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Pharmacological Effects of *Origanum Vulgare L.* in the Elevated Plus-Maze and Open Field Tests in the Rat

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ABSTRACT

Background and Objective: The medicinal plant *Origanum vulgare*. subsp. *vulgare* in the Lamiaceae family has been widely used by Iranian people for the treatment of respiratory, gastrointestinal and neurologic disorders. The present study was carried out to evaluate possible anxiolytic effects of aqueous extract of *O. vulgare* leaves and the flowering branches in rats.

Materials and Methods: Anxiolytic activity was assessed using the elevated plus-maze test. In addition, the possible sedative effect and muscle relaxant effect were evaluated using open field test and horizontal wire test, respectively. Single doses of *O. vulgare* extract(50, 100 and 200 m/kg) were administered intraperitoneally in male rats. Control rats were treated with an equal volume of saline, and the positive control rats with diazepam.

Results: In the elevated plus-maze, *O. vulgare* extract(200 mg/kg) increased percent of time spent on open arms($p<0.001$), and open arms entries($p<0.05$), compared with saline group. In addition, the extract decreased locomotor activity in the open field test($p<0.05$). However, unlike diazepam, the *O. vulgare* did not cause myorelaxant effect.

Conclusion: These results suggest that the aqueous extract of *O. vulgare* leaves and flowers has anxiolytic effect and may also have potential sedative effects.

1. Introduction

Anxiety disorders are marked by excessive fear and avoidance, often in response to specific objects or situations and in the absence of true danger (1). According to a recent epidemiological study, the lifetime prevalence of any anxiety disorder is 28.8%. Anxiety disorders are associated with impaired workplace performance and hefty economic costs, as well as an increased risk of cardiovascular morbidity and mortality (1,2). Anxiety disorders are widely treated with benzodiazepine anxiolytic agents. Although these drugs are relatively safe, they produce many undesirable side effects such as sedation, muscle relaxation, motor coordination deficits, memory/cognitive dysfunctions, and dependency/abuse liability (3-5). These side effects limit their clinical usefulness (6) and finding novel therapeutic agents for anxiety disorders is of major interest (7).

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Medicinal plants, as a group of natural substances could be a good source to find new remedies for these disorders. *Origanum vulgare* (oregano) belongs to the Origanum genus (Lamiaceae family) (8-11) which is native to warm temperate environments from Eurasia to the Mediterranean region (11,12). Origanum has many species, but two species *Origanum majorana L.* and *Origanum vulgare L.* have therapeutic properties. The latter is a common culinary herb used as spice in different parts of the world. The herb has been used in treatment of some conditions including colds, respiratory allergies, gastrointestinal disorders, diabetes mellitus, wound healing and as a tranquilizer, in alternative medicine for centuries. Origanum is also used in traditional medicine as diuretic, stimulant, antimicrobial, anti-inflammatory, and anticancer (10,13). Many of these activities have been attributed to compounds including carvacrol, thymol, rosmarinic acid, borneol, organol (A and B), ursolic acid, monoterpene hydrocarbons (limonene, terpinene, ocimene, caryophyllene, β-bisabolene and p-cymene) and monoterpene alcohols (linalool, 4-terpineol) (14-22). Although effects of its systemic administration have been evaluated in some anxiety models in mice, effects of *Origanum vulgare* has not been tested in anxiety model in rats, yet. Building on this foundation, the present study was conducted to determine whether the aqueous extract of *O. vulgare* leaves and the flowering branches produces anxiolytic-like effect in the elevated plus-maze test in rats. In addition, the effect of *O. vulgare* on locomotor activity was tested in the open-field and its myorelaxant effect was also evaluated using horizontal wire test.

