

# Effect of *Citrus aurantium* L. Essential Oil and Haloperidol on Anxiety in Male Mice

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## Abstract

Relationship between sociability and the amount of brain's dopamine is very well known. In this study, we have examined the effect of *Citrus aurantium* L. essential oil on anxiety and its interaction with dopaminergic pathways. 70 male mice were assigned into experimental, control, and sham groups. Essential oil of *Citrus aurantium* L. was injected intraperitoneally at doses of 0.5%, 2.5% and 5% for 5 days. Subcutaneous injection of haloperidol was administered on the fifth day, 30 minutes before the injection of the essential oil. The anxiety-related behavior of mice was then assessed by elevated plus-maze test. The result of this study showed that the injection of *Citrus aurantium* L. essential oil at doses of 2.5% and 5% increased significantly the time spent in the open arms (OAT) ( $p < 0.001$ ), also there was a significant increase in the number of entries into the open arms (OAE). Injection of different doses of the essential oil along with haloperidol significantly increased OAT ( $p < 0.001$ ). The results demonstrate that the essential oil of *Citrus aurantium* L. along with haloperidol medication reduces anxiety-related behaviors.

## Keywords

Anxiety, *Citrus aurantium* L., Essential Oil, Haloperidol, Mice

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## 1. Introduction

Anxiety can be defined as a reaction of threatening and/or stressful situations, which appears in forms of illness, pre-occupation and tension. Anxiety is usually a temporary phenomenon, but it should be treated as a serious illness if it lasts longer than usual or if it occurs in absence of threatening or stressful situations. If it appears acutely along with extreme fear, anxiety turns into what in psychiatry is called panic disorder [1]. The psycho-

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logical symptoms of anxiety include malaise, irritability, impatience, feeling of imminent danger, panic, agitation, fear, failure to keep calm, difficulty in concentrating, and sleep disorder [1].

Anxiety-related behaviors are closely affiliated with a set of areas in the brain. A network in the brain which includes amygdala, ventromedial prefrontal cortex (vmPFC), and hippocampus are recognized as the main areas in connection with anxiety disorders. Amygdala plays a critical role in danger detection by evaluating the environment for potential dangers. It prepares the body against dangers or threats by sending projections to areas related to autonomic responses as a means for regulating such activities as heart rate, blood pressure, respiration and transpiration. By controlling amygdala, ventromedial prefrontal cortex and hippocampus can inhibit fear. These areas, by providing necessary data and remembering past data, play an important part in inhibiting fear and anxiety [2]. Septo-Hippocampal System also has a significant role in overcoming anxiety, since the effects of anxiety-related behaviors correspond to temporary or permanent damages of septum and hippocampus [3]. Neurotransmitter systems that function in relation with aforesaid areas are also affiliated with anxiety processes. The relation between sociability and dopamine has already been recognized in the past studies. It is very common to use dopaminergic drugs such as amphetamines to improve social performances and confidence. A recent study shows that there is a close link between social status of people and adhesion of D2 and D3 dopamine receptors in striatum [4]. Some other studies also show how in people suffering from social anxiety, the adhesion of dopamine receptors in striatum is reduced [5]. Different drugs are used for treating different anxiety conditions. Some of these drugs reduce anxiety by affecting dopamine receptors and regulating brain activity. An old drug for mental illnesses, haloperidol is an inverse agonist of dopamine which is used for treating schizophrenia, acute mental or delirious conditions [6]. But other than synthetic drugs, there are some herbal medicines which have been noted in traditional medicine for their calming effects. Bitter orange, scientifically known as *Citrus aurantium* L., is a plant from Rutaceae family [7]. The flowers of this plant, known as “spring orange” in Iran, have primarily medical usages. *Citrus aurantium* L. has active ingredients with different effects. Synephrine and octopamine are two of the strongest active ingredients in *Citrus aurantium* L. The plant also contains flavonoids such as limonene, hesperidin, neohesperidin, naringin, and tangeretin. In addition, it also contains furanocoumarin [8]. In traditional medicine, the essence of *Citrus aurantium* L. is used for their sedative effects [9].

