is an annual, biennial or perennial aromatic herb, depending on the variety, which has been known since antiquity in Europe and Asia Minor. 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 (1, 2).

Fennel and its herbal drug preparations are used in public medicine for dyspeptic complaints such as mild, spasmodic gastric-intestinal complaints, bloating and flatulence (3-5), for pediatric colic and some respiratory disorders due to its anti-spasmodic effects (6). There has been many studies showing that different extracts of *Foeniculum vulgare* seeds have diuretic (7), antifungal (8, 9), antioxidant (10, 11), hepatoprotective (12), anti-inflammatory (13) effects. Essential oil of *Foeniculum vulgare* has recently been shown to have antinociceptive effects (13, 14). Trans-anethole and fenchone are the most important volatile components of the *Foeniculum vulgare* essential oil. The trans-anethol content reaches 84-90% in sweet fennel oil, whereas fenchone can be as high as 20% in bitter fennel oil (15). Chromotograhic analysis showed that fennel seeds also have methylchavicol, limonene, alpha-pinene, camphene, beta-pinene, beta-myrcene, alpha-copaen, alpha-phellandrene, 3-carene, camphor, cis-anethole (16-18). To find out which one of these components are responsible for antinociceptive activity of *Foeniculum vulgare* we tested some of the ingrediens, namely alpha-pinene, limonene, fenchone, trans-anethol and alpha-copaen, in tail-flick test, a commonly used pain model in mice.

Many of these components were reported to have various activities such as anti-inflammatory (19, 20), antimicrobial (21-23), antioxidant (24), anticarcinogen (25, 26). From all these constituents only limonene was shown to have analgesic activity shown in chemical nociception induced by intraperitoneal acetic-acid and in subplantar formalin test, but did not manifest a significant effect in hot-plate test (27). Orhan et al. (19) did not find an analgesic effect with alpha-pinene in p-benzoquinone-induced abdominal contractions in mice, but found a mild anti-inflammatory effect. The other compounds of *Foeniculum vulgare* essential oil have not been tested for analgesia yet.

In this study, alpha-pinene, limonene, fenchone, trans-anethol and alpha-copaen were investigated for their antinociceptive effects using tail-flick tests which is commonly employed as a pain model in mice.

Methods

Chemicals: (+)-Alpha-pinene, (+)-limonene, (-)-fenchone, trans-anethol and alpha-copaen were all in liquid form and each was used at the doses of 0.05, 0.1 and 0.2 $ml.kg^{-1}$, i.p. for tail-flick test. Alpha-pinene and fenchone were used at the dose of 0.2 $ml.kg^{-1}$, i.p. for rotarod test. Alpha-copaen was purchased from Fluka (Switzerland) and the others from Sigma-Aldrich (Steinheim,Germany). Morphine (10 $mg.kg^{-1}$), given subcutaneously, was used as a standard for comparison and obtained from Galen (Istanbul, Turkey).

Animals: The protocol for the study was approved by the Ethical Committee of Yuzuncu Yil University, Faculty of Medicine. Adult Balb/C mice (30 - 40 g, male) were obtained from Animal House of the Faculty of Medicine. Each experimental group included 5 animals and housed in a separate cage (48 x 35 x 22 cm) and kept at room temperature ($20 \pm 2\ ^0C$), with natural dark-light cycle and provided with pelleted food (Van Animal Feed Factory-Turkey) and water *ad libitum.*

Tail-flick test: Nociceptive response was assessed with a tail-flick apparatus (LSI Letica LE 7106, Spain) using a method initially described by D'Amour and Smith (28). 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. Mice responded to the focused heat stimulus by flicking or moving their tail from the path of the stimulus. The reaction time was automatically recorded. Beam intensity was adjusted to give a tail-flick latency of 7-8 sec in control animals. Measuring was terminated if the latency exceeded the end of time (20 sec) to avoid tissue damage. In all experiments nociceptive threshold was measured twice for each animal before the drug administration to find the base line latency, and 30, 90 and 150 min after the drug administration.