2. Materials and Methods

2.1. Animals

Male Wistar rats (200-250 g; Pasteur institute, Tehran, Iran) were used in this study. Tap water and rodent food pellets were available *ad libitum*. Animals were given one week adaptation before experiments at 22 ± 1°C with a 12-h light/dark cycle. Rats were allowed at least 2 h for adaptation to the new environment (*i.e.* laboratory) before drug administration. Experiments were carried out in a quiet room under dim light between 9:00 a.m. and 4:00 p.m. Drug or saline were administered in a random order. Six to ten rats were used in each treatment group. The animals were used only one time for behavioral testing. Naive rats were used for all behavioral experiments. All procedures were in accordance with the Shahid Beheshti University of Medical Sciences Guidelines for the Care and Use of Laboratory Animals and were approved by the local Research and Medical Ethics Committee.

2.2. Plant materials

The fresh whole herb of *O. vulgare* was collected from Clarr-dasht (at north of Iran) at August 2013. The identity of the herb was confirmed by Department of Pharmacognosy, School of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran, as *O. vulgare L.* subsp. *Vulgare.* A voucher specimen was kept in the herbarium of School of Pharmacy for future reference.

2.3. Preparation of the aqueous extract

Dried leaves and the flowering branches of *O. vulgare* were homogenized to a fine powder. Then, one hundred grams of powdered *O. vulgare* was macerated using 1000 ml of boiling distilled water, and allowed to infuse for 2 h at room temperature. The extract was filtered, then concentrated over the water bath, brought to dryness under vacuum, and stored at 4°C until use. The yield of the extract was 6.7% (w/w).

2.4. Drugs

Diazepam hydrochloride was used as the reference drug (10 mg/2 ml; Daru Pakhsh, Tehran, Iran). Doses of 1.3 or 3 mg/kg were chosen for diazepam for the behavioral experiments (See below) (23, 24). Moreover, different concentrations of *O. vulgare* extract were prepared by serial dilution from a stock solution of 50 mg/ml of the extract. The doses of *O. vulgare* and diazepam were obtained by a prior pilot study (unpublished data). Both diazepam and extract of *O. vulgare* were diluted with saline. All drug solutions were prepared freshly on the day of experiment and administered intraperitoneally (i.p.) at a volume of 1 ml/100 g body weight of animals.

2.5. Elevated plus-maze test

Anxiety was assessed using the rat elevated plus-maze test (EPM) (25). The apparatus consists of two open and two enclosed horizontal perpendicular arms (50×10 cm) positioned 40 cm above the floor. The junction of four arms forms a central square platform (10×10 cm). Each animal was placed in the junction of open and closed arms facing one of the closed arms and allowed to explore freely for 5 min. The sessions were recorded by a camera positioned right above the maze hanging from the ceiling. Data were obtained using Ethovision software (version 7), a video tracking system for automation of behavioral experiments (Noldus Information Technology, the Netherlands). During trial, the behavior of each rat was recorded as: (a) the number of entries into the open or closed arms and (b) the percent of time spent by rat on each arm (arm time ×100 / (300-center time)). Arm entries were defined as entry of all four paws into an arm. Animals were treated with diazepam at 1.3 mg/kg, the aqueous extract *of O. vulgare* (50, 100 and 200 mg/kg, i.p.) or saline, 30 min before test (n=6-10). After removal of rat, the apparatus was cleaned.

2.6. Open field test

The sedative activity was investigated by recording spontaneous locomotor activity of rat in an open field (26). Spontaneous locomotor activity was determined in individual rat which was placed in the center of an open field apparatus (45×45 cm), by a camera positioned right above the apparatus hanging from the ceiling. Data were obtained using Ethovision software (version 7) (see above). Locomotor activity was defined as total distance travelled (cm) by rat during a 30 min period after i.p. injection of either saline, diazepam (3 mg/kg) or *O. vulgare* extract (50,100 and 200 mg/kg) in groups of 6-8 rats. After each trial, the open field apparatus was cleaned.

