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# Study of the Anxiolytic Effect of *Ocimum Basilicum* Hydroalcoholic Extract in Mice.

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**ABSTRACT**

Traditional medicine literatures introduce basil (*Ocimum basilicum L*) as a sedative plant. This study aims at examining its anxiolytic effects scientifically. In present study, NMRI mice were divided into 8 groups (n=8). Basil hydro alcoholic extract anxiolytic effect was evaluated (25, 50, 100, 200, 400, 600 mg/Kg) using EPM model. Diazepam (1mg/Kg, IP) was used as a standard drug. Then, anxiety behaviour were recorded. One Way Anova with LSD post hock test were used for data analysis. Minimum significance level was $P<0.05$. In all basil extract groups, percentage of Open-Arm Time (OAT%) and Open-Arm Entry(OAE%) was reduced ($P<0.05$);this means the anxiety was decreased. In addition, Locomotor Activity(LA) decreased with higher dosage of basil hydro alcoholic extract ($P<0.05$). Having flavonoids, basil can affect the benzodiazepine receptors in the $GABA_A$ receptors and produce anxiolytic, muscle relaxing, and soporific effects.

**Keywords:** Anxiety, Basil, Diazepam, Elevated Plus Maze, Mice

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## INTRODUCTION

Anxiety disorders are of the major human problems that infect 25% of Americans. Typically, the chemical anxiolytic medications have side effects including: sense of dependence, drug withdrawal syndrome (in case of discontinuation in intake of medications), and drowsiness and lethargy. Therefore, studies are underway to find effective drugs with fewer side effects. Medicinal plants, used for treating different illnesses, are an option. As the combination of effective agents with other substances in herbal medications creates a biological balance, they are not accumulated in body and associated with few or no side effects; therefore, they have a considerable advantage over chemical drugs [1].

*Ocimum basilicum* (Ob) is of the family Lamiaceae (mints), used as a medicinal plant, culinary herb, and fresh vegetable. It is an herbaceous annual fragrant plant with stems 15-45 cm tall branching from the base, opposite, elliptic, sharp, and serrated edge leaves, and 4-6 stamens scented flowers that are white (and sometimes purple) in color, and arranged in a terminal spike. Its leaves and flower branches are used for their medicinal properties [2].

Ob contains 0.5% to 1.5% essential oil whose major constituents are methyl chavicol, linalool, camphor, EUGENOL, GERANIOL, CARVACROL and GERANIOL (OMID BEIGI, 2006) [3].

Different [medicinal] properties of *Ob* have been discovered and used for treating dyspepsia, bronchitis, dysentery and asthma, among many others (Singh et al., 1991). It is traditionally used for the treatment of anxiety, headache, migraine, neuralgia, inflammation, cough, cold, gastrointestinal problems, respiratory problems, fever, pain, spasm, flatulence, diabetes and hyper lipidemia [4]. In traditional medicine, it is also used for the treatment of cough, headache, diarrhea or constipation. It also has anti-parasitic and anti-cancer properties [5,6].

Since *Ob* grows widely in Iran, has a special place in the life of Iranians, and is used as an anxiolytic plant in traditional medicine [7], it is attempted here to scientifically examine its anxiolytic effect.

## METHODOLOGY

### Plant Material and Preparation of the Extract:

Plants were collected from Hamidieh. *Ob* was identified by Traditional Medicine and Material medical Research Center herbarium Jundishapur University of Medical Sciences (AJUMS), Ahvaz, Iran (Registration Number GP-94007) . To prepare hydro alcoholic extract of leaves, powdered leaves (300g) were macerated by 2000 mL of 70% ethanol (v/v) for 72 hours. The extract was then shaken and filtered, and the solvent was removed in a vacuum evaporator to obtain semisolid extract and then was placed in an oven in 60°C for 72 hours [8].

### Drugs:

Diazepam was purchased from Caspian Pharmaceutical Co (Rasht , Iran), respectively. The hydro alcoholic extract of olive fruit and drugs were diluted in saline.

