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Effect of Lemon Verbena on Memory of Male Rats

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Background: Lemon verbena (*Lippia citriodora*) has been known to have various pharmacologic activities.
Objectives: Lemon verbena leaves are used to make an herbal tea, which is traditionally used for treating spasms, common cold, severe abdominal pain, indigestion, insomnia, anxiety, and headache. Although it has been known to have various pharmacologic activities, no scientific study has been done to assess its effects yet. In this study aimed to assess the effect of the aqueous extract of lemon verbena on memory of male rats by using the passive avoidance task.
Materials and Methods: In this study, Wistar male rats (weight, 180-250 g) were recruited. Aqueous extract of lemon verbena leaves was prepared. A total of 40 Wistar male rats were randomly allocated to five groups (n = 8). Saline for control group and aqueous extract of lemon verbena in four experimental groups were administered intraperitoneally (respectively 10, 100, 500, and 1000 mg/kg) for seven days. Then passive avoidance learning test was used to evaluate learning and memory. On the seventh day, acquisition test was performed an hour after treatment and the retention test was performed on the next day.
Results: Analysis of data showed that in comparison to controls, treatment with the aqueous extract of lemon verbena (≥100 mg/kg) had decreased the step-through latency (STL) ($P < 0.001$). Moreover, treatment of the rats with the extract doses of ≥500 mg/kg had increased the total time spent in the dark compartment (TDC) in comparison to controls ($P < 0.001$).
Conclusions: These results indicated that aqueous extract of lemon verbena (≥100 mg/kg) has undesirable effects on memory; however, understanding the underling mechanisms needs further investigation.

Keywords: Vervain; Learning; Memory; Rats

1. Background

Previous studies have proven that the positive effects of flavonoids on memory (1-5). Flavonoids have antioxidant and neuroprotective effects (3, 6-9). It has been shown that intake of antioxidants could improve spatial learning and increase Long term potentiation (LTP) in rats with Alzheimer's disease (10). Reactive oxygen species (ROS) are the product of normal metabolism in a biological system. To avoid the undesirable effects of oxidative stress, the balance between production of ROS and antioxidants in a biological system is necessary. The imbalance between ROS and antioxidants leads to oxidative stress (11), which has harmful effects on the central nervous system and memory (12). Antioxidants such as vitamins A, C, and E, carotenoids, and plant polyphenols (flavonoids, phenolic acids) can act as an agent to inhibit ROS (13). Lemon verbena (*Lippia citroiodora*) is a perennial shrub belonging to the family of Verbenaceae. This plant is rich of flavonoids (14). In previous studies, antioxidant effects of Lemon verbena (Vervain) have been reported (15-17). Lemon verbena leaves are used to make herbal teas or are added to the conventional tea in place of actual lemon (as is common with Moroccan tea). It also is used to add a lemon flavor to fish and poultry dishes, salad dressings, vegetable marinades, jams, puddings, Greek yogurt, and beverages (18). It is traditionally used as folk remedy in treatments of asthma, spasms, common cold, fever, flatulence, severe abdominal pain , indigestion, insomnia, anxiety, and headache (19).

2. Objectives

Regarding the antioxidant effects of lemon verbena and its flavonoids-rich components, the aim of the present study was to evaluate the effect of the aqueous extract of lemon verbena on learning and memory by using the passive avoidance task in rats.

3. Materials and Methods

3.1. Preparation of the Extract

Dried leaves of lemon verbena were purchased from Mehr Giahe Kosar Company. The chopped leaves were soaked into 2 L of distilled water for 12 hours at room temperature. Then aqueous material was filtered, dried by hot air ovens (50 °C), and turned into powder. The powder of aqueous extract of lemon verbena was stored in a refrigerator. At the time of injection, the required amount of extract powder was weighed and dissolved in a certain amount of normal saline.

3.2. Animals and Experimental Groups

Male Wistar rats (weight range, 220-250 g) were purchased from Pasteur Institute of Iran. All animals were kept in a room under controlled conditions of 24 ± 1°C and 12/12 reversed light-dark cycle. They were housed in groups of three in polypropylene cages with access to water and food ad libitum. All procedures were done according to the guide for care and use of laboratory animals, published by the United States National Institutes of Health (NIH, Publication No. 85-23, revised 1985). The rats were randomly allocated to four experimental and one control groups of eight. Control group received 0.5 mL of intraperitoneal (IP) normal saline and the four experimental groups received IP lemon verbena aqueous extract of 10, 100, 500, and 1000 mg/kg, respectively, once a day for seven days. Then the rats were evaluated using passive avoidance learning test.

3.3. Passive Avoidance Learning

The apparatus for passive avoidance learning (PAL) consisted of two-chamber dark/light shuttle box (20 × 30 × 20 cm). The two chambers were connected by a guillotine door (7 × 9 cm). Floor of the dark chamber consisted of steel grids, used to deliver electric shocks. Electric shocks were delivered to the grid floor through a stimulator with a frequency of 50 Hz, duration of 1.5 seconds, and intensity of 0.2 mA. This test has three stages: habituation, acquisition, and retention. Basically, the procedure was done according to our previous studies (20-23).