2.7. Horizontal wire test

Immediately after open field test, rats in each treatment group including *O. vulgare*, diazepam and saline, were subjected to a horizontal wire test, as described by Hui *et al.*, with minor modifications (27). Briefly, rats were lifted by the tail and allowed to grasp a horizontally strung wire (2 mm diameter, 40 cm long, placed 60 cm above a table) with their forepaws. When the rat grasped the wire with both forepaws, its tail was gently released. The number of rats from each treatment group that did not grasp the wire with their forepaws or actively grasped the wire with at least one hind paw within 3 sec was recorded; myorelaxant agents are known to impair the ability of rat to grasp the wire. After each trial, the horizontal wire apparatus was cleaned.

2.8. Statistical analysis

Data were presented as mean ± SEM and were analyzed using GraphPad Prism software (version 5, Graphpad Software Inc., USA). Data from EPM test of diazepam was analyzed by *Student's* t-test. In other behavioral tests, data were analyzed using one-way analysis of variance (ANOVA) followed by *Dunnett's* test for multiple comparisons. A p value less than 0.05 ($p<0.05$) was considered significant.

3. Results

3.1. EPM test

Effects of *O. vulgare* or diazepam on rat behavior in the elevated plus-maze are summarized in Figure 1. As can be seen from the graph, normal rats typically avoided spending time on open arms, and entering into open arms. Instead, they had a tendency to stay more on closed arms, which were safer than the open arms. In contrast, diazepam-treated rats (1.3 mg/kg) spent more time on open arms and entered more into open arms than saline-treated group ($p < 0.05$) (Fig. 1, panel A). Likewise, *O. vulgare*-treated rats (at 200 mg/kg) spent more time on open arms ($p < 0.001$) and entered more into the open arms compared with control rats($p < 0.05$). However, no significant change was observed in the term of percent of time spent on open arms or number of entries into open arms at doses of 50 and 100 mg/kg of *O. vulgare* (Fig. 1, *panel B*). Moreover, as shown in the graph, diazepam ($p < 0.05$) and, the extract at doses of 100 and 200 mg/kg ($p<0.05$) decreased percent of time spent on closed arms, compared with

control. There was no significant difference between the *O. vulgare* extract or diazepam and control group for the number of closed arms entries, or total arm entries.

