

Alpha-Pinene as the Main Component of *Ducrosia anethifolia* (Boiss) Essential Oil is Responsible for its Effect on Locomotor Activity in Rats

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Abstract

Background: *Ducrosia anethifolia* (DA) is a medicinal plant traditionally used to treat a wide spectrum of illnesses. DA contains a series of antioxidant compounds, including alpha-pinene, which give it a sedative and relaxing effect. In spite of these effects of DA, the effects of DA essential oil on anxiety and locomotor activity in open field tests have not yet been studied. In the present study, the effects of DA essential oil and its main component of alpha-pinene on locomotor activity and anxiety were studied using open field tests.

Objectives: The purpose of this study was to investigate the effects of DA essential oil and its main component, alpha-pinene, on locomotor activity and anxiety behaviors using open field and antioxidant capacity tests in Wistar rats.

Methods: Sixty-three adult male Wistar rats (weighing 200 - 250 g) were divided into 9 groups: control, positive control (diazepam), essential oil treated groups (25, 50, 100, 200, and 500 mg/kg administered intraperitoneally, i.p.) and alpha-pinene groups (0.2 and 0.4 mg/kg, i.p.). For precisely 30 minutes after each injection in all groups, open field tests were used to assess behaviors such as rearing, line crossing, walling, grooming, and stretched attend posturing. In addition, oxidant and antioxidant parameters (malondialdehyde [MDA] and catalase [CAT]) were assessed in the rats' temporal lobes.

Results: DA (200 and 500 mg/kg doses) and alpha-pinene (0.2 and 0.4 mg/kg doses) significantly reduced locomotor activity, whereas doses of 25, 50, and 100 mg/kg of DA failed to show such an effect. Treatment with DA and alpha-pinene resulted in a significant decrease in MDA levels and a significant increase in CAT activity in comparison to controls.

Conclusions: The results of this study suggest that the main component of DA (alpha-pinene) is responsible for DA's ability to reduce locomotor activity and anxiety, which is indicative of CNS depressant activity. Moreover, it is possible that some of the motor suppression and sedation effects of the alpha-pinene in DA are due to the antioxidant capacity of this substance. However, further research and clinical evaluations are necessary to isolate and identify the other substances responsible for these activities in DA.

Keywords: *Ducrosia anethifolia* (Boiss), Alpha-Pinen, Locomotor Activity, Anxiety, Oxidative Stress, Rats

1. Background

The evolution of rodent locomotor activities and related behavior has become one of the most widely-used experiments to assess the effects of various experimental manipulations (1). In ethology, animal locomotion is any of a variety of movements or methods that animals use to move from one place to another (2). Movement is an essential skill required by all animals for a large variety of actions and is a complex neural process that occurs throughout the peripheral nervous system (PNS) and the central nervous system (CNS) (3). Locomotion control is generated by the spinal circuits necessary for movement, whereas the higher centers of the brain interact with the spinal cord to execute complex movements, such as posture and accu-

rate limb control (4).

The open field test is a very valuable and popular means of assessing animal locomotor activity (5, 6), and medicinal plants have long been used to treat disease and to facilitate the theoretical study of human physiology and pharmacology (7).

Ducrosia anethifolia (DA) (DC) Boiss. is a medicinal plant of the Apiaceae family that is widely used in Iran to treat various diseases (8). DA is known in Persian as *moshgak*, *roshgak*, or *moshkbu*. Phytochemical reports on DA essential oil have revealed that aliphatic aldehydes and other terpene compounds including limonene, citronellal, terpinolene, myrcene, alpha-pinene, pulegone, p-cymene, and pangolin are the main components of the aerial parts (9). These aromatic compounds are used as flavoring addi-

tives in foods and cosmetics (10). It has been documented that DA and some other related species have antioxidant, antimicrobial (11), antifungal, antimycobacterial (12), and sedative effects (10). DA is also used to promote relaxation and restful sleep (10). There are also some reports regarding the effects of the terpene constituents (a major component of DA essential oil), which are responsible for many of the pharmaceutical activities of medicinal plants, including antinociceptive (13), anti-inflammatory (14), anticonvulsant, anxiolytic, and analgesic effects (15).