### Animals:

Male mice (Weighting: 20-25 g) were obtained from the animal house of Jundishapur University of Medical Sciences, Ahvaz, Iran, and were kept in specific conditions, of constant 12-hour light/dark cycles at a controlled temperature of 23 ± 2°C. Standard pellet food and tap water were available ad libitum.

### Experimental Groups:

For investigation anxiety mice were divided eight groups; control group, positive control group received Diazepam (1mg/kg, IP) and groups treated with doses different by Ocimum basilicum (25,50,100,200,400,600 mg/kg, IP). Used to plus maze (EPM) model for evaluation anxiety behaviors

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**Elevation Plus-Maze Test:**

The plus-maze (EPM) test consisted of two open arms (35× 5 cm) and two closed arms (30 × 5 × 15 cm) emanating from a common central platform (5×5 cm). The entire apparatus was elevated to a height of 60 cm above floor level. At the beginning of the session, a mouse was placed at the center of the maze, with its head facing an open arm. The animals were allowed to explore the maze for 5 minutes, and the following parameters were recorded using Video Tracking: the time spent in open and closed arms and also the frequency of entry in to each arm type [9, 10].

**Statistical Analysis:**

Statistical analysis was performed using one-way ANOVA with post hoc LSD test. *P<0.05* was considered significant. All data are expressed as mean±S.D.

**RESULTS**

The results showed that OAT% was decreased significantly in Negative control group in comparison to other groups (Figure 1).

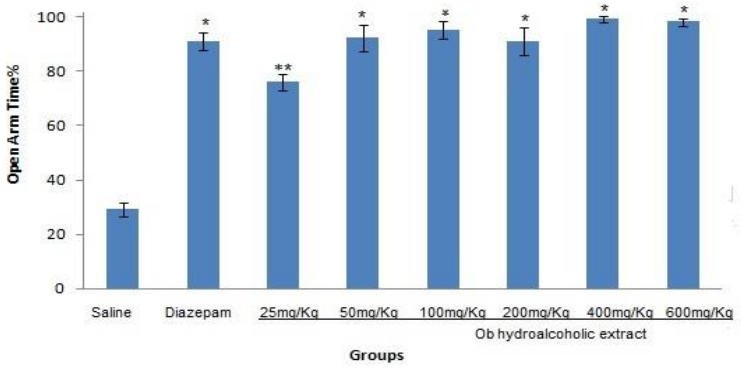


**Figure 1: Comparison of the effect of normal saline (intra peritoneal [injection]) and diazepam (intra peritoneal [injection]) and different doses of *Ob* hydro alcoholic extract (intra peritoneal [injection]) on OAT% in an NMRI mice.**

***** Significant difference with the group receiving 25 mg/kg of Ob hydro alcoholic extract and normal saline group (P<0.05).
** Significant difference with the normal saline group (P<0.05).

Also OAE% was decreased significantly Negative control group in comparison to other groups except the group receiving 25mg/Kg dose of *Ob* hydro alcoholic extract (Figure 2).

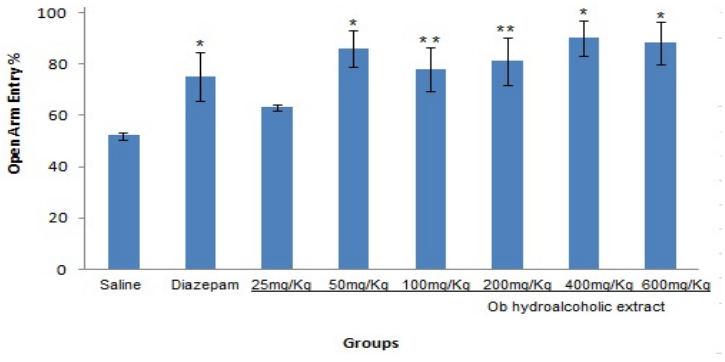


**Figure 2: Comparison of the effect of normal saline (intra peritoneal [injection]) and diazepam (intra peritoneal [injection]) and different doses of *Ob* hydro alcoholic extract (intra peritoneal [injection]) on OAE% in NMRI mice**

***** Significant difference with the group receiving 25 mg/kg of Ob hydro alcoholic extract and normal saline group (P<0.05).
** Significant difference with the normal saline group (P<0.05).

