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Anxiolytic Effect of *Ocimum basilicum* Extract in Rats Tested by Elevated Plus-Maze Task

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Abstract

Background: There are reports in traditional medicine about the effectiveness of *Ocimum basilicum* (OB) in the treatment of anxiety. The Elevated Plus-Maze (EPM) has been predominantly used to investigate anxiety levels in rodents.
Objectives: The purpose of the present study was to investigate the effectiveness of extract of OB on rat behavior in the EPM test.
Materials and Methods: Male Wistar rats weighing 220 - 250 g were used in the present study. Forty rats were divided into 4 groups: three OB groups (25, 50, 100 mg/kg oral administration of OB for 7 days) and a saline control group. One day after the last day of feeding, the animals' behavior in EPM was videotaped for 10 minutes. Then, their behavior scored for formal indexes of anxiety, such as the total distance covered by animals, the percentage of entries into and the time spent in open and closed arms.
Results: The results showed that after oral feeding of OB, the percentage of open arms entry and open arms time in EPM increased in the experimental groups. OB extract has no effect on the total distance covered by animals and number of closed arm entries.
Conclusions: Our results demonstrated that the extract of OB could induce anxiolytic effect in rats after 1 week oral administration. The effect of OB was not induced through changes in motor activity. Further investigations are necessary for pharmacological providing of OB and better understanding of its anxiolytic properties and neurobiological mechanisms.

Keywords: Elevated Plus-Maze, Rat, Anxiety, *Ocimum basilicum*

1. Background

Anxiety is a natural human reaction that involves both mind and body (1). Anxiety is defined by a diffuse, unpleasant, vague sense of apprehension. It is often concomitant by autonomic symptoms, such as perspiration, palpitations, headache, and tightness in the chest (2). Although, benzodiazepines have been known as an effective treatment of anxiety disorders, they have several undesirable side effects. Therefore, further research is necessary to find new anxiolytic drugs with less adverse effects (3-5).

Literature review revealed that the use of plants in the management of illnesses has been since time antiquity, and continuously grown over time as complementary medicine because they were readily and cheaply available healthcare alternatives (6). Drugs isolated from traditional plants may have possible therapeutic effects on anxiety. Research has been conducted to study natural anti-anxiety compounds for an alternative therapy (5).

Ocimum basilicum (basil) (OB) is a plant from genus *Ocimum* belonged to family *Lamiaceae* (7). The *Lamiaceae* family is one of the mostly used medicinal plants, a worldwide source of spices, and a consolidated source of extracts with strong antibacterial and antioxidant properties. These plants are used as spices and flavors for various food products as well as effective drugs for many applications in general medicine (8, 9). Sweet basil is used in Mediterranean cuisine and foods such as soup, cream cheese for sandwiches, and pasta dishes (10, 11). All aerial segment of the plant were used in medicine for remedy of cold and its sedative effect. It was also used to cure heartburn, soothe nerves, as well as manufacturing perfume (12, 13). It has been traditionally used for treatment of a variety of neurological disorders such as anxiety, headache, migraine, nerve pain, inflammation, cough, digestive disorders, chest and lung complaints, fever, insect bites, menstrual cramps, sinusitis and as carminative and antispasmodic (10, 11). The essential oil from OB is used in food, health, and cosmetic industries (14, 15). Essential oil of the plant is a combination of terpenoids and phenylpropanoids such as citral, eugenol, methyl eugenol, and methyl chavicol (16). Its major ingredients are monoterpene alcohols and phenols, amongst which are menthol, carvacrol, linalool, thymol, and eugenol (14, 15).

2. Objectives

The Elevated Plus-Maze (EPM) is one of the most widely used animal models of anxiety (17). EPM is a validated and reliable test for detecting both anxiolytic- and anxiogenic-

like effects of agents (18-20). There are no published reports in literature about the effect of the extract of OB on anxiety. On the basis of these considerations, this study was designed to characterize the anxiolytic-like activity of extract prepared from OB leaves, using an EPM test.

3. Materials and Methods

3.1. Plant Material

The aerial parts of *Ocimum basilicum* (basil) were purchased before flowering from local market and stored in at low temperature for further use.

3.1.1. Preparation of Extract

At first, basil was air-dried and milled. A total of 100 g of the milled flower was extracted with 80% ethanol. After the third day, the flower extract was separated from the flower with a cloth sieve. For complete separation of the leaf from the extract, filter paper was used to sieve the extract it into a bottle. The extract was then taken to the laboratory for the process of evaporation. The evaporation process involved the total removal of ethanol and water with which the extraction took place. The extract was concentrated using a rotary evaporator at 40°C. Then, it was dried at the laboratory temperature and dissolved in saline.