Rotarod test: Motor coordination of the mice were evaluated by using a rotarod apparatus (Degisim, Turkey) consisting of a bar with a diameter of 5.6 cm, subdivided into five compartments by a disc 19 cm in diameter. The bar rotated at a constant speed of 8 rpm. The motor coordination was assessed on the basis of the endurance time of the animals on the rotating rod. The day before the test, the animals were trained twice. On the day of the test only the mice able to stay balanced on the rotating rod between 60 and 120 sec (cut-of time) were selected. The performance time was measured before and 30, 90 and 150 min after drug treatment. The test was conducted between 9 a.m. and 1 p.m.

Statistical analysis: The results were expressed as mean ± standard error of the mean (S.E.M.). In tail-flick and rotarod tests the values after the drug administration were compared with those of the baseline latency values using repeated measures of ANOVA. To compare the analgesic effects of the drugs with those of morphine the analysis of variance (One-way ANOVA) with post-hoc Tukey's procedure for multiple comparisons was used. *P* values of less then 0.05 were considered significant.

Results

The results of the nociceptive threshold of tail-flick test of alpha-pinene, limonene, fenchone, trans-anethol and alpha-copaen are presented in Figure 1. In the control group, injected with physiological saline, there was no change in the nociceptive threshold 30, 90 and 150 min after the injection (9.72 ± 0.52, 8.40 ± 0.39, 9.56 ± 0.17 s, respectively) compared to the baseline latency measured before the injection (8.06 ± 0.15 s). Alpha-pinene produced significant analgesic effects at 0.05 $ml.kg^{-1}$ (11.56 ± 0.61, 10.48 ± 0.39, 11.32 ±0.37 vs. 7.96 ± 0.34 s), at 0.1 $ml.kg^{-1}$ (10.20 ± 0.96, 9.36 ± 0.38, 10.48 ± 0.42 vs. 6.96 ± 0.15 s), and at 0.2 $ml.kg^{-1}$ doses (11.02 ± 0.58, 9.62 ± 0.60, 13.50 ± 0.62 vs. 7.40 ± 0.36 s). Fenchone produced significant analgesic effects at 0.05 $ml.kg^{-1}$ (9.90 ± 0.42, 9.76 ± 0.33, 9.98 ± 0.50 vs. 7.22 ± 0.34 s), at 0.1 $ml.kg^{-1}$ (10.76 ± 0.84, 8.94 ± 0.48, 11.16 ± 0.36 vs. 7.06 ± 0.27 s), and at 0.2 $ml.kg^{-1}$ doses (10.74 ± 0.17, 8.76 ± 0.42, 10.44 ± 0.67 vs. 7.56 ± 0.34s). Analgesic effects of both alpha-pinene and fenchone lasted as long as 150 minute after the drug administration. Morphine significantly increased the pain threshold 30 and 90 min but not 150 min after the drug administration (15.69 ± 1.26, 18.08 ± 0.71, 11.00 ± 1.10 vs. 10.43 ± 0.51 s).

The analgesic effects of both alpha-pinene and fenchone were significantly less strong compared to that of morphine at 30 and 90 min after drug administration. Although the effect of morphine disappeared 150 min after the injection alpha-pinene and fenchone were still effective at this time point.

The motor coordination of mice treated with alpha-pinene or fenchone was not impaired 30, 90 and 150 min after the drug administration compared with that of the baseline latency (Table 1).

