

Assessment of aqueous extract of *Lemon verbena* on anxiety-like behavior in rats

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Abstract

Aim: The purpose of the present study was to evaluate the anxiolytic effects of the aqueous extract of *Lemon verbena* on rats. **Materials and Methods:** In this study, aqueous extract of *Lemon verbena* leaves was prepared, and then male Wistar rats (200-230 g) were divided randomly into six experimental groups. Each group received a single intraperitoneal (IP) injection of saline, aqueous extract of *Lemon verbena* (10 mg/kg, 100 mg/kg, 500 mg/kg, 1,000 mg/kg) or diazepam, respectively. The level of anxiety was asserted by an elevated plus maze (EPM) 20 min after treatment. **Statistical Analysis Used:** Results were analyzed by one-way analysis of variance (ANOVA) and Tukey's *post-hoc* test. The results were expressed as mean \pm standard error of mean (SEM). The differences were considered significant at $P < 0.05$. **Results:** The results of this study showed that there was a significant difference in the number of entrances into the open arms, spent time in the open arms and number of total entrances into the open and closed arms of the EPM between control, diazepam and extract-receiving groups (10 mg/kg, 100 mg/kg, 500 mg/kg, 1,000 mg/kg) and control. The numbers of entrances into the open arms, spent time in the open arms in extract receiving groups were significantly less than control group. **Conclusion:** These results indicated that acute administration of aqueous extract of *Lemon verbena* (≥ 10 mg/kg) increased anxiety-like behavior in rats in the EPM.

Key words: Anxiety, elevated plus maze, rat, *verbain*

INTRODUCTION

Anxiety is a common psychiatric disorder in society and includes an unpleasant feeling in a person. The effects of various plants have been studied to reduce anxiety, including *Melissa officinalis*^[1,2] and *Passiflora incarnata* Linneaus.^[3] Anxiolytic activity of these plants is dependent on the activity of flavonoids.^[2,3] Isolated flavonoids from plants that are used traditionally for tranquilizers have a selective affinity for central benzodiazepine receptors (BDZ-Rs).^[4,5] *Lemon verbena* (*Lippia citriodora*) is

a perennial shrub belonging to the family of *Verbenaceae*. This plant is rich in flavonoids^[6] and its antioxidant effect has been proved by several studies.^[7-9] *Lemon verbena* leaves are used in herbal tea or in standard tea in place of actual lemon (as is common with Moroccan tea).^[10] *Lemon verbena* has also been used traditionally to reduce stress and anxiety;^[11] however, no scientific study has been done to prove this claim. The aim of the present study was to evaluate the effect of aqueous extract of *Lemon verbena* on anxiety-like behavior in male rats by using the elevated plus maze (EPM).

MATERIALS AND METHODS

Preparation of the extract

Dried leaves of *Lemon verbena* were purchased from Mehr Giahe Kosar Company (Mashhad, Iran). The chopped leaves were soaked in 2 L of distilled water for 12 h at room temperature. Then the aqueous material was filtered, dried

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by hot air ovens (50°C), and turned into a 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.

Animals and experimental groups

Male Wistar rats (weighing 220-250 g) were bought from the Pasteur Institute of Iran. All animals were kept in a room under controlled conditions of $24 \pm 1^\circ\text{C}$ and 12/12 reversed light-dark cycle. They were housed in triple groups in polypropylene cages with free access to water and food *ad libitum*. All procedures were done according to the guide for the caring and using of laboratory animals published by United States Nation Institutes of Health (NIH Publication No. 85-23, revised 1985). The rats were randomly divided into four experimental groups, one control group, and one diazepam-receiving group of eight ($n = 8$). The control group received 0.5 mL of intraperitoneal (IP) normal saline and the four experimental groups received IP *Lemon verbena* aqueous extract of 10 mg/kg, 100 mg/kg, 500 mg/kg, and 1000 mg/kg, and the diazepam-receiving group received 0.3 mg/kg of IP diazepam.

Elevated plus maze model

The EPM has been accepted as an animal model for testing anxiolytic substances.^[12-15] The EPM apparatus consists of two closed arms ($50 \times 10 \times 40$ cm) and two open arms (50×10 cm). The maze is elevated 50 cm from the floor.^[16] Twenty minutes after the treatment with normal saline (for the control group) or the *Lemon verbena* extract (10 mg/kg, 100 mg/kg, 500 mg/kg, 1000 mg/kg), diazepam (0.3 mg/kg) was injected IP, rats were placed at the center of the maze so that their heads were toward the open arms. The number of entrances into the open arms, time spent in the open arms, and number of total entrances into the open and closed arms were recorded for 10 min by using closed circuit camera. The tests were performed between 9 AM and noon. An increase in both the number of entrances into the open arms and time spent in the open arms are indicators of anxiety reduction in rats.^[17]

Statistical analysis

The 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 \pm standard error of mean (SEM). The differences were considered significant at $P < 0.05$.

RESULTS

The results of this study showed that there was a significant difference between the number of entrances into the open arms [$F(5, 42) = 20.399$; $P < 0.001$] [Figure 1], spent time

in the open arms [$F(5, 42) = 14.916$; $P < 0.001$] [Figure 2], and total number of entrances into the open and closed arms [$F(5, 42) = 22.166$; $P < 0.001$] [Figure 3] of extract-receiving groups (10 mg/kg, 100 mg/kg, 500 mg/kg, 1000 mg/kg), the control group, and the diazepam-receiving group ($P < 0.001$).