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In addition LA was increased significantly in comparison to other groups receiving 25,50 mg/Kg dose of *Ob* hydro alcoholic extract (Figure 3).

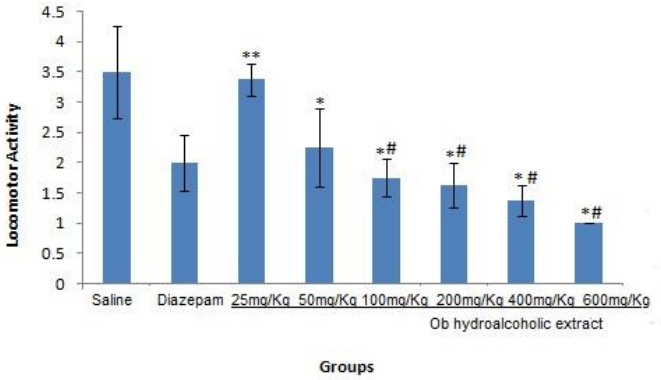


**Figure 3: Comparison of the effect of normal saline (intra peritoneal [injection]) and diazepam (intra peritoneal [injection]) and different doses of *Ob* hydro alcoholic extract (intra peritoneal [injection]) on LA in NMRI mice**

*Significant difference with normal saline group(P<0.05).
**Significant difference with diazepam group(P<0.05).
# Significant difference with the group receiving 25 mg/kg of Ob hydro alcoholic extract(P<0.05).

No significant difference were seen between diazepam group and the group receiving 50 mg/kg dose of *Ob* hydro alcoholic extract in OAT%,OAE% and LA (Figure 4,5,6).

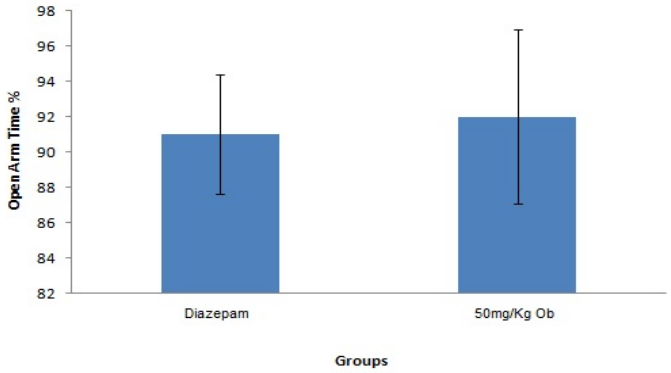


**Figure 4: Comparison of the effect of diazepam (intra peritoneal [injection]) and 50mg/Kg dose of *Ob* hydro alcoholic extract (intra peritoneal [injection]) on OAT% in NMRI mice**

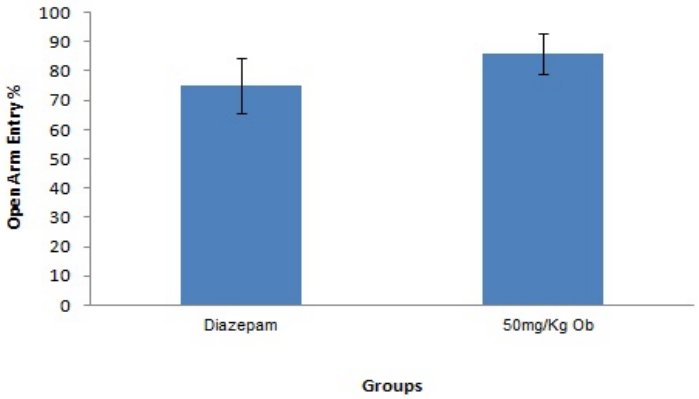


**Figure 5: Comparison of the effect of diazepam (intra peritoneal [injection]) and 50mg/Kg dose of *Ob* hydro alcoholic extract (intra peritoneal [injection]) on OAE% in NMRI mice**