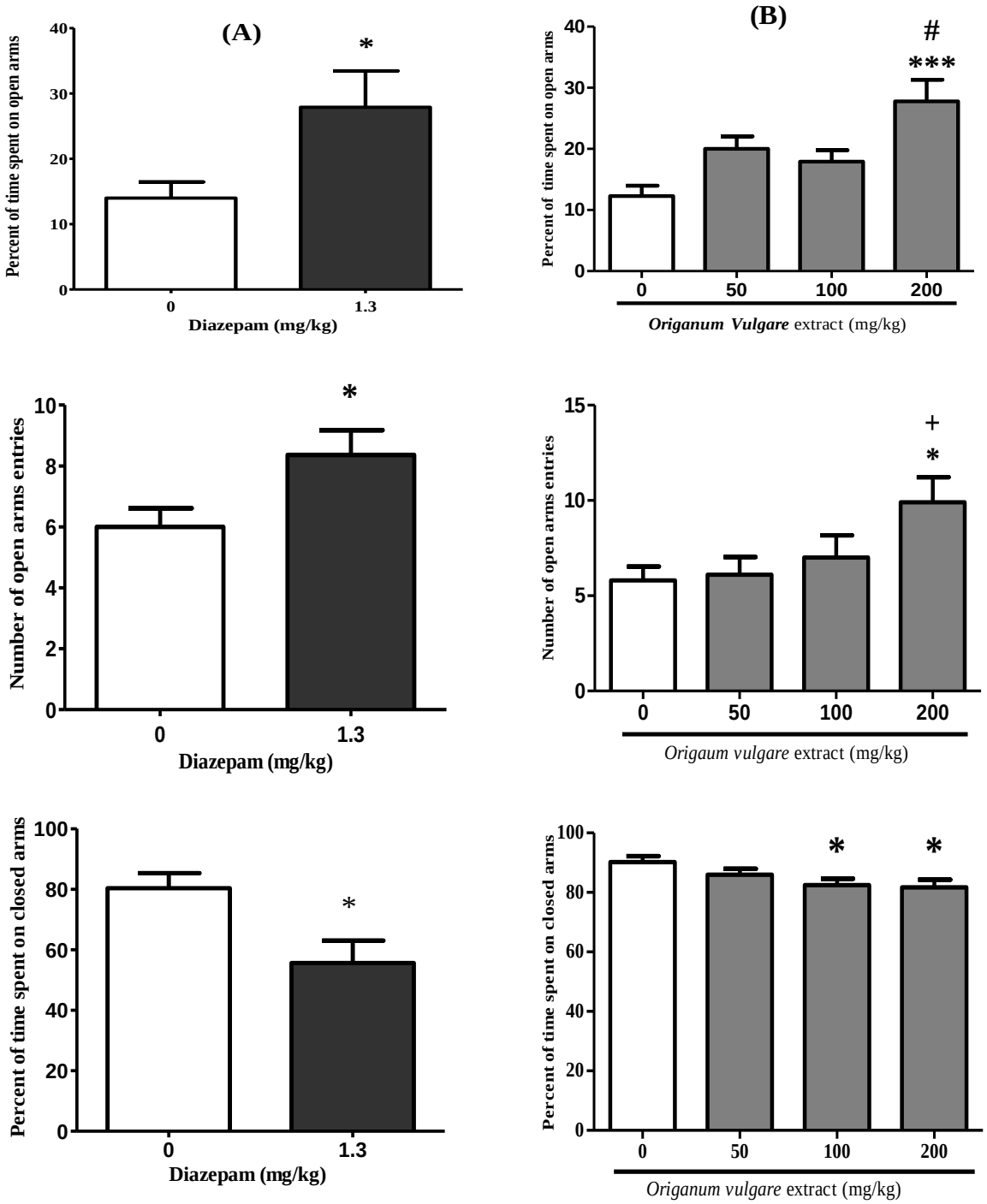

Fig. 1: Effects of diazepam (***A***) or *origanum vulgare* ***(B)*** on anxiety parameters in the elevated plus-maze test (EPM). Rats were injected intraperitoneally with diazepam or extract of *O. vulgare* , and 30 min later, percent of time spent on open arms (***upper***)), number of open arms entries (***middle***) and percent of time spent on closed arms (***lower***) were measured for 5 min. Bars represent the mean ± S.E.M, with n=6-10. (0)=Control group. *p< 0.05, **p<0.01 compared to control rats, $^{\#}$p<0.05 compared to *O. vulgare* at 100 mg/kg, and + p<0.05 compared to *O. vulgare* at 50 mg/kg.

3.2. Open field test

In the open field, diazepam (3 mg/kg) produced a significant reduction in locomotor activity ($p<0.05$) (Fig. 2). Moreover, *O. vulgare* at a dose of 200 mg/kg caused a significant decrease in locomotor activity, compared with saline-treated group ($p<0.05$) (Fig. 2). However, there was no significant change in the term of distance travelled at 50 and 100 mg/kg of the extract.