In this study, we have investigated the anti-anxiety effect of *Citrus aurantium* L. essential oil and its interference with haloperidol as an inverse agonist of dopamine on male mice.

## 2. Materials and Methods

This study was done on 70 male albino mice weighed 22 to 28 g supplied by Pasteur Institute. The mice were kept in the animal room of the Medical Faculty of Baghiatallah University. The temperature of the animal room was 22°C to 24°C and the mice were kept under a 12-hour light-dark cycle. The mice had complete access to sufficient food and water, and their cages were cleaned every two days. The terms and conditions of keeping laboratory animals and handling were applied completely during the experiment according to the current law of animal care of Medical Sciences Research Center of Medical Sciences Azad University.

### 2.1. Essential Oil Preparation

Collected *Citrus aurantium* L. flowers were dried in darkness and pulverized. 300 g of the dried powder of *Citrus aurantium* L. was put in a 1000 cc balloon and distilled water was added to make up the volume to 1000 cc. then, the balloon was put on the heater and connected to the Clevenger apparatus for 2 hours. 2 drops of n-hexane was added to the tube. Essential oil was collected and dewatered using sodium sulfate. Then, it was put in open vials and due to exposure to the air the n-hexane vaporized. The vials were completely covered by aluminum foil and kept in a cool place. The essential oil was obtained at different densities of 0.5%, 2.5% and 5%.

Haloperidol (2 mg/kg) was supplied by Kimiadarou Company. Haloperidol was applied through subcutaneous injection using sodium chloride 9% (normal saline) sterile.

### 2.2. EPM Test

The plus-maze apparatus was made of Plexiglas and consisted of two open (30 × 5 cm) and two closed (30 × 5 × 15 cm) arms. The arms extended from a central platform of 5 × 5 cm. The apparatus was mounted on a Plexiglas base raising it 38.5 cm above the floor. The test consisted in placing a mouse in the center of the apparatus (facing a closed arm) and allowing it to freely explore. All experiments recorded using personal camcorder. The

number of entries into the open arms and the time spent in these arms were scored for a 5-min test period. An entry was defined as placing all four paws within the boundaries of the arm. The following measures were obtained from the test: the total number of arm entries; the percentage of arm entries into the open arms; the time spent in the open arms expressed as a percentage of the time spent in both the open and closed arms. Anxiolytic activity was indicated by increases in time spent in open arms or in number of open arm entries. Total number of entries into either type of arm was used as a measure of overall motor activity.

### 2.3. Treatment Methods

The animals were injected intraperitoneally with the *Citrus aurantium* L. essential oil for 5 days. On the fifth day and thirty minutes before applying *Citrus aurantium* L. essential oil, haloperidol (2 mg/kg) was applied to the experimental groups through subcutaneous injection. Thirty minutes after the injection of haloperidol, all the groups were assessed for anxiety-related behavior by elevated plus-maze test.