3.2. Animal

Male Wistar rats weighing 220 - 250 g were purchased from Razi institute, Tehran, Iran. Forty animals were divided into 4 groups: three OB groups (25, 50, 100 mg/kg administration for 7 days by feeding) and one saline group. They were housed in groups of 4 per cage under a 12:12 dark/light cycle (lights on at 07:00 AM) at 22 ± 2°C and given free access to food and water. Rats were randomly assigned to different treatment groups (n = 10). Each animal was tested only once. All experiments were carried out in a quiet room under controlled light conditions between 10:00 AM and 2:00 PM. Furthermore, all experiments were conducted in accordance with international standards of animal welfare recommended by the society for neuroscience (21).

3.3. Behavioral Test

3.3.1. Elevated Plus-Maze Test

EPM is one of the most commonly used models to assess anxiety in small rodents. Its design was similar to that originally described by Lister (22). In summary, the apparatus is composed of two open (50 cm × 10 cm× 1 cm) and two enclosed (50 cm × 10 cm × 50 cm) arms that radiate from a central platform (10 cm ×10 cm) to form a plus sign (Figure 1). The plus-maze was elevated to a height of 50 cm above the floor level by a single central support. Oral administration of OB extract was done in 3 doses (25, 50, 100 mg/kg prescription for one week by feeding). One day after the last day of feeding, animals' behavior in the experimental sessions (10 minutes) was recorded by a video camera located above the maze, interfaced with a monitor and a computer in an adjacent room. The recorded behavior in the computer was subsequently scored for conventional indexes of anxiety. These indexes were the total distance covered by animals, the time spent in, and the number of entries into two kinds of arms within the test session.

3.4. Statistical Analysis

The difference between the means was determined by 1-way ANOVA followed by Tukey post hoc analysis. Results are expressed as Mean ± SEM. If the P value was under 0.05, results would be considered statistically significant.

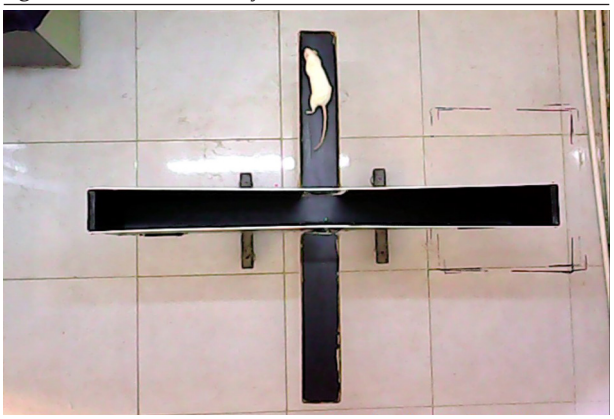
4. Results

The total distance travelled by the OB extract treated groups within the 10-minute test was not significantly ($P > 0.05$) different from that of the control group (Figure 2). The effects of different doses of the OB extract on the duration of time spent in the open arms are shown in Figure 3. One-way ANOVA indicated that OB treated groups spent more time in the open arms compared to the control group. Tukey post hoc test analysis showed that extract-treated groups under the doses of 50 and 100 mg/kg, spent more time in the open arms ($P < 0.01$).

The effects of different doses of hydroalcoholic extract of OB on the percentage of animal entries into the open arms are shown in Figure 4. One-way ANOVA indicated that compared to the control group, extract of OB caused an increase in the percentage of animal entries into the open arms. Also, Tukey post hoc test analysis showed that, OB has a significant increase in the percentage of animal entries into the open arms in concentrations of 50 mg/kg ($P < 0.05$) and 100 mg/kg ($P < 0.01$), but not at 25 mg/kg in comparison to the control group.

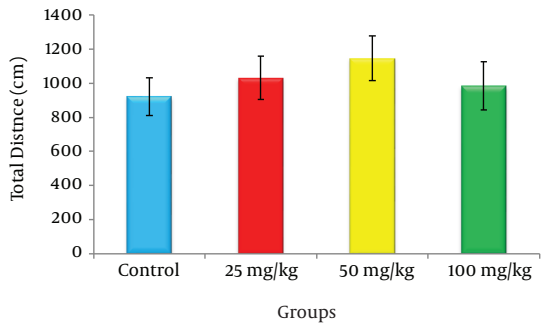
The number of entries into the closed arms was not significantly different between the OB treated and control groups (Figure 5).

