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Original Article

Effects of the Aqueous Extract of Lavender on Spatial Learning and Memory of Rats Undergoing Pentylenetetrazol Kindling

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Abstract

Background and Aim: Since memory impairment is one of the most common complaints in patients with epilepsy, development of new ways to improve this dysfunction is of high significance. The present study aimed to evaluate the impacts of the aqueous extract of lavender (AEL) on spatial learning and memory in the pentylenetetrazol (PTZ) kindling model of epilepsy.

Materials and Methods: Forty male rats were randomly divided into 5 groups, including the sham, control, and experimental groups. Pretreatment of the control(C), sham(S) and experimental (E) groups included a, 13 injections of PTZ (i.p. every other day); b, 20 days of normal saline and AEL in doses 100mg/kg, 200mgkg and 400 mg/kg in S and E groups (i.p. every day) respectively. Finally, the spatial memory and learning were examined by the Morris water maze (4 trials/day in five consecutive days) and the probe test was performed in the 6th day.

Results: There were no significant differences between the treatment groups with the sham and control groups in terms of time latency, the distance traveled, and movement speed to find the hidden platform($P>0.05$). In contrast, 200 mg/kg of AEL remarkably reduced the time latency to find the platform ($P=0.02$).

Conclusion: The aqueous extract of lavender in moderate doses can lead to improved spatial retrieval memory in kindled rats, although it does not have any effect on spatial learning. Given the effect of AEL on learning and memory, further dose- dependent studies are required to reveal the possible effects of different doses of AEL.

Keywords: Epilepsy, *Lavandula officinalis*, Memory and learning tests, Spatial memory

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Introduction

Memory impairment is one of the most complaints in patients with epilepsy (1). Several factors such as the

location of brain involvement, age of the onset, and the type of seizure are effective on the memory impairment caused by epilepsy (2). Due to the fact that the temporal lobe is central to memory formation and maintenance (3), people with temporal lobe epilepsy experience memory impairment even after the seizure is completely controlled (4).

Memory is the ability of the brain to store information and recall them (5), and learning is defined as changing the behavior of living beings through practice (6). In the process of learning and memory formation, different parts of the brain such as the cerebral cortex, amygdala and especially hippocampus are very important (7).

In information processing and memory formation, the hippocampus plays an important role in associating multifaceted complex information and creating new memory paths (8). It has been found that the CA3 region of the hippocampus is central to memory formation and spatial learning (9). Spatial memory helps animals remember where they acquired information and where to apply that information (10). Working memory is only retained for as long as the animal is performing an action, and then it is lost because the existing information is no longer needed. The hippocampus is necessary for processing the information related to space recognition and working memory (11). One of the methods of evaluating spatial memory and learning in rats is the use of the Morris water maze(MVM) (12).

Lavandula officinalis is a flowering plant belonging to the Lamiaceae family. Extracts of this plant are used as herbal medicines (13). According to recent studies, this drug has beneficial effects (antidepressant, anticonvulsant, antispasmodic) on the central nervous system (CNS) (14). Rahmati *et al*. indicated that the hydroalcoholic extract of *Lavandula officinalis* could significantly improve memory impairment, anxiety, and depression (15). Moreover, Abbasi Maleki conducted a study in which he showed considerable antidepressant effects of Lavandula officinalis extracts and claimed that this drug could have a similar anti-depressant effect on fluoxetine which is commonly used as an antidepressant drug (16). According to recent evidence, the extract of this plant has anti-acetylcholinesterase (AchE) activity and thus its use increases the level of acetylcholine in the brain (17). This property of *Lavandula officinalis* can be important in the treatment of dementia diseases such as Alzheimer's (18). In this case, Kashani evaluated the effects of an aqueous extract of lavender (AEL) on spatial memory of rats with Alzheimer's disease (AD) and revealed that AEL could significantly reverse spatial learning deficits in AD rats (19).

In addition to the beneficial effects of *Lavandula officinalis* on the improvement of memory and the learning process (20-22), there is evidence about the anticonvulsant and neuronal protection effects of this plant (23). However, the effects of *Lavandula officinalis* extract on memory impairment in epileptic patients are not well understood. Hence, we evaluated the effects of an aqueous extract of Lavender (AEL) on spatial learning and memory of kindled rats.