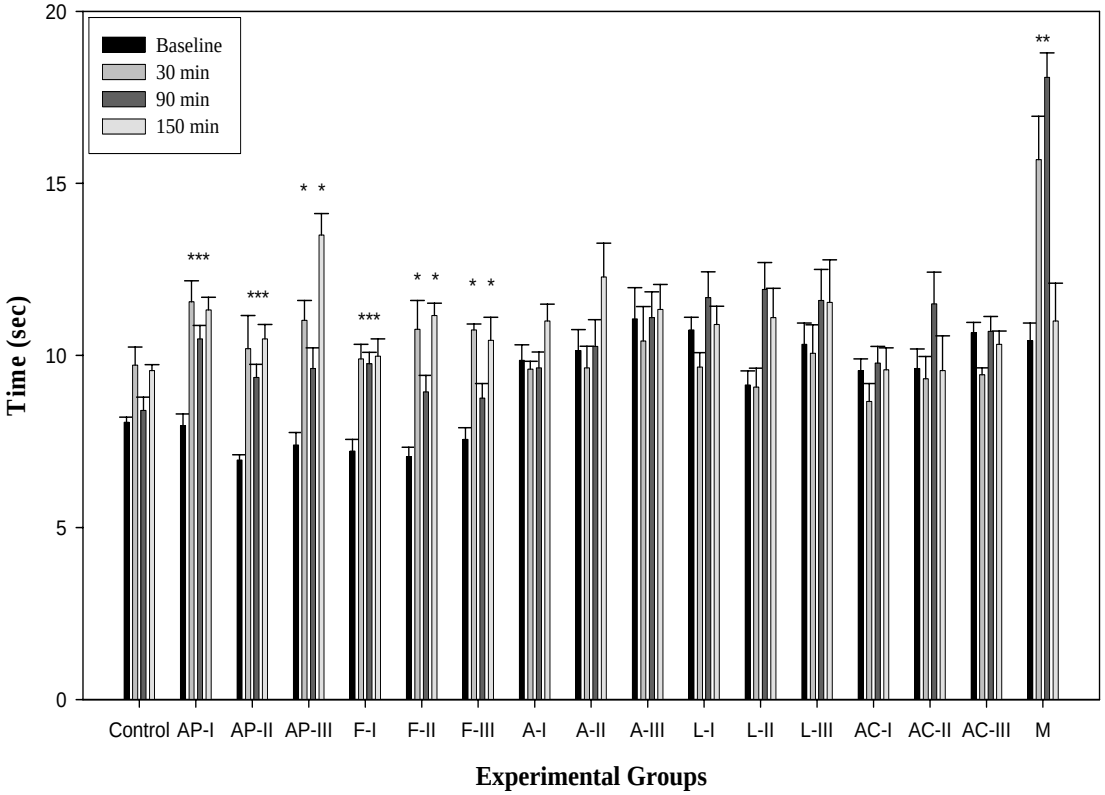

Figure 1. Effects of alpha-pinene (AP), fenchone (F), trans-anethol(A), limonen (L) and alpha-copaen (AC) at the doses of 0.05 (I), 0.1 (II) and 0.2 (III) ml/kg, i.p. on tail-flick latency in mice. M, morphine 10 $mg.kg^{-1}$, s.c. Significance in comparison with baseline latency: *p<0.05, repeated measures of ANOVA.

Discussion

In the present study we investigated antinociceptive effects of the major constituents of *Foeniculum vulgare* in tail-flick test, a commonly used pain model in mice. Out of 5 constituents tested, namely alpha-pinene, limonene, fenchone, trans-anethol and alpha-copaen, only alpha-pinene and fenchone showed a significant analgesic activity.

Table 1. Effects of alpha-pinene and fenchone on rotarod test in mice (*n=5*).

Treatment	Time (min)	Endurance time on rotarod (s)(mean ± SEM)
Control (physiologic saline)	0	120 ± 0
	30	120 ± 0
	90	103 ± 17
	150	120 ± 0
Alpha-pinene	0	120 ± 0
	30	120 ± 0
	90	118 ± 2
	150	120 ± 0
Fenchone	0	120 ± 0
	30	120 ± 0
	90	120 ± 0
	150	119.4 ± 0.6
Morphine	0	120 ± 0
	30	57.8 ± 16.7 *
	90	104.5 ± 15.5
	150	120 ± 0

*p<0.05, repeated measures of ANOVA.