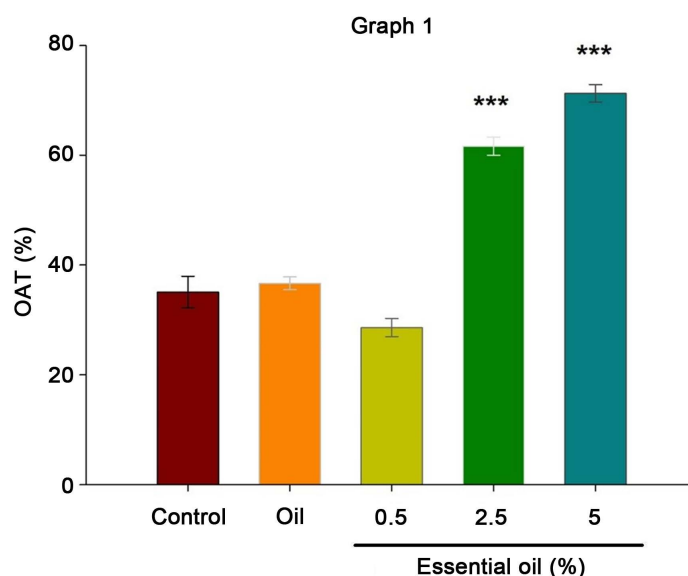
### 2.4. Statistical Analysis

One-way analysis of variance was then employed in order to draw a comparison between experimental, control, and sham groups. A follow-up test (Tukey) was also applied in a significant level ( $P < 0.05$ ) to analyze the results in different groups. The statistical analyses were done using SPSS v.17 software.

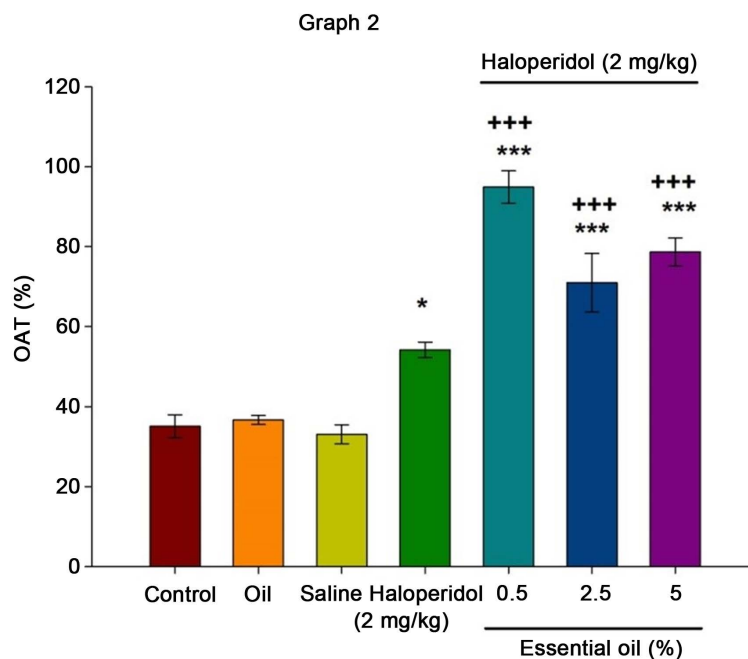
## 3. Results

**Graph 1** shows that the intraperitoneal injection of essential oil of *Citrus aurantium* L. (at doses of 0.5%, 2.5%, and 5%) increased the time spent in the open arms. In terms of the time spent in the open arms, there is a significant difference between the groups that were injected with doses of 2.5% and 5% and the control groups ( $P < 0.001$ ).

**Graph 2** demonstrates that subcutaneous injection of haloperidol (2 mg/kg) results in the increase of time spent in open arms, which shows a significant difference from control group ( $P < 0.05$ ). Injection of haloperidol and *Citrus aurantium* L. essential oil (at doses of 0.5, 2.5 and 5 percent) together increases the time spent in the open arms significantly ( $P < 0.001$ ). The percentage of time spent in the open arms was significantly higher in groups that received haloperidol and the essential oil together groups receiving only haloperidol ( $P < 0.001$ ).



**Graph 1.** Comparison between experimental groups (received of *Citrus aurantium* L. essential oil at doses of 0.5%, 2.5%, and 5%), control group and sham group (received olive oil) of antianxiety effect of *Citrus aurantium* L. essential oil, in terms of the time spent in the open arms. Mean  $\pm$  S.E.M.,  $n = 7$ . \*\*\* $P < 0.001$  versus control and sham groups. OAT spent time in open arms.



**Graph 2.** Comparison between experimental groups (received of *Citrus aurantium* L. essential oil at doses of 0.5, 2.5, and 5 percent, along with haloperidol), control group and sham group (received olive oil) antianxiety effect of *Citrus aurantium* L. essential oil, in terms of the time spent in the open arms. Mean  $\pm$  S.E.M., n = 7. \*P < 0.05 and \*\*\*P < 0.001 versus control and sham groups. +++++P < 0.001 versus haloperidol group. OAT is the spent time in open arms.