Materials and Methods

Drugs and Chemicals

Pentylenetetrazol (PTZ) was purchased from Sigma, India. It was dissolved in saline (0.9% NaCl) and administered (i.p.).

Extract Preparation

The lavender plant was provided by Agricultural Jihad of Arak University, Iran, after the approval of a botanical expert. This plant was dried in the shade and powdered by an electric mill. Then, 30 grams of this powder was transferred to the percolator after initial moistening with distilled water, and the percolation process continued at room temperature for 48 hours. Subsequently, the extract was filtered and concentrated with a rotary evaporator using a vacuum system and Whitman paper of 0.45 and 0.22 micrometers. The extract was dried at 40°C and stored at 4°C.

Animals

In total, forty male Wistar rats (Pasteur Breeding Center, Tehran, Iran) with 200-250 g of body weight at arrival were used. The animals were housed in laboratory-controlled conditions (12 h light/dark cycles, 7:00–19:00 light and 19:00–7:00 dark, temperature 22 ± 2 °C) at Arak University of Medical Sciences. They had free access to food and water.

All the research procedures and animal care were approved by the Ethics Committee of Arak University of Medical Sciences (# IR.ARAKMU.REC.1395.114).

To evaluate the effects of the aqueous extract of Lavender (AEL) on memory impairment, all the rats

were treated by PTZ, and then the kindled rats were randomly divided into five groups(n=8). In contrast to the control group that received only PTZ and was maintained in the same condition, animals in the sham group received normal saline. Moreover, according to recent publications, the three most common doses which in recent studies exhibited antiepileptic effects (24, 25) and also improved memory (15,19, 20) impairment were selected for treatment in the experimental groups(n=24), and the rats in these groups received 100, 200, and 400 mg/kg of AEL (intraperitoneal; i.p.).

Kindling to induce

Kindling was induced using a sub-convulsive dose of PTZ (37.5 mg/kg, i.p.) that was administered every other day for 26 days (13 injections). Following every injection, the animals were maintained in a Plexiglas chamber (30 cm × 30 cm × 30 cm), and convulsive behavior was observed and recorded for 30 minutes (26). Convulsive responses were categorized as it was already explained. The rats were regarded completely kindled when seizure attacks (stage five) happened following every injection for three consecutive injections. The recording parameters were seizure stage, latency to the onset of stage two and five seizures, and stage five duration (27).

Evaluation of Spatial Learning and Memory

To evaluate cognitive impairment, we used the Morris water maze (MVM) test, which consists of a circular water tank (diameter 1.36 m, ~60 cm deep) filled with opaque water (~40 cm deep, 29°C). The maze was randomly divided into four areas (north-south-east-west) and a hidden platform was submerged below the water's surface in the center of the target quadrant. The animals underwent 5 days of training trials (4 training sessions a day) and one-day probe trials to assess spatial *learnin* and spatial retrieval memory, respectively. The experiments were performed in a relatively dark room, where visible signs had been installed on its four sides and the animal could use these signs to find the location of the hidden platform. At the beginning of the training trial, the rats were placed on the platform for 10 seconds and then were allowed to swim in water at one of the four quadrants. The animals were allowed to find the mounting platform in the 90th second when they discovered it. They were kept on the platform for 15 seconds. If the test rats did not find the hidden platform within the 90 seconds, they were rescued and gently placed on the platform for 15 seconds. Time latency and swimming distance were used in the training session to locate the hidden platform in order to evaluate spatial learning.

Twenty-four hours after the end of the training, the platform was removed from the pool and the rats were allowed to search for it, over a 90-second period in the probe trial (the 6th day) [26]. The time spent in the target quadrant was meant to assess spatial retrieval memory. Swimming behaviors were monitored during all the trials by a video camera mounted overhead, and the path length in the target quadrant with the proportion of time spent in the target quadrant was automatically recorded using a video tracking system (21).

Statistical Tests

Statistical analyses were performed using GraphPad Prism (Version 6). One and two-way ANOVA were used to perform statistical tests, and Tukey's test was used to compare a variable among the groups at different times. The obtained data were reported as mean ± standard error (significance level $P<0.05$).

