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Lavandula angustifolia aqueous extract ameliorates anxiety and depressive-like behaviors in chronic mild stress-treated male rats

Mir Behrad Aghazadeh Ghadim, Asma Neisy, Mohsen Sisakht and Zahra Khoshdel*

Department of Medical Biochemistry, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

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The study aims to show the possible anxiolytic and antidepressant-like properties of an oral lavender's flower aqueous extract (LAE) in chronic mild stress (CMS) model of rats. Test and control rats received LAE (200 or 400 mg/ kg) and distilled water respectively. Tests of Sucrose preference (SP), elevated plus maze (EPM), and open field (OFT) were used to evaluate rats' behavioral changes . The percentage of SP in the CMS group was 2.6 times less than that of the unstressed group ($p<0.05$). However, administration of LAE (200 and 400 mg / kg) increased SP of CMS rats by 2.0 and 3.01 times respectively in comparison to those of the stressed animals. EPM analysis revealed that 5-week CMS exposure significantly reduced the number of entries in open arm (0.8 times) as compared to unstressed rats (6.6 times). LAE (200 and 400 mg / kg) reversed CMS by increasing both the total time spent (1.73 and 1.23% respectively) and the number of entries in open arm (5 and 5.7 times respectively) compared to those related to unstressed group ($p< 0.05$). CMS decreased the number of entries and time spent in the central zone of OFT. Administration of LAE (200 and 400 mg/ kg) to stressed rats enhanced the total distance traveled respectively by 3.30 and 2.65% than the control rats. Taken together, oral lavender aqueous extracts showed ameliorating effects on the depression and anxiety-related behaviors in rats.

Key words: Depression, lavender, chronic mild stress, anxiety, aqueous extract.

INTRODUCTION

Depression, the silent mental illness, is expanding rapidly and can be considered as one of the most common diseases in the world (Adams and Adams, 1991; Pollard et al., 1975). It is defined as a negative emotional experience that is closely associated with behavioral and psychological changes. At clinical levels, depression often disrupts the social engagement process and the patient retires from usual activities (Adams and Adams, 1991). Recent evidence has shown that long-term and chronic exposure to stress factors might lead to an alternation of some genes that are expressed in the hippocampus and eventually increase the incidence of depression (Caspi et al., 2003; Kaufman et al., 2006; Kendler et al., 2005). However, the mechanism has not been elaborated on so far, which complicates the discovery of an effective treatment for the illness.

Nevertheless, over the past decades, several categories of helpful drugs including amitriptyline and imipramine,

*Corresponding author. E-mail: khoshdelz@sums.ac.ir. Tel/ Fax: +987132303029.

which are classified as tricyclics antidepressants, and also selective serotonin reuptake inhibitors (SSRIs), e.g. Fluoxetine, have been discovered and prescribed to patients. However, extensive research has shown that many of them can exert unpleasant side effects such as hypotension, insomnia, sexual dysfunction, myoclonus and daytime sedation (Butler and Pilkington, 2013; Cohen, 1990; Remick and Froese, 1990). Thus, the need for drugs with fewer complications and easier access has shifted the attentions to herbal medicine (Sewell and Rafieian-Kopaei, 2014); among them St John's wort (Linde et al., 1996), valerian, Zhu sha (Wing, 2001) and lavender are the most widely used groups of antidepressant agents for psychiatric problems. Lavender (*Lavandula angustifolia)* is a genus of 47 known species of flowering plants in the mint family, Lamiaceae. It is originally from the Mediterranean area and because of the special aroma, it is known as “the queen of the garden of fragrances” (Chen and Chen, 2015). It has a long history of usage as a treatment for different types of neurological diseases in folk medicine (Cavanagh and Wilkinson, 2002; Gorji, 2003; Gyllenhaal et al., 2000; Vakili and Gorji, 2006). It was used as tea infusion (that is its aqueous extract) in traditional medicine to ameliorate insomnia, depression and nervous disorders. The aqueous extract contains phenolic components, e.g. hydroxycinnamic acids and flavone glycosides (Harborne and Williams, 2002) which appear to have antioxidant properties (Zheng and Wang, 2001). While the effects of lavender oil on humans and rodents’ nervous system is now well established (Bradley et al., 2007), its aqueous extract has not been thoroughly studied. Therefore, the present study examines the anxiolytic and antidepressant-like effects of chronic consumption of the lavender aqueous extract on the chronic mild stress (CMS) induced depression by using SPT, EPM and OFT on the rodent model of depression.