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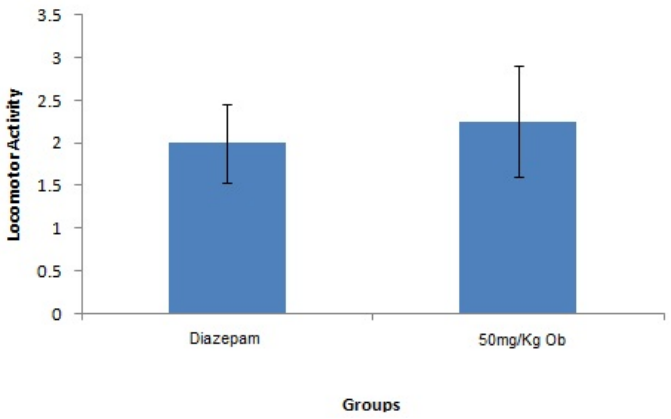


**Figure 6: Comparison of the effect of diazepam (intra peritoneal [injection]) and 50mg/Kg dose of *Ob* hydro alcoholic extract (intra peritoneal [injection]) on LA in NMRI mice**

## DISCUSSION

Anxiety is an adaptive response to physiological, mental and social stressful stimuli. The pathological anxiety is the most common disorder that interferes with daily life and concerns the patients [11]. This is a common mental disorder in human that evokes an unknown danger in the patient [12].

Many chemical drugs such as benzodiazepines (e.g. Alprazolam and Diazepam) have been used for treating or controlling anxiety. In spite of having some advantages, they have side effects including drug dependence, which appears after discontinuing the use of medication. Therefore, they should not be consumed for a long time [13]. Because of this, tendency towards drugs with fewer side effects, including herbal medications, has been increased these days.

Since *Ob* grows widely in Iran, has a special place in the life of Iranians, and is used as an anxiolytic plant in traditional medicine [7], it is attempted here to scientifically examine its anxiolytic effect.

Results show that the injection of higher dose of *Ob* hydro alcoholic extract dose into small white mouse causes higher OAT% and OAE% as compared to the negative normal saline group. However, no significant difference was observed with the positive diazepam group. In addition, LA follows a falling trend with the injection of higher dose of *Ob* hydro alcoholic extract as compared to the negative normal saline group. Again, no significant difference was observed with the positive diazepam group. The effect of *Ob* on different behavioral activities has not been investigated directly, yet the various studies have been carried out on other medical aspects of this plant.

Sasha et al. (2012) studied the effect of *Ob* methanolic extract on benzene hemato toxicity and mentioned that its mono terpenes reduce this problem [14].

Fathiazad et al. (2012) reported that the presence of flavonoid and phenol (methyl chavicol) in the *Ob* ethanolic extract might prevent the isoproterenol-induced myocardial infarction (15). Karinovic et al. (2011) studied the antioxidant capacity of *Ob* extracts and realized that the aqueous extract had greater antioxidant effect than alcoholic extract [5].

Sakr et al. (2013) studied the effect of *Ob* extract on cadmium-induced testicular changes and concluded that the presence of flavonoids, such as caffeic acid and p-coumaric acid, in *Ob* extract prevents toxicity of cadmium [6].

Abdoli et al. (2012) studied the antidepressant effect of *Ob* methanolic extract on the mice under forced swimming test (FST) and electromagnetic field. The results showed that the antidepressant effect of *Ob* is due to the phenolic compositions of flavonoids and phenolic acids [16].