DISCUSSION

The fear of height induces anxiety in the rats when they are placed on the EPM. Anxiolytic agents increase the number of entrances and spent time in the open arms by the animals.^[18] In this study, results indicated that treatment with aqueous extract of *Lemon verbena* with doses ≥ 100 mg/kg caused a decrease in the number of entrances and spent time in the open arms by the animal.

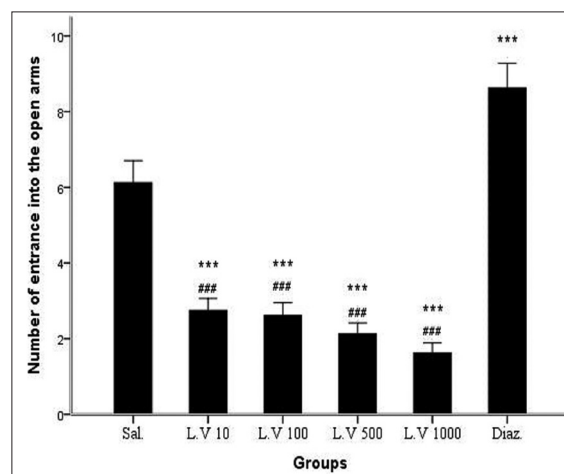


Figure 1: The number of entrances into the open arms in the effect of *Lemon verbena* extract (10, 100, 500, 1000 mg/kg) on anxiety. *** $P < 0.001$, (ANOVA), in comparison to saline. *** $P < 0.001$ in comparison to Diazepam. Values represent the means \pm SEM. ($n = 8$)

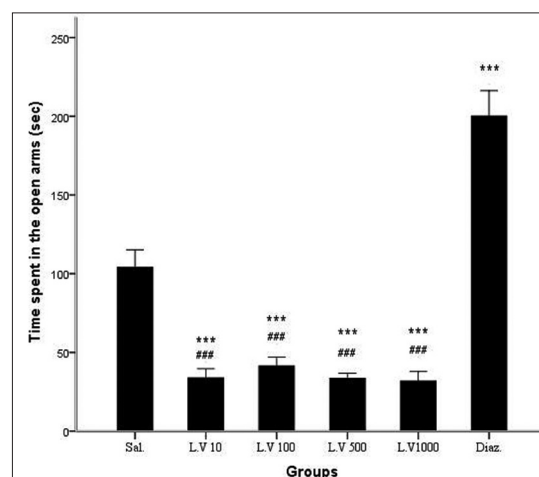


Figure 2: Time spent in the open arms in the effect of *Lemon verbena* extract (10 mg/kg, 100 mg/kg, 500 mg/kg, 1000 mg/kg) on anxiety. *** $P < 0.001$, (ANOVA), in comparison to saline. *** $P < 0.001$ in comparison to Diazepam. Values represent the means \pm SEM. ($n = 8$)

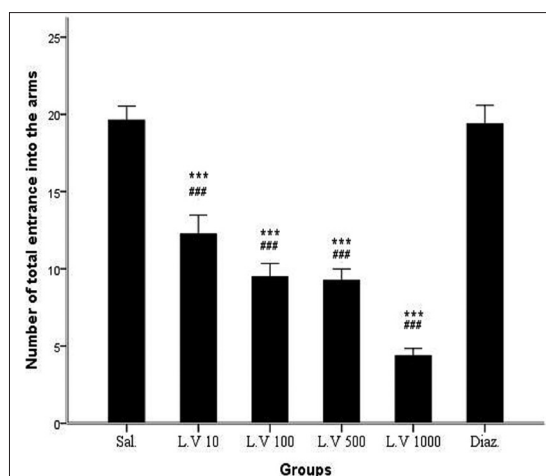


Figure 3: Total number of entrance into the open and close arms in the effect of *Lemon verbena* extract (10 mg/kg, 100 mg/kg, 500 mg/kg, 1000 mg/kg) on anxiety. *** $P < 0.001$, (ANOVA), in comparison to saline. ### $P < 0.001$ in comparison to Diazepam. Values represent the means \pm SEM. ($n = 8$)

However, there was a reduction in the motor activity of rats. On the other hand, observations showed that administration of doses of aqueous extract of *Lemon verbena* dependently caused oversleeping in rats. Previous studies showed that flavonoids caused a significant reduction of walking time and increased the number of sleeping animals.^[19] Flavonoids have a selective affinity for central benzodiazepine receptors (BDZ-Rs).^[4,5] Benzodiazepines in low doses cause anxiolytic effect, including diazepam (0.3 mg/kg), but with high doses show a more hypnotic effect, so it is possible that the doses less than 10 mg/kg of extract induce anxiolytic behavior in rats.

CONCLUSION

These results indicate that acute administration of aqueous extract of *Lemon verbena* with doses 10 mg/kg, 100 mg/kg, 500 mg/kg and 1,000 mg/kg would increase anxiety-like behaviors in rat in EPM.

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