Results and Discussion

Spatial Learning

The results of the present study showed that there were no significant differences between the treatment group and the sham as well as control groups in terms of the time spent ($F(3, 20) = 0.41$, $P=0.74$, Fig 1), the distance traveled ($F(3.20)) = 0.40$, $P=0.74$, Fig 2) and movement speed ($F(3.20)) = 0.40$, $P=0.74$, Fig 3) to find the hidden platform.

Spatial Retrieval Memory

After the learning trials, a probe test was performed by removing the hidden platform in the MVM in order to

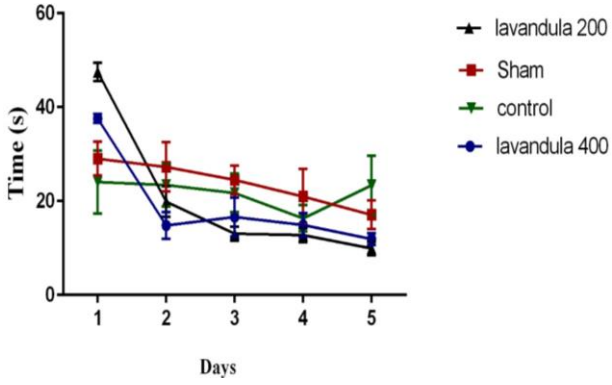


Figure 1. A Comparison of the Learning Process Based on the Time Latency Traveled in Different Groups.

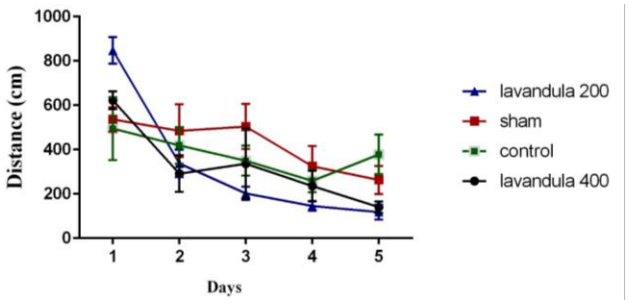


Figure 2. A Comparison of the Learning Process Based on the Distance Traveled in Different Groups.

measure the spatial retrieval memory. At this stage, the time spent in the quadrant where the platform was located was evaluated. The results revealed a significant statistical distinction between the groups regarding the memory process (F (3,23)) =43.3, P=0.03, Fig 4). The comparison between the groups using the Tukey's post-doc test showed that in contrast to 400mg/kg, 200 mg/kg of AEL had a significant difference with the control group in terms of the average time latency on the platform (P=0.02).

The comparison of the results showed that there was a remarkable statistical difference between different groups in terms of the memory process (F (3,23) =43.3, P=0.03). Moreover, receiving 200 mg/kg of lavender significantly improved recall memory (P=0.02).

Our results showed that although AEL did not have any effect on spatial learning, it could improve spatial retrieval memory in kindled animals in the middle dose. Research on the effects of lavender on learning and memory can be divided into two parts. In one part, effects of lavender have been evaluated on healthy animals, and research has evaluated the effects of this drug on animals with diseases. Rabiei *et al*. (20) investigated the effect of the ethanolic extract of Lavandula on learning and memory in normal rats and

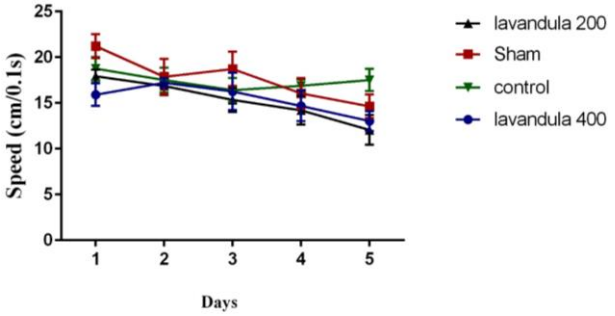
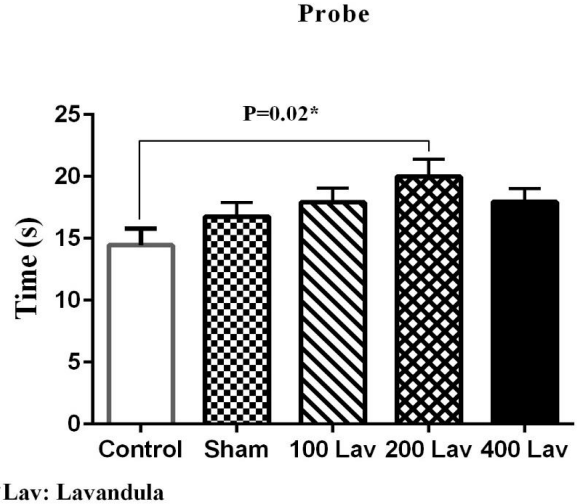