MATERIALS AND METHODS

Preparation of lavender aqueous extracts (LAE)

A sample was provided as dried flowers of *L. angustifolia* Mill from a herbarium store. The taxonomic identity of the plant was authenticated by herbarium of the Faculty of Pharmacy of Shiraz University of Medical Sciences, Shiraz, Iran. To prepare the LAE, 100 grams of dried lavender (*L. angustifolia* Mill) purple flowers were mixed with one liter of distilled water at 90°C. The mixture was then stirred for three hours in a packed container; filtered and lyophilized to give LAE powder. The lyophilized LAE was kept protected from light in a desiccator at 4°C until use (Kageyama et al., 2012; Sellami et al., 2013). 200 or 400 mg / kg bw of LAE powder was dissolved in distilled water just before use. These doses were selected based on the previous studies (Alnamer et al., 2012; Rahmati et al., 2017).

Drug administration

To prepare 200 and 400 mg / kg dosage of LAE, 200 or 400 mg powder was dissolved in 1 ml distilled water, respectively, just before use. All the rats in unstressed and stressed groups were treated orally at a volume of 1.0 ml with LAE at doses of 200 and 400 mg / kg. The unstressed control and stressed control groups only received distilled water.

Animals

A total number of 48 adult male Sprague Dawley rats weighing 280 ± 10 g were provided by the Animal laboratory Center of Shiraz University of Medical Sciences, Shiraz, Iran. The animals were kept on a 12 h light / dark cycle, at a constant temperature (23 ± 1℃) and humidity (50 ± 5%), with free access to food and tap water; however, these conditions were subject to change during the deprivation periods required in the chronic mild stress (CMS) procedure. Moreover, behavioral tests were performed during the dark phase at 20:00–5:00. All the experimental protocols were approved by the Institutional Animal Ethics Committee of Shiraz University of Medical Sciences (Code: IR. SUMS.REC. 1397.861). Before starting the experiment in order to minimize nonspecific stress responses during the experiment, the animals were allowed to adapt to the laboratory environment for one week. Then, they were randomly divided into 2 groups: CMS and unstressed. The unstressed rats were maintained under normal conditions, and during the stress procedure, CMS rats were housed separately in a different room. Next, each group was subdivided into three groups (8 rats in each group): (i) Unstressed rats were exposed to distilled water *ad libitum,* (ii) unstressed rats which were orally treated with LAE at a dose of 200 mg / kg, and (iii) unstressed rats which were orally treated at a dose of 400 mg / kg of LAE – each of the three groups was housed in a separate cage; (iv) CMS rats which were orally treated with distilled water, (v) CMS rats which were orally treated with 200 mg / kg of LAE, and (vi) CMS rats which were orally treated with 400 mg / kg of LAE – each rat was housed in a separate cage. During the 5-week period of treatment, water or respective dose of LAE (200 and 400 mg / kg) was orally administered to rats, everyday 1 h before exposure to CMS procedure. All animals were weighed weekly once after the acclimation and regularly over the next 5 weeks.

Chronic mild stress procedure

The chronic stress procedure in this study was adapted from Willner et al. (1997). The protocol involved exposure to a variety of unpredictable mild stressors over a five-week period, and the stressors program was randomly scheduled for each week. Stressors in this study were: two periods (18 h) of grouped caging (four per cage), two periods (6 h) of tilted caging, two periods (20 h) of food and water deprivation, one period (18 h) of wet cage (200 ml of water spilled in each cage,) two periods (3 h) of stroboscopic lightning (a flashlight flickers at 300 flashes/min in dark room) one period (48 h) of continuous light, two periods (3 h) of white noise (a non-tuned radio on high volume)

Sucrose preference test (SPT)

Anhedonia or inability to feel pleasure, a distaste to drink sucrose solution (sweetened water), was evaluated using an SPT. For a two-bottle sucrose preference test, all the stress and control rats were trained to drink a 2% sucrose solution during one-hour-long sessions for 5 days (from 10.00 am until 11.00 am). The positions of the sucrose and water bottles were changed in each training session, to omit any preference to one side. A week after the last training session, the CMS period was started, and at the end of each week of CMS period, the rats were deprived of food and water

for 20 h and SPT was assessed pursuant to Willner et al. (1987). SPT was calculated by using the following formula:

% Preference = [(Sucrose preference/ Total fluid intake) ×100].

The OFT procedure

The test was carried out in a 72 × 72×36 cm black Plexiglass square as the apparatus. The apparatus floor was divided into 16 equals 18 × 18 cm squares. Four central squares were used because normal rats had high locomotor activity and crossed the lines of the test chamber many times during a test session (Fuchs and Flügge, 2006; Redmond et al., 1997). All rats, individually, were subjected to an open field test 24 h after the last chronic stress procedure. The test began by placing the rats at the center arena of the open field apparatus. The test used a CCD camera (2 frames/s) and tracking software (EthoVision XT 11Noldus Information Technology B.V., Wageningen, and the Netherlands). The OFT provides a measure of locomotion, exploration, and anxiety. In detail, exploration: The number of central square entries and the duration of time spent in the central square (Hilakivi-Clarke et al., 1990; Sáenz et al., 2006); Thigmotaxis: Tendency to the walls and corners (Simon et al., 1994; Treit and Fundytus, 1988); Total distance traveled (centimeter) were measured during a 15 min test (Walsh and Cummins, 1976).