Although antinociceptive effect of neither alpha-pinene nor fenchone was as strong as that of morphine their effects lasted 150 min while the effect of morphine did not last as long. The compounds causing locomotor disruption or having sedative effects may cause a false positive result in some tests measuring analgesia (29). Therefore we have also tested the highest analgesic doses of alpha-pinene and fenchone for their effect on motor coordination by rotarod test. In this study neither alpha-pinene nor fenchone caused motor incoordination at the maximal doses used. Thus the analgesic effect of alpha-pinene and fenchone is not likely to be through its effect on locomotor function or its sedative effect. Although the present study found an analgesic effects with alpha-pinene Orhan et al. (19) did not find so in p-benzoquinone-induced abdominal contractions in mice, but found a mild anti-inflammatory effect. The discrepancy between these two studies could be due to different pain models employed. Fenchone has not been studied for analgesia before and reported to have analgesic effect here first time. Limonene, which did not change pain threshold in tail-flick test in the current study, was shown to cause a significant inhibition on chemical nociception induced by intraperitoneal acetic-acid and in subplantar formalin test, both of which are inflammatory pain models (27). However the researchers did not find an antinociceptive effect with the same compound in hot-plate test model of pain, which uses a thermal stimulation like in tail-flick test. As seen from these studies a compound effective in one pain model may not exert similar effect in a different pain model, which could be related to the different mechanisms relaying the pain sensation and the different site of action of the chemicals.

Among the major compounds of *Foeniculum vulgare* essential oil screened herein, only alpha-pinene and fenchone exhibited antinociceptive activity in tail-flick model of pain in mice without inducing motor incoordination.

References

1. Hansel R, Keller K, Rimpler H. Foeniculum. Hagers Handbuch der Pharmazeutishen. New York: Springer; 1993:156-81.
2. Farrel KT. Spices, condiments, and seasonings. Westport: AVI Publishing; 1985:106-9.
3. Czygane FC. Fenchel: Teedrogen. In: Wichtl M, ed. Wissenscaftliche Verlagsgesellscaht 2nd ed. Stuttgard:; 1989:171-3.
4. Forster HB. Spasmolytische wirkung pflanzhlicher carminativa. Z Al Med. 1983;59:1327-33.
5. Forster HB, Niklas H, Lutz S. Antispasmotic effects of some medicinal plants. Planta Med. 1980;40:309-19.
6. Reynolds JEF. Essential oils and aromatic carminatives. Martindale-The Extra Pharmacopeia. 28 ed. London: Royal Pharmaceutical Society; 1982:670-6.
7. Wright CI, Van-Buren L, Kroner CI, Koning MM. Herbal medicines as diuretics: a review of the scientific evidence. Journal of ethnopharmacology. 2007;114(1):1-31.
8. Soylu S, Yigitbas H, Soylu EM, Kurt S. Antifungal effects of essential oils from oregano and fennel on Sclerotinia sclerotiorum. Journal of applied microbiology. 2007;103(4):1021-30.
9. Ozcan MM, Chalchat JC, Arslan D, Ates A, Unver A. Comparative essential oil composition and antifungal effect of bitter fennel (Foeniculum vulgare ssp. piperitum) fruit oils obtained during different vegetation. Journal of medicinal food. 2006;9(4):552-61.
10. Satyanarayana S, Sushruta K, Sarma GS, Srinivas N, Subba Raju GV. Antioxidant activity of the aqueous extracts of spicy food additives--evaluation and comparison with ascorbic acid in in-vitro systems. Journal of herbal pharmacotherapy. 2004;4(2):1-10.
11. Ruberto G, Baratta MT, Deans SG, Dorman HJ. Antioxidant and antimicrobial activity of Foeniculum vulgare and Crithmum maritimum essential oils. Planta Med. 2000;66(8):687-93.
12. Ozbek H, Ugras S, Dulger H, et al. Hepatoprotective effect of Foeniculum vulgare essential oil. Fitoterapia. 2003;74(3):317-9.
13. Choi EM, Hwang JK. Antiinflammatory, analgesic and antioxidant activities of the fruit of Foeniculum vulgare. Fitoterapia. 2004;75(6):557-65.
14. Ozbek H, Tas A, Ozgokce F, Selcuk N, Alp S, Karagoz S. Evaluation of median lethal dose and analgesic activity of Foeniculum vulgare Miller essential oil. Intl J Pharm. 2006;2(2):181-3.
15. Bernath N, Nemeth E, Kattaa A, Hethelyi E. Morphological and chemical evaluation of fennel (Foeniculum vulgare Mill.) population of different origin. J Eseent Oil Res. 1996;8:247-53.
16. Mimica-Dukic N, Kujundzic S, Sokovic M, Couladis M. Essential oil composition and antifungal activity of Foeniculum vulgare Mill obtained by different distillation conditions. Phytother Res. 2003;17(4):368-71.