In first stage, immediately after treatment, each rat was placed in the light chamber and after 20 seconds, the door was raised and the rats were allowed to explore for 30 seconds in the dark chamber. This action was repeated after 30 minutes. In second stage, 30 minutes after first stage, each rat was placed in the light chamber and after 20 seconds, the door was opened, and the rat was allowed to move freely into the dark chamber. Step-through latency (STL) in the first acquisition trial (STLa) was recorded. Upon entry into the dark chamber, the door was closed and the rat was given an electrical shock. After 30 seconds, the rat was returned to its home cage. Two minutes later, the rat was again placed in the light chamber. If the rats did not enter the dark chamber during 120 seconds, acquisition trial was completed. The number of trials was recorded. In the next day and for the third stage, the retention test was performed one hour after treatment. During the retention test, the rat were placed in the light chamber and allowed to explore for 20 seconds and then, the guillotine door was opened. STL into the dark compartment (STLr), the total time spent in the dark compartment (TDC) and the number of total entrance into the dark compartment (EN) was recorded for 300 seconds.

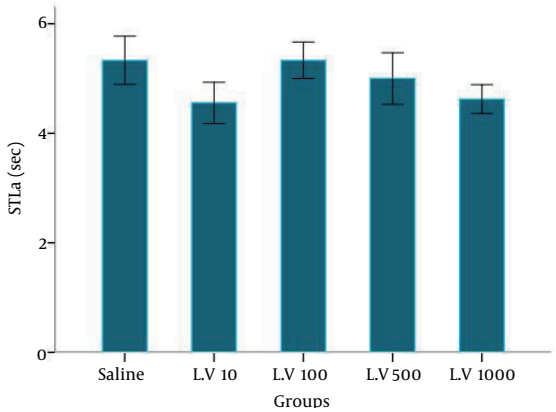
3.4. Statistical Analysis

Obtained results for different groups were analyzed by one-way ANOVA and if applicable, by Tukey's post-hoc test. The results were expressed as mean ± standard error of mean (SEM). The differences were considered significant at $P < 0.05$.

4. Results

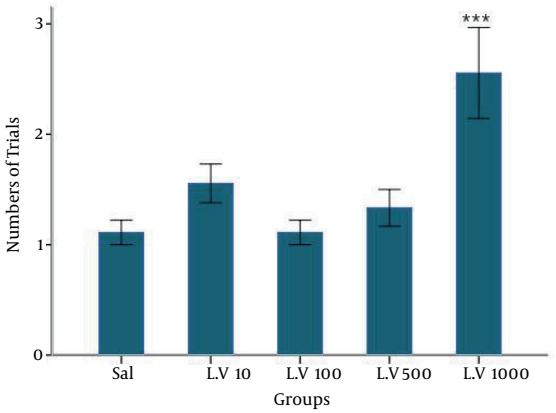
The results of this study showed that there was no significant difference among experimental groups in the STLa (STL in the first acquisition trial) [$F(4, 39) = 0.913$ and $P = 0.466$] (Figure 1). The number of trials to acquisition was different between groups. It increased by administration of extract with dose of 1000 mg/kg [$F(4, 39) = 7{,}147$ and $P < 0.001$] (Figure 2). There was a significant difference in the STLr (STL in the retention test) among groups [$F(4, 39) = 13.982$ and $P < 0.001$]. STLr in extract-receiving groups (100, 500, and 1000 mg/kg) was significantly shorter than control group (Figure 3). The TDC of animals receiving extracts of 500 and 1000 mg/kg was longer than control group [$F(4, 39) = 5.417$, $P < 0.05$; and $F(4, 39) = 5.417$, $P < 0.001$, respectively] (Figure 4). There was no significant difference among experimental groups in EN [$F(4, 39) = 1.304$ and $P = 0.286$] (Figure 5).

Figure 1. Step-Through Latency in the First Acquisition Trial in Rats Receiving Lemon Verbena Extract (10, 100, 500, and 1000 mg/kg) in comparison to Saline-Treated Rat on the Passive Avoidance Test



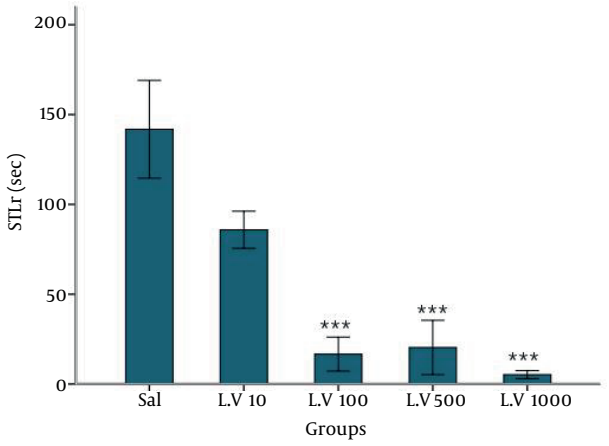
Values represent mean ± SEM (n = 8).