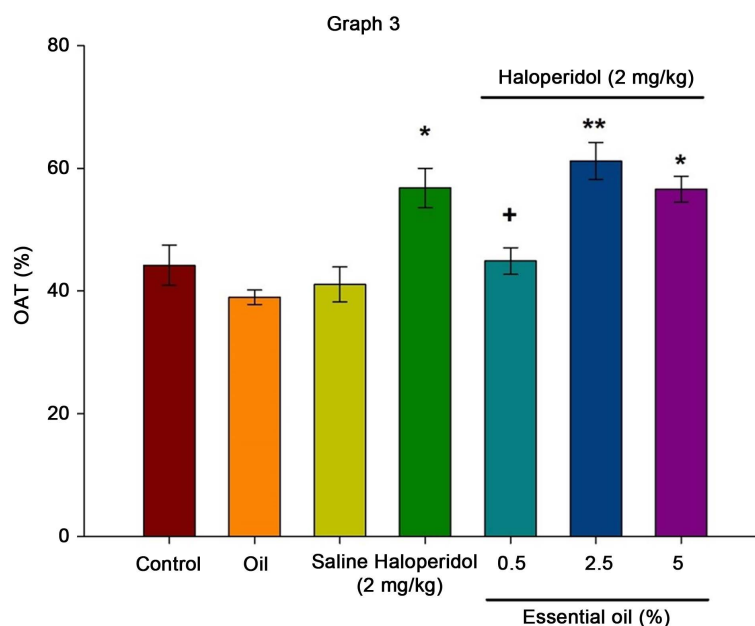
As demonstrated in **Graph 3**, the subcutaneous injection of haloperidol (2 mg/kg) is also effective in increasing the number of entries to the open arms, which shows a significant difference from control group ( $P < 0.05$ ). Applying haloperidol and of *Citrus aurantium* L. essential oil (at doses of 2.5% and 5%) together leads to the increase of number of entries to the open arms which shows a significant difference from the control group when applied at doses of 2.5 percent ( $P < 0.01$ ), and at dose of 5 percent ( $P < 0.05$ ).

#### 4. Discussion

The results of this study show that of *Citrus aurantium* L. essential oil can, increase the time spent in the open arms, as well as the number of entries to the open arms dose dependently. These results demonstrate that of *Citrus aurantium* L. essential oil can reduce the amount of anxiety in mice significantly, compare to control and sham groups. The best result emerged in groups receiving the highest dosage (5%).

Past studies have focused on different effects of *Citrus aurantium* L. essential oil. The ingredients of *Citrus aurantium* L. essential oil and also of the leaves of this plant contain alkaloid, linalool, linalyl acetate, myrcene, limonene, and limonoid, as well as a high level of flavonoids; these ingredient sare richer in the flowers of the plant than in its leaves [10] [11]. A study shows that linalool can play a critical role in reducing induced seizures in Syrian hamsters [12] [13]. Komiya demonstrates in his research that limonene has anti-anxiety effects on Syrian hamsters [14]. Limonene also has sedative and anticonvulsant effects, as tested on animals [12].

In addition, flavonoids have wide physiological effects such as prevention of lipoproteins oxidation with low molecular weight and stability of immune cells, and therefore they have extensive usage in treating mental illnesses, viral infections, inflation and allergies [15]. Mahmoodi shows that flavonoids in *Citrus aurantium* L. have sedative effects and can help patients suffering from depression [16]. Past studies attest that the limonene in *Citrus aurantium* L. enter the brain through peripheral circulation and causes the simultaneous reduction of neurons in the central nervous system and therefore has anti-anxiety effects [17]. Coumarin is another ingredient in *Citrus aurantium* L. which has anti-stress and sedative effects [16]. The linalool in *Citrus aurantium* L. also