Figure 1. Elevated Plus-Maze System



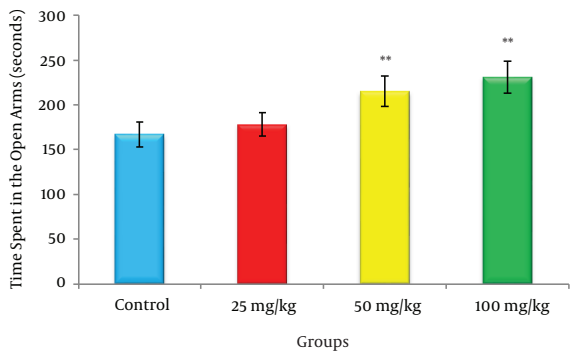
The maze contains two open arms and two closed (wall-sheltered) ones.

Figure 2. The Effects of *Ocimum basilicum* Extract (25, 50, 100 mg/kg) on the Total Distance Travelled by the Animals Within the 10 Minutes Test Session



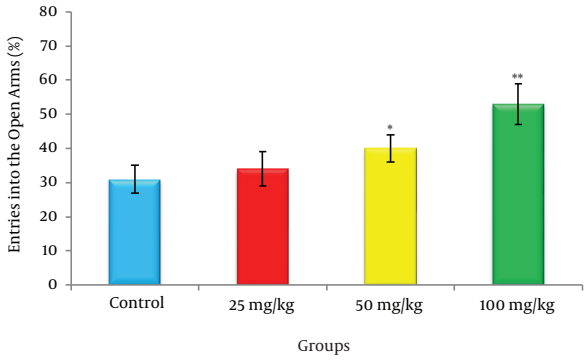
Data are expressed as mean ± SEM. Comparisons were made using ANVOA followed by post hoc Tukey multiple comparisons test (n = 10, in each group).

Figure 3. The Effects of *Ocimum basilicum* Extract (25, 50, 100 mg/kg) on the Time Spent by Animals in the Open Arms Within the 10 Minutes Test Session



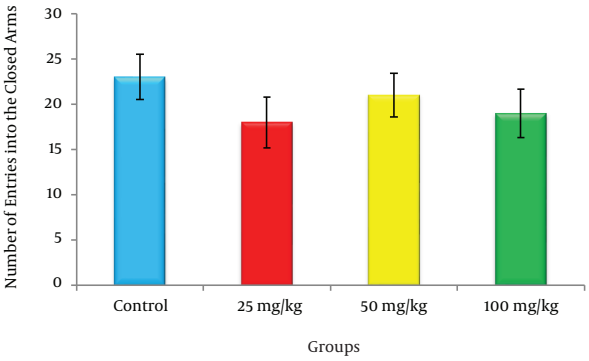
Data are expressed as mean ± SEM. Comparisons were made using ANVOA followed by post hoc Tukey multiple comparisons test (n = 10). *: P < 0.05. **: P < 0.01.

Figure 4. The Effects of *Ocimum basilicum* Extract (25, 50, 100 mg/kg) on the Percentage of Animal Entries Into the Open Arms of the EPM Within the 10 Minutes of Test Period



Data are expressed as mean ± SEM. Comparisons were made using ANVOA followed by post hoc Tukey multiple comparisons test (n = 10). *: P < 0.05. **: P < 0.01.

Figure 5. The Effects of *Ocimum basilicum* Extract (25, 50, 100 mg/kg) on the Number of Closed Arm Animal Entries During the 10 Minutes Test Session



Data are expressed as mean ± SEM. Comparisons were made using ANVOA followed by post hoc Tukey multiple comparisons test (n = 10).

5. Discussion

Our experiment studied the behavioral effects of the hydroalcoholic extract from the OB. The results of the present study demonstrated that the extract of OB increased both the percentage of entries and the percentage of the time spent of rats in the open arms of the maze. In other words, the extract was able to produce anxiolytic effect in rats after one week oral administration. The effect of OB was not produced by changes in motor behavior, because the total distance travelled by the animals was not altered. An increment in the time and the percentage of the entrances into the open arms without changing locomotors behavior is a potent sign for anxiolytic effect of the OB extract (19, 23-28).