Figure 2. The Number of Trials in the Study of the Effect of Lemon Verbena Extract (10, 100, 500, and 1000 mg/kg) on the Passive Avoidance Test



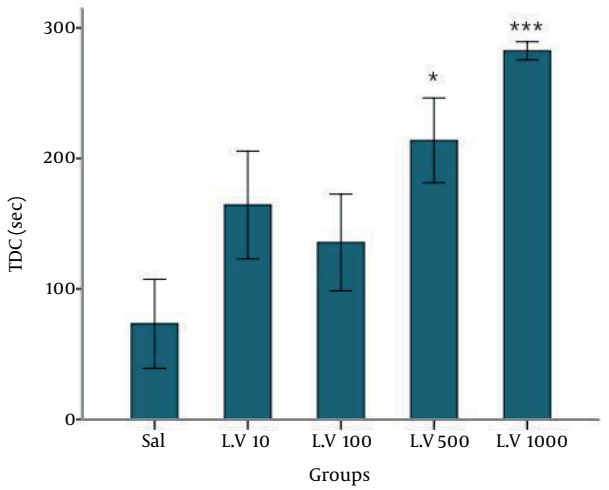
** * $P < 0.001$, (ANOVA), as compared to other groups; values represent mean ± SEM (n = 8).

Figure 3. Step-Through Latency in the Retention Test in the Study of the Effect of Lemon Verbena Extract (10, 100, 500, and 1000 mg/kg) on the Passive Avoidance Test



** * P < 0.001 (ANOVA), as compared to saline and L.V 10 treated groups; values represent mean ± SEM (n = 8).

Figure 4. Time Spent in the Dark Compartment (TDC) in the Study of the Effect of Lemon Verbena Extract (10, 100, 500, and 1000 mg/kg) on the Passive Avoidance Test

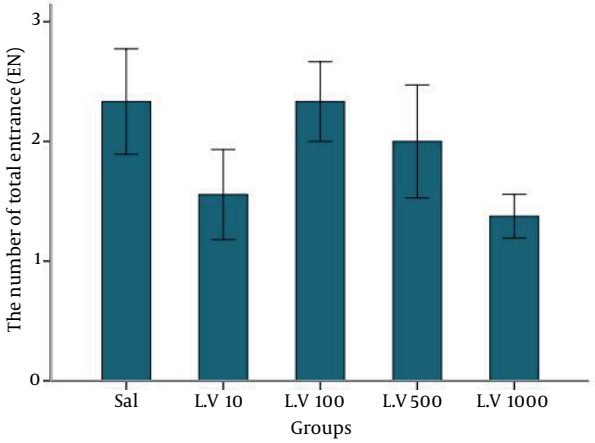


*** P < 0.001, compare to saline, L.V 10 and L.V 100 groups; * P < 0.05 (ANOVA), as compared to saline-treated rat. Values represent mean ± SEM (n = 8).

5. Discussion

The results of this study showed that treatment with aqueous extract of lemon verbena with doses > 100 mg/kg could decrease STLr and could increase TDC with doses > 500 mg/kg. However, there was not any reduction in the movement of rats. Theses effect means that lemon verbena aqueous extract exerts negative effect on memory. Terpenoids inhibit acetyl cholinesterase enzyme and therefore, increase acetylcholine (Ach) in brain (24).

Figure 5. The Number of total Entrance in the Study of the Effect of Lemon Verbena Extract (10, 100, 500, and 1000 mg/kg) in Comparison With Saline-Treated Rat on the Passive Avoidance Test



Values represent the mean ± SEM. (n = 8).

The previous studies have indicated the effect of cholinergic system (25-27) as well as flavonoids (1-3, 6) and antioxidants (7) on improvement of memory performance. With regard to flavonoids and terpenoids (14) components of lemon verbena, it is a strong antioxidant (15-17, 28); therefore, it is expected to increase the memory. This unexpected result might be explained by ROS, which contribute to molecular signaling (29). For example, in a study, tempol deteriorated impaired learning and memory in healthy rats, but improved the memory performance in diabetic rats on the passive avoidance task (30). This inconsistency in effects of tempol was related to its stronger antioxidant effect (31) and removal of excess ROS (30). Hence, it is possible that removal of excess ROS by lemon verbena could take down normal cell signaling pathways of memory processing in the healthy rats. Doses of 100, 500, and 1000 mg/kg might cause an improvement in memory performance in diabetic rats or doses between 10 and 100 mg/kg might improve the memory performance in healthy rats. More research on this issue would improve our knowledge about the involved mechanisms.

In conclusion, our results revealed that chronic administration of lemon verbena aqueous extract with doses of more than 100 mg/kg would decrease memory performance in passive avoidance test.

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Authors' Contributions

Study concept and design, analysis and interpretation of data, and critical revision of the manuscript for important intellectual content: Mojgan Veisi, Siamak Shahidi,

Alireza Komaki, and Abdolrahman Sarihi; acquisition of data, statistical analysis, and rafting the manuscript: Mojgan Veisi and Siamak Shahidi; administrative, technical, and material support: Siamak Shahidi, Alireza Komaki, and Abdolrahman Sarihi; and study supervision: Siamak Shahidi.

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