The effects of aqueous extract of *Origanum vulgare* on learning and memory in male rats

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**Abstract**

**Introduction:** The effectiveness of antioxidants on learning and memory improvement has been shown, previously. Due to the high level of antioxidants, available in *Origanum vulgare*, the present experiment aimed to examine the effect of aqueous extract of *O. vulgare* on passive avoidance learning (PAL) in male Wistar rats.

**Methods:** This study was performed on 30 male Wistar rats weighing 250 to 290 g. The rats were randomly assigned into five groups (n=6), as follows: the control, sham (saline), and three groups treated with different doses of *O. vulgare* extract (150, 250, and 350 mg/kg). The saline or extract was administered via daily oral gavage for 14 days. The groups were then subjected to the passive avoidance task, and their behaviors were recorded. The rats’ locomotor activity was also measured using the open field test.

**Results:** The number of trials to acquisition was significantly lower in the "*O. vulgare* (350 mg/kg)" group than the control group. The step-through latency and the time spent in the dark compartment in the retention test, was significantly higher and lower in the "*O. vulgare* (250 and 350 mg/kg)" groups than the control group, respectively. No significant differences were found in the distances traveled among the experimental groups in the open field test.

**Conclusion:** Aqueous extract of *O. vulgare* can enhance learning and memory. The high levels of antioxidants in *O. vulgare* extract may be responsible for its effectiveness in learning and memory.

**Implication for health policy/practice/research/medical education:**
The results of this study showed that the aqueous extract of *Origanum vulgare* enhanced learning and memory in rats. Hence, this plant might be useful in patients with Alzheimer’s disease. One of the mechanisms underlying the effectiveness of *O. vulgare* on learning and memory may be related to its antioxidant properties.


**Introduction**

Plant extracts are attractive candidates for treating brain function abnormalities and metabolic disorders of individuals with specific pathological conditions, which is due to their inherent antioxidant property as well as beneficial effects on human health (1-3).

*Origanum vulgare* is native to Europe, Northern Africa, America, and Asia (4) and is widely distributed in these regions. It is commonly used as a spice in many diets and also as a therapeutic agent for several diseases, including colds, coughs, and digestive disorders (5). In addition, *O. vulgare* is rich in phenol and ester compounds (1) and also exerts strong antimicrobial and antioxidant activities (6). The high levels of carvacrol, flavonoid, and phenolic acid contents of *O. vulgare* (7,8) are responsible for its antioxidant activity (9-11). Moreover, these phenolic antioxidants have diverse biological activities, as they exhibit anti-ulcer, anti-inflammatory, anti-diabetic, antiviral, cytotoxic, and antitumor effects (12), and are also associated with the health benefits of *O. vulgare*.

Indeed, the antioxidants derived from natural sources have recently been regarded, since different forms of active oxygen are thought to be harmful to human health. In particular, the aqueous extract of *O. vulgare* is known to have antioxidant activity (13). Rosmarinic acid, eriocitrin, apigenin-7-O-glucoside, and oreganol are the most...
important bioactive constituents in *O. vulgare* (14,15).

An important characteristic of animals and humans is the ability to alter their behavior based on experience, owing to their learning and memory capabilities. Learning is the acquisition of a skill or knowledge, whereas memory is the maintenance and storage of information. Learning and memory are closely related concepts and should not be considered separately (16,17). Learning and memory are regarded as the most important functional levels of the central nervous system, as they are involved in encoding, storing, retaining, and recalling information (18).

Many recent studies have focused on the role of medicinal plants in learning and memory. Therefore, this study was conducted to evaluate the effects of the aqueous extract of *O. vulgare* on passive avoidance learning (PAL).

**Materials and Methods**

**Animals**

Thirty male Wistar rats weighing 250 to 290 g were prepared from the Hamadan University of Medical Sciences. The animals were housed in a temperature-controlled room with a 12-hour light–dark cycle.

**Preparation of *O. vulgare* extract**

*Origanum vulgare* leaves were collected in the spring from Sanandaj, Kurdistan, Iran. The leaves were dehydrated and powdered. Then, the powder was refluxed with distilled hot water (<50°C) by a ratio of 1/100 for a full day. The solution was filtered using a filter paper, and after reducing the pressure by a Rotary evaporator (40°C), the compound was condensed. Evaporation yielded a semi-solid mass. A stock suspension of the mixture was made by dissolving 5 g of the compound in 100 mL of distilled water to achieve a solution with a concentration of 50 mg/mL. Other concentrations were prepared from this stock suspension by diluting this solution with an appropriate amount of distilled water (19).