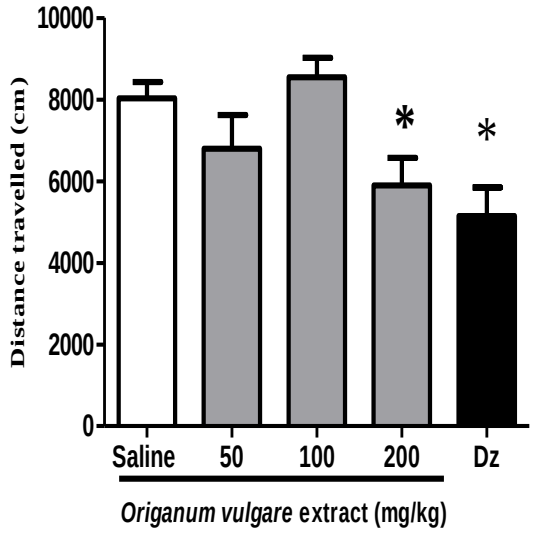

Fig. 2: Effects of diazepam and *Origanum vulgare* on spontaneous locomotor activity. Immediately after injection of diazepam (3 mg/kg) or extract of *O. vulgare*, locomotor activity was measured as distance (cm) travelled by rat for 30 min. Bars represent the mean ± S.E.M., with n=6-8. * $p<0.05$ compared to control rats.

3.3. Horizontal wire test

Diazepam (3 mg/kg) produced an impairment in performance of rats to grasp the wire compared with control rats ($p<0.001$). However, there was no significant difference between performance of rats in *O. vulgare* and control groups in the wire test (Fig. 3).

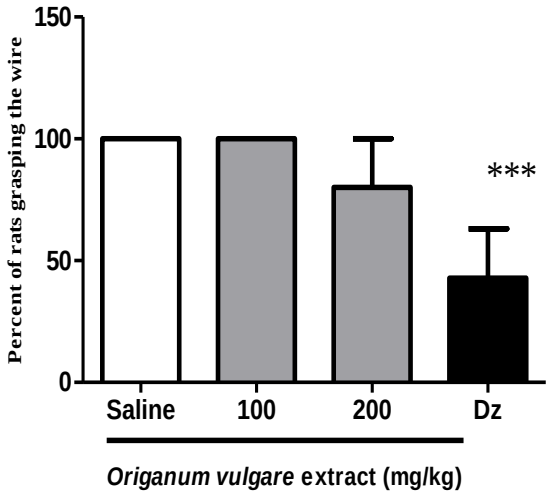

Fig. 3: Effects of diazepam (3 mg/kg, i.p.) or *Origanum vulgare* at 50, 100, and 200 mg/kg (i.p.) on the percentage of rats failing the horizontal wire test. Bars represent the mean ± S.E.M., with n=6-8. *** $p < 0.001$ compared to control rats.

4. Discussion

The present study demonstrated some behavioral effects of *O. vulgare* in rats, which are compatible with anxiolytic-like and sedative effects of this medicinal plant. The EPM is one of the most widely indicated models for study of animal anxiety. This test uses natural stimuli, i.e., the fear of a new, brightly-lit open space and the fear of balancing on a relatively narrow raised platform (28, 29). This test has been validated for study of anxiety in both of rats and mice (25, 30). So, we used this test to assess anxiolytic potential of aqueous extract of *O. vulgare* in rat. In EPM test, parameters of anxiety are time spent on open arms and open arms entries, during a given period. These parameters are sensitive to anxiolytic agents such as benzodiazepines which act via $GABA_A$ receptor-complex. So, we used diazepam as the positive control. Our findings revealed that aqueous extract of *O. vulgare* produced similar changes in EPM. Rats treated by extract of *O. vulgare* at 200 mg/kg entered the open arms more and resided there longer than control rats. These findings definitely indicate that *O. vulgare* exerts an anxiolytic-like activity in rats.

In the open field test, we found that aqueous extract of *O. vulgare* at 200 mg/kg caused a reduction in spontaneous locomotor activity of rats. In this test, reduction in locomotion may be indicative of sedative effect. Sedation is a common side effect of the prescribed anxiolytic agents such as diazepam, because of its depressant effect on locomotor activity (31, 32). Other causes of decrease in locomotor activity may be motor impairment or skeletal muscle relaxation (23). In horizontal wire test we found that diazepam at its sedative dose (3 mg/kg) produced significant myorelaxant effect. This effect did not occur with its anxiolytic dose, i.e. 1.3 mg/kg (unpublished data). On the contrary, *O. vulgare* at doses used did not show myorelaxant effect (Fig. 3). So, this may provide a pharmacological advantage of *O. vulgare* over benzodiazepine drugs such as diazepam.