**Graph 3.** Comparison between experimental groups (received of *Citrus aurantium* L. essential oil at doses of 0.5, 2.5, and 5%, along with Haloperidol), control group and sham group (received olive oil) in antianxiety effect of *Citrus aurantium* L. essential oil, in terms of the number of entries to the open arms. Mean  $\pm$  S.E.M.,  $n = 7$ . \* $P < 0.05$  and \*\* $P < 0.01$  versus control and sham groups. + $P < 0.5$  versus haloperidol group. OAE is the number of entries to the open arms.

inhibits the release of acetylcholine from synaptic terminals and reduces the CNS activity and causes relaxation [18]. Shabanian *et al.* (1387) show in their study that *Citrus aurantium* L. and diazepam reduce the anxiety in patients before surgical operations and therefore they can be used as an effective pre-medication for surgery [19]. Another study on the anti-anxiety and sedative effects of *Citrus aurantium* L. with a possible effect on the enzymatic metabolism of barbiturates in mice shows that *Citrus aurantium* L. is an anxiolytic compound which prolongs the time of sleep [20]. Lehrner's research (2000) demonstrates that the release of the essence of *Citrus aurantium* L. in the dental waiting room reduces the anxiety in patients [21]. The current study also showed that *Citrus aurantium* L. reduces the anxiety-related behaviors in mice, and the strongest result came with the highest dose of 5%. According to the results of this study, as well as those of the past studies, it is clear that the essential oil of *Citrus aurantium* L. and its various ingredients reduce the anxiety by effecting the areas in the brain that correspond to the processes of stress and anxiety. The results of this study confirm those of the past studies.

Haloperidol was also used as an inverse agonist of dopamine in order to compare the effects of *Citrus aurantium* L. with dopaminergic pathways. The dopaminergic system in the brain is one of the primary neurotransmitter systems that is much affected by mental stimuli [19]. As reported, stress inducers lead to the release of dopamine in various areas of the brain including prefrontal cortex and stimulate the cognitive and behavioral processes [22]. Different doses of agonists and antagonists of dopamine (haloperidol, sulpiride) on rats have shown reduction of time spent in the open arms, as well as the number of entries to the open arms, in comparison to the control group, and have anxiety effect in fact [23]. According to the studies done, haloperidol significantly reduces the anxiety in two behavioral tests on mice under treatment [24]. The results of the current study showed that the injection of haloperidol, either alone or along with *Citrus aurantium* L., increases the time spent in the open arms, as well as the number of entries to the open arms, which shows a significant difference from the sham and control groups. Some of the past studies have focused on the various effects of *Citrus aurantium* L.: Carvalho *et al.* (2002) have investigated the anti-anxiety effects of the essence of *Citrus aurantium* L. through elevated plus maze and open field tests, with positive results [20]; Khoori *et al.* (1385), investigating the effects of *Citrus aurantium* L. on electrophysiological properties of atrioventricular node (node-AV) in rabbits, have shown that *Citrus aurantium* L. has antiarrhythmic effects on the heart and also has a protective role

against depressant effects of ouabain on node-AV [25]. Karla *et al.* (2006), experimenting on rats, have shown that haloperidol, by blocking the dopamine D2 receptors, can help treating schizophrenia [26]. A study done by Stowe (2008) demonstrates that using agonists of dopamine can prevent the Parkinson disease [27]. Hosseini *et al.* (2011), by inducing electroshocks and depression, have investigated the anti-depressant effects of ethanol extract of *Citrus aurantium* L. and have shown that it reduces depressant effects [28]. Anticonvulsant effects of *Citrus aurantium* L. have been reported in a study by Abbas Nezhad [29].

## 5. Conclusion

The current study shows that the essential oil of *Citrus aurantium* L. can reduce the anxiety-related behaviors in male mice. In drawing a comparison with haloperidol with essential oil of *Citrus aurantium* L., the anti-anxiety effect of *Citrus aurantium* L. is in part due to affecting dopaminergic pathways.

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