Figure 3. A Comparison of the Trend of Animal Movement Speed Changes in Different Groups.

reported that the alcoholic extract of this plant in doses of 100, 200, and 400 mg/kg could significantly improve learning and memory. Moreover, Manganiello-Terra *et al*. (28), evaluated the effect of lavender essential oil (LEO) on the memory updating of conditioned contextual fear and indicated that LEO not only could intensify memory extinction but also hindered memory updating. Furthermore, there are many reports about the effects of this plant on memory and learning (29-31). However, these studies focused on memory improvement in healthy conditions, while the effects of this herbal medicine on an animal's model of the neurocognitive disorder might be influenced by the pathophysiology of the disorder (32, 33). Thus, it is necessary to investigate possible effects of this plant on secondary memory impairment in patients with underlying disorders.

Recently, Shashank M Patil *et al*. (34) indicated neuromodulator effects of Lavandula and its possible effects on epilepsy, depression, and anxiety. Moreover, Rahnama-ye Bashm *et al*. (35) investigated the effects of Lavandula on short and long-term memory in diabetic-induced memory impairment. They reported that the use of lavender could lead to memory enhancement only in a dose of 400 mg/kg, but lower doses did not have any significant effect on memory. In contrast to the results of the study conducted by Rahmati *et al*., we found that AEL could significantly improve spatial retrieval memory in kindled animals only in 200mg/kg, and this drug did not have any



*Lav: Lavandula

Figure 4. A Comparison of the Time Latency in the Quadrant Where the Platform is Located in the Probe Test.

significant effect on the learning process. Differences might be related to different types of the underlying disease, the type of lavender extract prepared (alcoholic or aqueous), and types of behavioral tests for memory evaluation. Furthermore, Mushtaq *et al*. (36) investigated the effect of the lavender alcohol extract on scopolamine-induced dementia. They found that the use of lavender alcohol extract could lead to memory improvement by reducing the amount of acetylcholinesterase and also due to its protective effects on oxidative stress in the brain.

On the other hand, in line with the results of this research, it was indicated in a study by Kashani *et al*. (37) on the role of lavender aqueous extract on the spatial memory of animals with Alzheimer's disease that receiving lavender only in a dose of 200 mg leads to the improvement of the spatial memory in animals with Alzheimer's disease. Furthermore, Soheili *et al*. (38) indicated that the aqueous extract of lavender has dose-dependent effects on memory impairment in patients with Alzheimer's disease.

As it has been reported in a study by Shashank M Patil *et al*. (34), phytoconstituents that are the major component of Lavandula have antiepileptic and anxiolytic effects through modulating human GABAAR-beta3, human serotonin transporter, and human potassium channel KCSA-FAB. However, kindling by PTZ is associated with neuronal damage (39, 40). Moreover, lavender exhibited a dose-dependent effect on memory and learning (30, 36). Thus, considering the effective role of oxidative stress in the process of memory impairment caused by kindling (41, 42) and also given the protective effects of this plant on oxidative stress (43, 44), it seems that higher doses of this plant might have significant improving effects on kindling-induced memory impairment. However, lower doses have no enhancing effects on the memory of kindled animals. Furthermore, it seems that the existing differences concerning the possible role of lavender in learning and memory might be related to the difference in dosage and forms of the prepared extract (aqueous or alcoholic) of Lavandula, behavioral tests for learning evaluation, and the pathophysiology of underlying disorders.

We suggest that further studies focus on the molecular mechanism of AEL on kindling-induced memory impairment and examine possible effects of higher doses of this herbal medicine by different tests of memory.

Conclusion

The aqueous extract of lavender has no effect on spatial learning. However, its use in moderate doses can lead to improved spatial retrieval memory in kindled rats. Given the effect of AEL on learning and memory, further dose-dependent studies are required to reveal the possible effects of different doses of AEL.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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