2. Objectives

Hajhashemi et al. (10) reported DA's anxiolytic effects; however, there is no acceptable data on its locomotor activity or that of its constituents collected through open field tests. It has been demonstrated that the brain utilizes the largest amount of oxygen of the bodily organs and is therefore subject to increased oxidative stress. Therefore, this study was conducted using field tests and examination of rat temporal lobes in order to clarify the effects of DA essential oil and its main component of alpha-pinene on locomotor activity and oxidative stress in male rats.

3. Methods

3.1. Plant Material

Aerial parts (flowers and leaves) of DA were collected from the Lalehzar mountainous area (altitude of 2,800 m) in the Kerman province of Iran in July. The voucher specimens were deposited at the herbarium of the Shahid Bahonar University of Kerman (Code number: 1371). For distillation, the material was dried at room temperature and used. The DA essential oil was isolated for four h through hydrodistillation, then dried over 14% anhydrous sodium sulfate and stored in a refrigerator (4°C).

3.2. Drugs

Alpha-pinene and diazepam were purchased from Sigma Aldrich Chemical Co. The drugs were dissolved in saline solution (0.9%) and administered intraperitoneally (i.p.).

3.3. Animals

Sixty-three adult male Wistar rats (weighing 200 - 250 g) were prepared from the animal house of the Shahid Bahonar University of Kerman. The animals were housed in a room under a 12-hour light/dark cycle and fixed temperature ($22 \pm 2^\circ\text{C}$). Food and water were given ad libitum. All experimental procedures were approved by our institutional animal ethics committee (EC/94).

3.4. Motor Activity Measurements

Motor activity was measured using the open field test, which consisted of a black painted wooden cage ($40 \times 60 \times 60$ cm) equipped with a video camera placed above the cage, and acquired data were processed by maze router software (16). The animals were randomly selected and divided into 9 groups ($n = 7$): control, positive control (diazepam 2 mg/kg), essential oil-treated groups (25, 50, 100, and 200 mg/kg, i.p.) and alpha-pinene treated groups (0.2 and 0.4 mg/kg, i.p.). Open field test activities rearing, line crossing, walling, grooming, and stretched attend posturing were recorded for precisely 30 min after each injection in all groups.

3.5. Biochemical Measurements

After behavioral assessment, the animals were sacrificed under deep anesthesia by carbon monoxide, and the temporal lobes were dissected and stored at -80°C until the day of assay.

3.5.1. Brain Lipid Peroxidation

Lipid peroxidation products, such as MDA, are considered reliable indicators of oxidative damage (17). The rats' temporal lobe tissue (0.5 g) was homogenized in 10 mL of 0.1% trichloroacetic acid (TCA) and centrifuged (15,000 rpm for 15 minutes), then 4.0 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA was added to a 1.0 mL aliquot of the supernatant heated at 95°C for 30 minutes and finally cooled in an ice bath. The absorbance of the supernatant was recorded at 532 nm (Biochrom WPA Biowave II UV/Visible Spectrophotometer). The TBA reactive substances (TBARS) content was estimated according to its extinction coefficient ($155\text{mM}^{-1}\text{cm}^{-1}$) and expressed in units (U). One "U" is defined as the μmol of MDA produced in min per mg of protein (17).

3.5.2. Evaluation of Catalase (CAT) Activity

CAT activity was assayed according to the method of Dhindsa and Matowe, then estimated by monitoring the decrease in absorbance of H_2O_2 within 30 seconds at 240 nm. The reaction mixture contained 50 mM of potassium phosphate buffer (pH 7.0), 15 mM of H_2O_2 , and 100 μl of enzyme extract (18). The specific activity of CAT was expressed in terms of U/mg of temporal lobe tissue.