Collectively, our results showed that the extract of *O. vulgare* produced anxiolytic and sedative effects at doses of 200 mg/kg, without producing myorelaxant effect in animal model of rat. This is the first report on anxiolytic effects of systemic administration of *O. vulgare* in the elevated-plus maze in animal model. These results represent a central depressant role for the extract. The authors suggest that it is possible that similar or identical compounds acting through different receptors or receptor subtypes could be responsible for the observed central effects of *O. vulgare* in rat. Results of our study were partly supported by previous studies. Mechan *et al* evaluated effects of oregano on light/dark box and Marble-burying test, and some anxiety-related biochemical parameters in the brain of mice. The oregano extract was demonstrated to inhibit the reuptake and degradation of the monoamine neurotransmitters in a dose-dependent manner, and microdialysis experiments in rats revealed an elevation of extracellular serotonin levels in the brain. Furthermore, following administration of oregano extract, behavioral responses were observed in mice that parallel the beneficial effects exhibited by monoamine-enhancing compounds when used in human subjects (33). This study focused on anxiolytic effects of a specific oregano extract, and sedative effect was not tested. This study supported the observed anxiolytic activity in the present study.

Carvacrol is an important ingredient in *O.vulgare*. Accordingly, in a study the behavioral effects of single intracerebroventricular doses of carvacrol (5-isopropyl-2-methylphenol) in animal models EPM, open field, rotarod and barbiturate-induced sleeping time tests were evaluated in mice (34). The results showed that carvacrol had no effect on the spontaneous motor activity in the rotarod test nor in the number of squares crossed in the open-field test. However, carvacrol decreased the number of grooming in the open-field test. In the plus maze test, carvacrol significantly increased all the observed parameters in the EPM test and flumazenil was able to reverse the effects of diazepam and carvacrol (34). However, unlike our findings in the open field, locomotor activity was not depressed (Fig. 2). Also, recently, a group of researchers investigated the anxiolytic-like activities of carvacrol at single doses (i.p.) compared to those of the vehicle, buspirone and diazepam. Carvacrol increased times pent in the open arms and the number of open arms entries in the EPM, as well the time spent in the light box and the number of entries to light box in the light/dark box and the and the number of marbles buried in the marble-burying tests. The anxiolytic-like activity of carvacrol was not associated with psychomotor retardation in the open field test and in the rotarod test, contrarily with what happened with diazepam (35).

Besides carvacrol, oregano has many other active phytochemicals such as thymol, alpha-pinene, rosmarinic acid, chlorogenic acid, and the flavonoid naringin (10). Authors suggest that flavonoids present in *O. vulgare* may be responsible for the observed anxiolytic and sedative effects. Many scientists believed that this effect is due to the affinity of flavonoids for the benzodiazepine receptors in the CNS. Furthermore, a sedative effect on central nervous system has been shown for some flavonoids (36, 37). Besides, according to some studies (33), it is also probable that carvacrol in the extract be responsible for the anxiolytic effect of the plant. Moreover, inhibitory effects on degradation and reuptake of monoamine transmitter (See above) could

partly explain the mechanism of the observed effects in the present study. Nevertheless, the active ingredient(s) responsible for the observed central nervous system effects of *O. vulgare* has to be isolated and identified in forthcoming studies.

In summary, our results showed that the aqueous extract of *O. vulgare* leaves and heading flower has anxiolytic-like and sedative effects in rats with no myorelaxant effect. However, further detailed studies are needed to clarify the underlying anxiolytic and sedative mechanism(s) and the active phytoconstituent(s) involved in the observed behavioral effects of *O. vulgare* in rat.

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Conflict of interest: The authors declare that they have no conflict of interest.

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