Sarahroodi et al. (2012) showed that the administration of ethyl acetate extract of *Ob* not only is effective on recalling and memorizing capabilities but also prevents brain ischemia [4].

Oliveira et al. (2009) studied the antiepileptic effect of the volatile oils of *Ob* and attributed it to the presence of such substances such as linalool in the bail plant [17].

Husseinzadeh et al. (2011) studied the effect of different fractions of *Ob* extract on morphine withdrawal syndrome in mice. Results showed that *Ob* extract, especially its non-polar components, can reduce morphine withdrawal syndrome [2].

Khaki et al. (2010) studied the effect of *Ob* on apoptosis rate in the uterus tissue of rats under magnetic fields. Results proved that the presence of natural antioxidants in *Ob* neutralizes the devastating effects of electromagnetic radiation [18].

Many studies have been also carried out on the anxiety and its formation and treatment mechanisms. Pourmehdi Rad et al. (2009) examined the anxiolytic effect of the hydroalcoholic extract of chamomile on male and female mature mice in the presence and absence of gonads and concluded that some flavonoid compounds in chamomile may have anxiolytic effects by connecting to the position of benzodiazepines in GABA receptors. This plant contains phytoestrogens, which function as a weak agonist of estrogen and progesterone, and thus exhibits anxiolytic effect [19].

Bochani et al. (2011) studied the effect of lead on rats. They observed that lead causes anxiety through inhibiting NMDA receptor, calcium flow in the voltage-sensitive calcium canals, and neurotransmitters release process. It also arouses anxiety by interfering with the stimulation of glutamate and GABA receptors in hippocampus [20].

Zahedi Khorasani et al. (2006) studied the effect of hydro alcoholic extract of the aerial parts of Achillea millefolium in easing the anxiety of NMARI mice and concluded that this plant is able to impose its anxiolytic effect, as like as its painkilling effect, through interfering with the opioid system [1].

Khajehpour et al. (2014) studied the involvement of adrenergic system in the anxiolytic effect of hydroalcoholic extract of valerian root in mature male mice and concluded that this plant imposes its anxiolytic effect directly and indirectly through affecting GABA receptors, and neurotransmitters of serotonin and adenosine [21].

Also, Khodami et al. (2011) studied the anxiolytic effect of the alcoholic extract of Dracocephalu polychaetum bornm on male rates and hypothesized the possible anxiolytic effect of its flavonoids on $GABA_A$ receptors [22].

Pourabbas et al (2011) studied the anxiolytic effect of Foeniculum vulgare on female rats and discussed the role of a GABAergic system and estrogen receptors in anxiety reduction [23].

The above studies indicate that flavonoids, as active ingredients of medicinal plants, are among the factors with anxiolytic properties in plants and can impose such an effect through interacting with $GABA_A$ receptors. This interpretation confirms the anxiolytic effect of the hydroalcoholic extract of *Ob*.

The studies on the anxiety behaviors of laboratory animals consider the increase of OAT and OAE factors without changes in L.A factor as a mere anxiolytic effect. However, the results show a gradual reduction of L.A following the increased dose of *Ob* injected to laboratory animals without any significant difference with Diazepam. Studies show that Diazepam affects L.A and reduces it through imposing soporific and muscle relaxing effects [24]. It was also observed that the administration of muscimol (the exclusive antagonist of $GABA_A$ receptor) considerably reduces morphine-induced locomotor activity and significantly antagonizes the effect of increase in morphine-induced locomotor activity [25]. Another study showed that the excitation of benzodiazepine receptors in $GABA_A$ causes a suitable antispasmodic effect and reduces L.A [26]. All above items confirm L.A reduction obtained in this study.

## CONCLUSION

This study showed that *Ob* has anti-anxiety, antispasmodic, and soporific properties with the effects similar to Diazepam. Therefore, these effects are probably formed by bonding with the benzodiazepine receptor in $GABA_A$.

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