3.6. Statistical Analysis

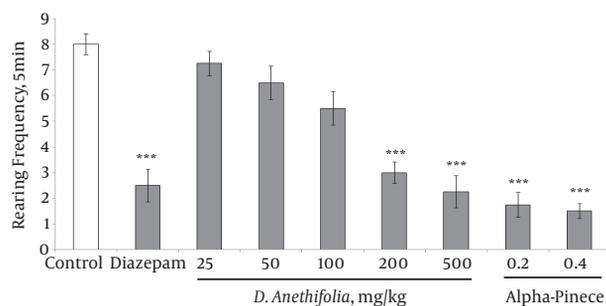
All data are represented in terms of mean \pm SEM. Results obtained from the various tasks and comparison among groups was made by analysis of variance (ANOVA), followed by the Tukey test. P values < 0.05 were considered statistically significant.

4. Results

4.1. Rearing Frequency

The rearing frequencies of animals in groups treated with diazepam, 200 and 500 mg/kg of essential oil, and alpha-pinene (0.2 and 0.4 mg/kg) were significantly ($P < 0.001$) lower than that in the control group (Figure 1).

Figure 1. Comparison of the Frequency of Rearing in the Open Field Maze Test in the Different Experimental Groups

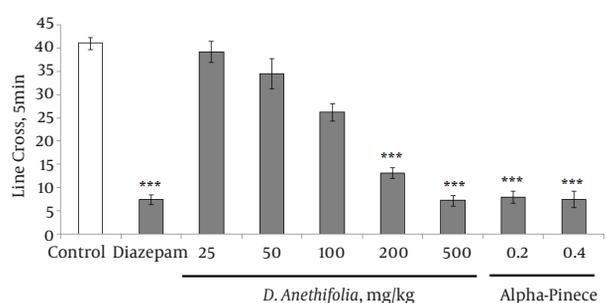


Data are represented in terms of mean \pm SEM (n = 7), ***P < 0.001 compared with the untreated control group.

4.2. Line Crossing

The frequency of line crossing (all four paws crossing a line) of rats in the control group and in groups dosed with 25, 50, and 100 mg/kg of essential oil was not significantly different. The frequency of line crossing in rats treated with 200 and 500 mg/kg of essential oil and alpha-pinene at doses of 0.2 and 0.4 mg/kg was significantly ($P < 0.001$) lower than in the control group and in groups treated with 25, 50, and 100 mg/kg of essential oil; these data are represented in Figure 2.

Figure 2. Comparison of the Frequency of Line Crosses in the Open Field Maze in the Different Experimental Groups

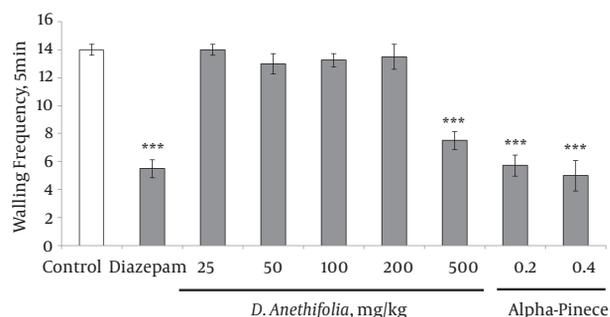


Data are represented in terms of mean \pm SEM (n = 7), ***P < 0.001 compared with the untreated control group.

4.3. Walling Measurements

Walling frequencies of the 9 groups are shown in Figure 3. The walling frequencies of the experimental groups treated with DA essential oil at doses of 500 mg/kg and with alpha-pinene at doses of 0.2 and 0.4 mg/kg were significantly lower than that of the control group.

Figure 3. Comparison of the Frequency of Walling in the Open Field Maze Test in the Different Experimental Groups

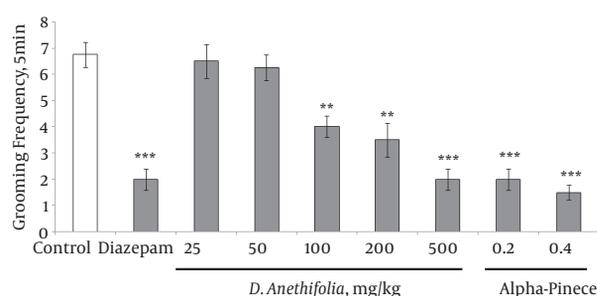


Data are represented in terms of mean \pm SEM (n=7), ***P<0.001 compared with the untreated control group.