**Experimental design**

The animals (30 rats) were separated into five groups (n = 6 per group), as follows: the control, sham (saline), and three groups treated with different doses of the *O. vulgare* extract (150, 250, and 350 mg/kg). The saline or *O. vulgare* extract was administered once a day (at about 08:00 AM) for 14 days by oral gavage. The experimental timeline is shown in Figure 1.

**Open field test**

Locomotor activity was assessed by an open field test, which was designed to measure behavioral responses, including locomotor and exploratory behaviors (20). The test apparatus consisted of a clear, square-shaped, plexiglas box (76 x 76 cm) enclosed by 42-cm-high matte walls, and its bottom consisted of 25 equal parts. For animal locomotion analysis, the rats were removed from their cages and located in the middle of the open field apparatus (one at a time). The area was entirely restricted by a white curtain to reduce extra-maze cues. On the test day, the caged rats remained in the experimental room for 30 minutes, and then each rat was placed in the middle of the apparatus, and the needed data were immediately recorded. To examine possible effects of the extract on locomotor activity, the total distance traveled (cm) during 10 minutes of the test period was measured using a Maze Router (21).

**Passive avoidance task**

**Step-through passive avoidance task**

We used the step-through passive avoidance task to evaluate PAL and memory (22-25). The shuttle box apparatus consisted of a light compartment (20 x 20 x 30 cm) made of transparent plastic and a black compartment made of black opaque plastic (20 x 20 x 30 cm). The bottom of both compartments consisted of stainless steel rods 3 mm in diameter spaced by a distance of 1 cm. A shock generator (Behbood Pardaz Co., Iran) electrified the floor of the black compartment. There was a rectangular opening (6 x 8 cm) between the light and black compartment, which was closed by a matte guillotine door.

**Passive avoidance training**

The procedure was performed as described previously (25-28). In brief, after the rat entered the black chamber unconsciously (step-through latency, STLa), an electric shock was applied, and the animal was returned to its cage after 30 seconds. This procedure was repeated after 2 minutes. Each time the rat reentered the black compartment, a shock was applied. The training was finished, when the animal stayed in the light compartment for 120 continuous seconds (25-27). The number of trials to acquisition (NTa; entries into the black compartment) was noted.

The rats that did not cross into the black chamber
The effect of *Origanum vulgare* on memory
during the training period, except for the practice during
the adaptation phase, were excluded from the experiment.
A 3-second shock was delivered after 2 minutes, only if
the same animal entered the black chamber following the
first foot shock. The animals were immediately removed
from the black compartment and transferred to their
cages (29,30).

**Retention test**

Twenty-four hours after training, the trained rats were
subjected to the retention test with the same experimental
procedure; however, no shock was applied to the grid
floor after entering the animals to the black compartment
(31,32). During the retention test, the animals were
allowed to access the black compartment or STLr and the time spent in the
black compartment (TDC) over a 10-minute cycle were
recorded.

**Data analysis**

Statistical analyses were performed using the SPSS
software. One-way analysis of variance (ANOVA) was
used for multiple comparisons, followed by the Tukey’s
test. Values are expressed as mean ± SEM, and the
significance level was considered at 0.05.

**Results**

**Effects of *O. vulgare* on body weight**

Although the rats’ body weight increased during the
experiment in all groups, the *O. vulgare* extract did not
have a significant effect on the body weight of the subjects
compared with the control group (Figure 2).

**Effects of *O. vulgare* on locomotor activity**

An open field test was used to assess motor activity as a
reaction to an unknown environment. Figure 3 shows
the total distance traveled (in centimeters) during the
open field test in all experimental groups. No significant
differences were observed in the distance traveled among
the experimental groups (*P > 0.05*), which indicates that
the locomotor activity and exploratory action of the rats
in all groups were not differentiable.

**Effects of *O. vulgare* on acquisition in the passive avoidance
task**

No significant difference was found in the STLa between
the studied groups (*P = 0.6716*). This observation showed
that the exploratory behavior of the animals in the
black compartment was not differentiable. A significant
difference was observed in the NTa between the groups
(Figure 4). Specifically, the NTa in the "*O. vulgare* (350
mg)" group was significantly lower than that of the saline
group (*P < 0.05*).

**Effects of *O. vulgare* on retention in the passive avoidance
task**

Twenty-four hours after training, the retention test was
performed. There were significant differences in the
STLr between the studied groups (Figure 5A). The STLr
values in the "*O. vulgare* (350 mg)" and "*O. vulgare* (250
mg)" groups were significantly higher than the control

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**Figure 2.** Effects of the aqueous extract of *Origanum vulgare* (ORVU) on
the first and last body weight measurements in all experimental groups. Each column and bar represents the mean ± SEM.

