ANTINOCICEPTIVE ACTIVITY OF ALPHA-PINENE AND FENCHONE

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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.

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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). Chromatographic 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 ingredients, 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 ± 2 °C), 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-off 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).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (min)</th>
<th>Endurance time on rotarod (s) (mean ± SEM)</th>
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<td>Alpha-pinene</td>
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<td>Morphine</td>
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<td>30</td>
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*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