Elevated plus-maze (EPM) apparatus and procedure

The elevated plus-maze allows a rapid screening of potential anxiolytic drugs (Lister, 1987; Rodgers et al., 1997). The maze was made of two opposite 50 × 10 cm open and two 50 × 10 × 40 cm closed arms. Central platform and the arms were made of grey Plexiglass, while the walls of the enclosed arms were made of black Plexiglass. The test was performed within the first half of the dark phase of their light/dark cycle by placing the animals on the center of the plus maze, and they were allowed to explore the apparatus freely for 5 min; while the frequency and duration of entries to both open and enclosed arms were recorded using a CCD camera (2 frames/s) placed above the apparatus. Then, the data were encoded using the tracking software (EthoVision XT 11Noldus Information Technology B.V., Wageningen, and the Netherlands). The maze was cleaned all over with alcohol after each test. A number of standard factors were recorded for more analysis**,** including Open arm frequency: the number of rat entry into the open, unprotected arms. Open arm duration: the total time the rat spent in the open arms (Cruz et al., 1994; Lister, 1987).

Statistical analysis

All results are expressed as mean ± SEM. Analysis of variance (ANOVA) was conducted using computer software (SPSS Inc, USA version 16) for the comparison across the experimental conditions. The Tukey test was used for post hoc analysis. Differences were considered to be statistically significant if P< 0.05. One-way ANOVA was performed after normal distribution of data was verified by the Shapiro-wilk test.

RESULTS

Effect of CMS and two doses of LAE treatment on the sucrose preference test (SPT)

As a main index for evaluating anhedonia, the sucrose consumption of each group of rats was measured at week 0- and 5-weeks post CMS and CMS accompanied by drug treatment. Figure 1A represents the time course of the level of sucrose intake during the entire period of 6 weeks, involving baseline, and 5 weeks of stress regimen without any drug administration and further five weeks of stress procedure accompanied by chronic treatment of two doses of LAE. As can be seen, there was no significant difference between the groups in baseline on sucrose intake (Week 0 p> 0.05 Figure 1A). In the rats subjected to CMS regimen for 5 weeks, the sucrose intake of this group of rats was significantly lower than the unstressed group. By the third week of CMS procedure, sucrose consumption started to significantly decrease and a progressive decline in the sucrose consumption reflecting inability to feel pleasure. Figure 1B shows that average sucrose consumption of the stressed control group (STC) rats was almost 22.40% which was 2.6 times less than the unstressed control group (P< 0.05) Figure 1B also indicates that administration of two doses of LAE (200 and 400 mg /kg) for 5 weeks to the animals continuously exposed to CMS resulted in a significant reduction in anhedonia, as measured by sucrose intake. LAE consumption has dose-dependently increased the sucrose preference, 2.0 and 3.01 times in the stressed + LAE 200 mg /kg and stressed + LAE 400 mg /kg test groups in comparison to the STC group, respectively. There was no significant difference between the control group and the rats treated with two doses of LAE during 5 weeks. All data were expressed as the mean ± SEM of each group. One-way ANOVA was performed, and Tukey test was used for post hoc analysis. A P-value of <0.05 was considered statically significant.

Effect of CMS and two doses of LAE on the rats' behaviors in the OFT

The OFT results are shown in Figure 2A to D. After 5 weeks of the CMS procedure, the total movement distance and the percentage of time to reach the central area significantly decreased, whereas total immobility time increased significantly compared to that of the control rats. Furthermore, the frequency of entry to the center as well as total movement distance of CMS rats was reversed by treatment with two doses of 200 and 400 mg /kg of LAE. As can be seen, administration of 200 and 400 mg / kg of LAE significantly enhanced the frequency of entry to the center as well as the time spent in it, compared to the STC group (P<0.05). This increase was in a dose-dependent manner. The average entry to the center was 10% for the ST + LAE 400 mg /kg group which was 1.42 and 6.25 times more than the ST + LAE 200 and the STC rats, respectively. Additionally, the most surprising aspect of Figure 2A is that treatment of LAE in a dose of 400 mg / kg strongly suppressed the effect of CMS because no significant difference was found in