According to the literature, benzodiazepines are the major class of compounds used in anxiety (3). However, as benzodiazepines present a narrow safety margin between their anxiolytic effect and unwanted side effects, many researchers has prompted to evaluate new compounds (4, 5, 29, 30). Given the above mentioned problem, this study aimed to determine the anxiolytic-like activity of the hydroalcoholic extract produced from OB. Herbs had been utilized for medicinal goals long before recorded history (31) and their utilization in medicate is still well-disseminated around the world (32, 33). Many herbs exert known pharmaceutical effects on the CNS and are able to act on chronic conditions such as anxiety that do not respond

well to conventional medicinal treatments (34). Various kinds of plant medicines have been used as anxiolytics in different regions of the world (5). Some parts of OB are traditionally used as antispasmodic, aromatic, carminative, digestive, galactogogue, stomachic, and tonic agents (35-37). They have also been used as a traditional medicine to treat various ailments such as migraine, insomnia, depression, nausea, abdominal cramps, poor digestion, gonorrhea, and persistent diarrhea (38). These therapeutic effects are caused by some properties of this plant. These properties include its hypoglycemic, hypolipidemic (39), antiulcerogenic (40), antimicrobial (41), chemopreventive (42), antimutagenic (43), antioxidant (44), and antihypertensive (45). Externally, they have been used for the treatment of skin infections (46).

Several studies have been conducted to explore multiple neural substrates and mechanisms that contribute to the etiology of anxiety, among them the imbalance between oxidation and antioxidant defense system has gained much attention (47). Some studies have demonstrated the role of oxidative stress in the anxiety of rodents (48-50). Also, many reports suggest that the perturbation of antioxidant defense is important in the process of emotional disorders such as depression and anxiety (47). According to these reports, the induction of oxidative stress in mice CNS occurs concomitantly with anxiety (50). High degree of anxiety has been positively associated with the increase in reactive oxygen species (ROS) level. In another study, oxidative stress in hippocampus of adult rats was shown to be anxiogenic (51, 52). Moreover, the increase in anxiety-like behavior is reversed by antioxidant tempol treatment, suggesting direct involvement of oxidative stress in mediating anxiety-like behavior of rats (53).

Previous studies have shown that OB contains a high degree of antioxidant activity (54), which is attributed to its terpenoids, polyphenols, and flavonoids like quercetin, kaempferol, myricetin, tannins like catechin, (55) and essential oils like eugenol and methyl chavicol (56). It has been mentioned that the antioxidant activity of plants might be due to their phenolic compounds. The phenols with linalool are the major components of OB (57). In the present study, OB extract decreased the level of anxiety in animals. In connection to this finding, an effect of linalool inhalation has been shown to reduce anxiety (58). Also, it has been reported that linalool prevents glutamate (the main excitatory neurotransmitter) from binding to its receptors in the neocortex of rats (59). The presence of linalool, linalyl acetate, in the plant extract supports the claim that the extract has sedative effect (60). According to another study, it has been shown that kaempferol induce anxiolytic activities in the elevated plus-maze test in mice (61). Quercetin decreases corticotrophin releasing factor (CRF) expression in the brain, which is commonly implicated in the high anxiety (62). CRF release from hypothalamus and consequent secretion of adrenocorticotropic hormone form the anterior pituitary and glucocorticoid from the adrenal cortex are the major endocrine response to stress (63). It is possible that these compounds play essential role in anxiolytic properties of OB in EPM test.

In summary, our data provide direct evidence that oral administration of OB extract may have anxiolytic effects in rats. Possibly, the anxiolytic activity observed in this work was not only dependent on the polyphenols, flavonoid, or essential oil content, but also related to other substances with antioxidant activities. Further investigations are necessary for providing pharmacological products of OB and better understanding of anxiolytic properties and neurobiological mechanisms of OB extract.

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Footnotes

Authors' Contribution: Study concept and design: Alireza Komaki, Zahra Nemati, and Siamak Shahidi; acquisition of data: Zahra Nemati, Samaneh Oveisi, and Alireza Komaki; analysis and interpretation of data: Alireza Komaki, Samaneh Oveisi, and Zahra Nemati; drafting of the manuscript: Alireza Komaki and Siamak Shahidi; critical revision of the manuscript for important intellectual content: Alireza Komaki and Zahra Nemati; statistical analysis: Alireza Komaki and Siamak Shahidi; administrative, technical, and material support: Alireza Komaki, Samaneh Oveisi, and Zahra Nemati; and study supervision: Alireza Komaki and Siamak Shahidi.

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