4.4. Grooming

Figure 4 shows that the grooming frequency of experimental animals (doses of 100, 200, and 500 mg/kg of essential oil and 0.2 and 0.4 mg/kg of alpha-pinene) was significantly ($P < 0.001$) lower than that of the control group.

Figure 4. The Effect of DA Essential Oil and Alpha-Pinene on Grooming Frequency in Open Field Maze Tests in Rats



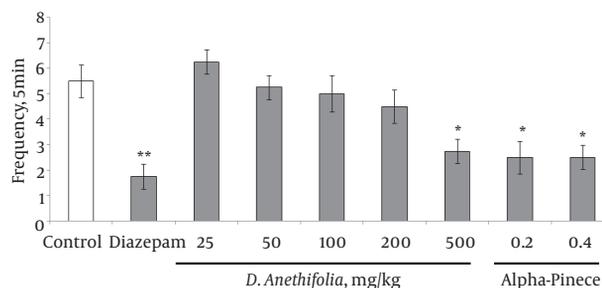
Data are represented in terms of mean \pm SEM (n = 7), **P<0.01 and ***P < 0.001 compared with the untreated control group.

4.5. Stretched Attend Posturing

The frequency of stretched attend posturing of the rats is depicted in Figure 5. Mean values recorded were 5 ± 0.50 for controls, 1 ± 0.75 for the diazepam group, and $2 \pm$

0.75, 2 ± 0.50 , and 2 ± 0.25 for the groups treated with 500 mg/kg of essential oil and with 0.2 and 0.4 mg/kg of alpha-pinene, respectively. Frequencies in the experimental animals (doses of 500 mg/kg of essential oil and 0.2 and 0.4 mg/kg of alpha-pinene) were significantly ($P < 0.05$) lower than in the control group.

Figure 5. Effect of DA Essential Oil and Alpha-Pinene on Stretched Attend Posturing in Open Field Tests in Rats

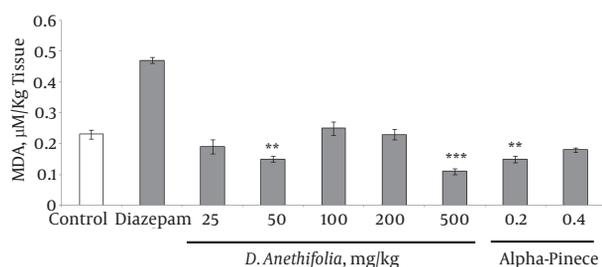


Data are represented in terms of mean \pm SEM ($n = 7$), * $P < 0.05$ and ** $P < 0.01$ compared with the untreated control group.

4.6. MDA Levels

Injection of DA essential oil at doses of 50 and 500 mg/kg and alpha-pinene at 0.2 mg/kg significantly decreased temporal lobe MDA levels compared with the control group (Figure 6).

Figure 6. Effect of DC Essential Oil and Alpha-Pinene on Brain Temporal Lobe MDA Levels

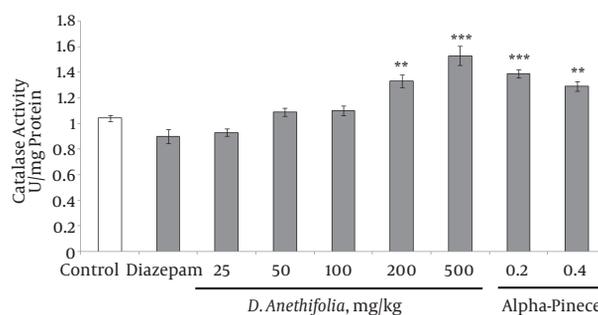


Data are represented in terms of mean \pm SEM ($n = 7$), *** $P < 0.001$ and ** $P < 0.01$ compared with the untreated control group.