**Figure 3.** Effects of aqueous extract of *Origanum vulgare* (ORVU)
on the total distance traveled in the open field test in all experimental
groups. Each column and bar represents the mean ± SEM. No significant
differences were observed among the studied groups.

**Figure 4.** Effects of aqueous extract of *Origanum vulgare* (ORVU) on
the number of trials to acquisition during the passive avoidance learning task
in all groups. Each bar represents the mean ± SEM. *P < 0.05 compared
with the control group.
animals \((P < 0.05)\). In addition, a significant difference was found in the time spent in the dark compartment among the groups (Figure 5B). The time spent in the dark compartment in the “\(O.\ vulgare\) (350 mg)” and “\(O.\ vulgare\) (250 mg)” groups was significantly lower than that of the control group \((P < 0.01)\). The time spent in the dark compartment in the “\(O.\ vulgare\) (350 mg)” group was significantly lower than that of the “\(O.\ vulgare\) (150 mg)” group \((P < 0.0001)\).

Discussion

The present study investigated the effects of aqueous extract of \(O.\ vulgare\) on learning and memory in male rats. Our results demonstrated that the aqueous extract of \(O.\ vulgare\) had a significant effect on learning and memory using a passive avoidance task. The \(O.\ vulgare\)-induced reduction in the NTa in the passive avoidance task demonstrated an increase in the acquisition. Moreover, an enhancement in the STLr and a reduction in the TDC in the \(O.\ vulgare\) groups during the retention test indicated the effectiveness of \(O.\ vulgare\) on memory retention \((24,33,34)\). Interestingly, no significant difference was found in the distance traveled among the experimental groups in the open field test, which demonstrated that the locomotor activity and exploratory behavior of the animals were not changed among the groups. In general, the aqueous extract of \(O.\ vulgare\) (250 and 350 mg/kg) increased PAL in comparison to the control group. However, no significant effects were found on the body weight, locomotion, and exploratory behavior of the animals.

Medicinal plants have been consumed by human beings to treat several disorders, such as pediatric diseases, neurological dysfunctions, pain, digestive disorders, hormonal imbalances, etc \((35,36)\). The role of medicinal plants and their extracts in cognitive function disorders, neurological dysfunctions, pain, digestive symptoms are impaired after oxidative stress, and oxidative stress contributes to cognitive impairment following oxidative brain damage \((40)\). Furthermore, antioxidants can effectively enhance learning and memory \((24)\). Hence, one of the possible mechanisms underlying the improvement effects of \(O.\ vulgare\) on learning and memory may be related to its antioxidant properties. It has been shown that some combinations of \(O.\ vulgare\) decrease lipid peroxidation in neural cells \((14)\). Rosmarinic acid, eriocitrin, apigenin-7-O-glucoside, ursolic acid, and oreganol A and B are the most important bioactive constituents of \(O.\ vulgare\) \((14,15)\). Rosmarinic and ursolic acid are the main components of the \(O.\ vulgare\) with antioxidative properties. Thus, using \(O.\ vulgare\) may increase the antioxidant capacity of the consumers. In molecular-based studies, ursolic acid and rosmarinic acid are supportive against amyloid-beta (Aβ)-induced reactive oxygen species production. Several \textit{in vitro} studies also have shown that the Aβ protein elevates free radical synthesis and oxidative degradation of lipids in neurons. However, vitamin E pretreatment and using ursolic acid prevented the Aβ-induced neurotoxicity \((41)\).

Another potential mechanism underlying the effectiveness of \(O.\ vulgare\) on learning and memory may be related to the increased levels of acetylcholine in the hippocampus following the consumption of \(O.\ vulgare\), as ursolic acid is known as a potent acetylcholinesterase inhibitor \((42)\). Therefore, the antioxidant and acetylcholinesterase inhibition properties of \(O.\ vulgare\) may be involved in the observed effectiveness of \(O.\ vulgare\) on learning and memory.
Conclusion
In summary, the aqueous extract of *O. vulgar* enhanced learning and memory in rats. Hence, this plant might be useful in patients with Alzheimer’s disease. One of the mechanisms underlying the effectiveness of *O. vulgar* on learning and memory may be related to its antioxidant properties. Further studies are required for a better understanding of the neurobiological effects of *O. vulgar* extract on the cognitive process.

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Authors’ contributions
AK designed, analyzed the data, and revised the manuscript. AG was responsible for performing the experimental procedures. FT and NF provided assistance in the study design. AG and SAK participated in plant extraction and all animal interventions. SAK and SS participated in the draft preparation. The paper was read and approved by all authors.

Conflict of interests
The authors declare no conflict of interest.

Ethical considerations
All procedures were approved by the ethics committee of Hamadan University of Medical Sciences (Approval code: IR.UMSHA.REC.1394.305).

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