17. Diaz-Maroto MC, Diaz-Maroto Hidalgo IJ, Sanchez-Palomo E, Perez-Coello MS. Volatile components and key odorants of fennel (Foeniculum vulgare Mill.) and thyme (Thymus vulgaris L.) oil extracts obtained by simultaneous distillation-extraction and supercritical fluid extraction. Journal of agricultural and food chemistry. 2005;53(13):5385-9.
18. Simandi B, Deak A, Ronyai E, et al. Supercritical carbon dioxide extraction and fractionation of fennel oil. Journal of agricultural and food chemistry. 1999;47(4):1635-40.
19. Orhan I, Kupeli E, Aslan M, Kartal M, Yesilada E. Bioassay-guided evaluation of anti-inflammatory and antinociceptive activities of pistachio, Pistacia vera L. Journal of ethnopharmacology. 2006;105(1-2):235-40.
20. Martin S, Padilla E, Ocete MA, Galvez J, Jimenez J, Zarzuelo A. Anti-inflammatory activity of the essential oil of Bupleurum fruticescens. Planta Med. 1993;59(6):533-6.
21. Hammer KA, Carson CF, Riley TV. Antifungal activity of the components of Melaleuca alternifolia (tea tree) oil. Journal of applied microbiology. 2003;95(4):853-60.
22. Macchioni F, Cioni PL, Flamini G, et al. Acaricidal activity of pine essential oils and their main components against Tyrophagus putrescentiae, a stored food mite. Journal of agricultural and food chemistry. 2002;50(16):4586-8.
23. Lee HS. Acaricidal activity of constituents identified in Foeniculum vulgare fruit oil against Dermatophagoides spp. (Acari: Pyroglyphidae). Journal of agricultural and food chemistry. 2004;52(10):2887-9.
24. Wei A, Shibamoto T. Antioxidant activities and volatile constituents of various essential oils. Journal of agricultural and food chemistry. 2007;55(5):1737-42.
25. Giri RK, Parija T, Das BR. d-limonene chemoprevention of hepatocarcinogenesis in AKR mice: inhibition of c-jun and c-myc. Oncology reports. 1999;6(5):1123-7.
26. Crowell PL, Siar Ayoubi A, Burke YD. Antitumorigenic effects of limonene and perillyl alcohol against pancreatic and breast cancer. Advances in experimental medicine and biology. 1996;401:131-6.
27. do Amaral JF, Silva MI, Neto MR, et al. Antinociceptive effect of the monoterpene R-(+)-limonene in mice. Biological & pharmaceutical bulletin. 2007;30(7):1217-20.
28. D'Amour FE, Smith DL. A method for determining loss of pain sessation. Pharmacol J Exp Ther. 1941;72:74-9.
29. Chernov HI, Wilson DE, Fowler F, Plummer AJ. Non-specificity of the mouse writhing test. Arch Int Pharmacodyn Ther. 1967;167:171-8.