4.7. The Effect of DC Essential Oil and Alpha-Pinene on Catalase

Following the injection of DC essential oil at doses of 50 and 500 mg/kg and alpha-pinene at a 0.2 mg/kg, the CAT activity in the temporal lobe of the brain was significantly higher than in the control group (Figure 7).

Figure 7. Effect of DA Essential Oil and Alpha-Pinene on CAT Activity in the Temporal Lobe of the Brain



Data are represented in terms of mean \pm SEM ($n = 7$), *** $P < 0.001$, ** $P < 0.01$ compared with the untreated control group.

5. Discussion

The effects of DA essential oil and its main component (alpha-pinene) on locomotor activity were evaluated in the present study using the open field maze test in Wistar rats. Results showed that DA essential oil and alpha-pinene significantly reduced the frequency of rearing, walling, and grooming behaviors, as well as the number of lines crossed in the experimental animals. Additionally, the results showed that DA and alpha-pinene significantly decreased oxidative stress factors.

According to the above mentioned results, it can be concluded that DA essential oil has a potential inhibitory effect on locomotion. In addition, according to our data and the results of previous studies, alpha-pinene is the most effective compound present in DA. Antioxidant therapy is a method for treating oxidative stress-related diseases and includes either natural antioxidant enzymes or factors that increase the functioning of these enzymes (19). Reports have shown that natural drugs such as DA have antioxidant properties because of the presence of alpha-pinene (20), citronellal (15), γ -terpinene, myrcene, and limonene (21).

According to the results of GC/MS analysis, DA essential oil consists of about 20 compounds, with the most important component being alpha-pinene (12.4%), which may play the greatest role in DA's inhibitory psychomotor effects (10). DA has been used in different parts of Iran to treat several ailments, such as body aches, headaches, and the treatment of colic and colds (22). Phytochemical analyses have demonstrated that DA contains a wide spectrum of bioactive compounds and terpenoids (10). In addition, the analgesic, sedative, and hypnotic properties of DA are due to the presence of alpha-pinene, citronellal (23), γ -terpinene, myrcene, and limonene (24). DA es-

essential oil may lead to a decreased alertness and CNS depression, resulting in reduced locomotor activity, which has been shown to result in anxiolytic, sedative, and analgesic effects (25). It has been reported that plants containing flavonoids, sterols, tannins, and saponin have anxiolytic activity and sedative effects, and these phytochemicals are also found in DA (26). The anxiolytic and sedative effects of several plants containing alpha-pinene have also been described, and alpha-pinene may be responsible for many of these observed effects (27). Alpha-pinene has a promoting effect on gamma-aminobutyric acid (GABA)-A receptors and increases the postsynaptic GABA-dependent chloride flows in GABA-A receptors (28). It seems that the inhibitory effect of this essential oil may act on the brain through the stimulation of GABA receptors (29). Benzodiazepine drugs, such as diazepam, significantly decrease locomotor activity and produce anxiolytic effects (30) via interaction with GABA receptors (31). This may directly activate GABA receptors or may lead to a decrease in the rate of firing of the main excitatory neurotransmitters in the brain (32).

Citronellal, citronellol, myrcene, and β -pinene are monoterpenes that are present in DA and have N-methyl-D-aspartate (NMDA) receptor antagonist activities (33). In animal models of anxiety, NMDA receptor antagonists have been shown to have anxiolytic effects and to affect various aspects of emotionality, although they have not been tested extensively (34).

Our results show that DA essential oil (500 mg/kg) and its main component, alpha-pinene, have a significant effect on locomotor activity and anxiety in open field tests. The antioxidants of the essential oil and alpha-pinene could be one reason for the above mentioned results. The other reason may be related to the interaction of some of the main compounds of DA with neurotransmitters, such as GABA and glutamic acid.

Footnotes

Authors' Contribution: Study concept, analysis, and critical revision of the manuscript for important intellectual content, Mehdi Abasnejad, Saeed Esmaeili-Mahani, and Ali Mostafavi; interpretation of data and drafting of the manuscript, Mahnaz Zamyad; Study supervision, Mehdi Abasnejad.

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