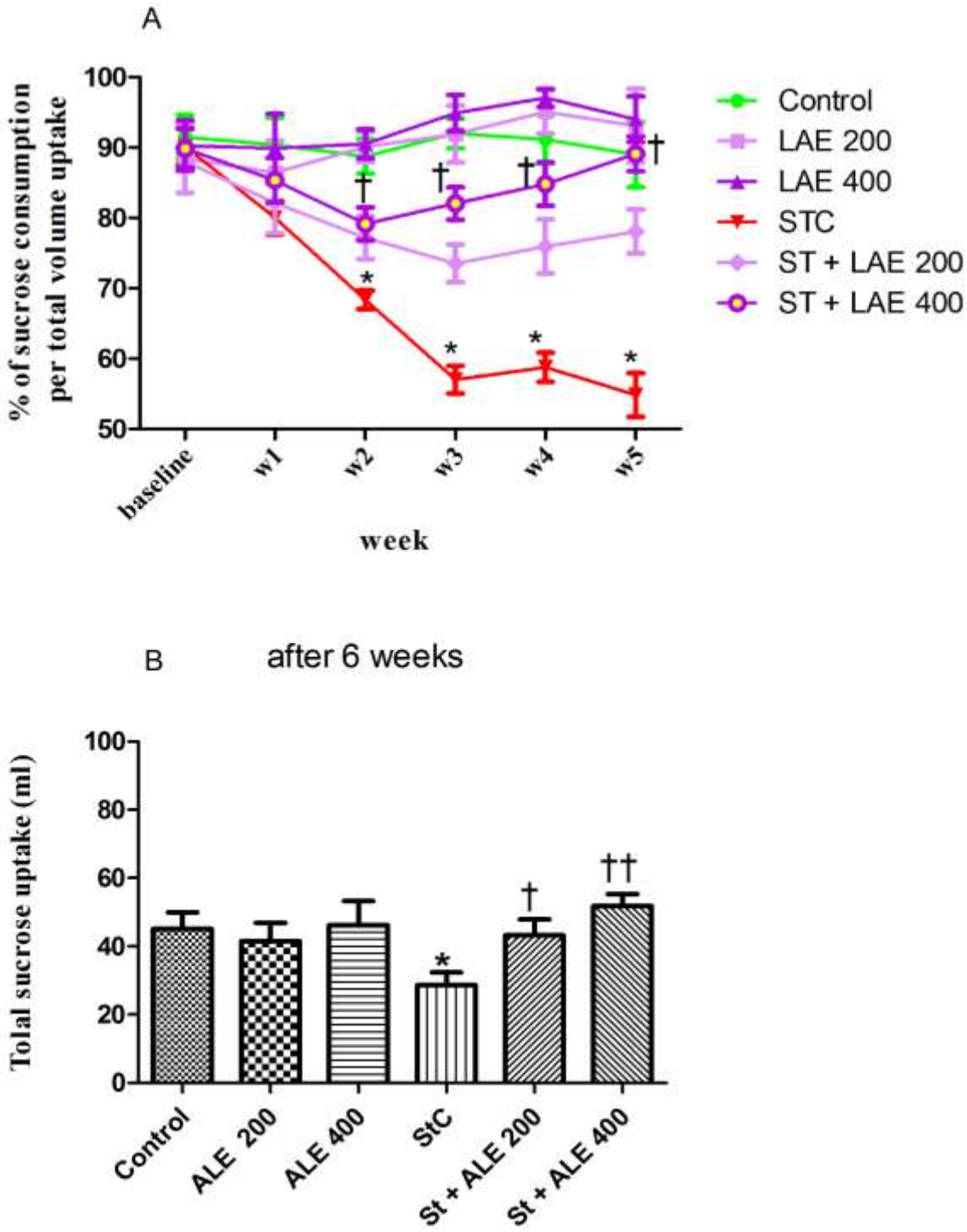

Figure 1. Effect of CMS and two doses of LAE treatment on the sucrose preference test. (A) Represents the time course of the level of sucrose intake during the entire period of 6 weeks, involving baseline, and 5 weeks of stress regimen without any drug administration and further five weeks of stress procedure accompanied by chronic treatment of two doses of LAE. Data represent the mean ± SEM, n=8. * P<0.05 versus control, †, P<0.05 versus CMS (one-way ANOVA, Tukey post hoc tests). Figure 1B shows the average sucrose consumption of stressed control group (STC) rats was 2.6 times less than the unstressed control group (P< 0.05). (B) also indicates that administration of two doses of LAE (200 and 400 mg / kg) for 5 weeks to the animals continuously exposed to CMS resulted in a significant reduction in anhedonia, as measured by sucrose intake. The sucrose intake could be reversed significantly compared to the CMS group. Data represent the mean ± SEM, n=8, * P<0.05, compared to the control group, †, †† P<0.05 and P<0.001 respectively compared to STC (one way ANOVA, Tukey post hoc tests).

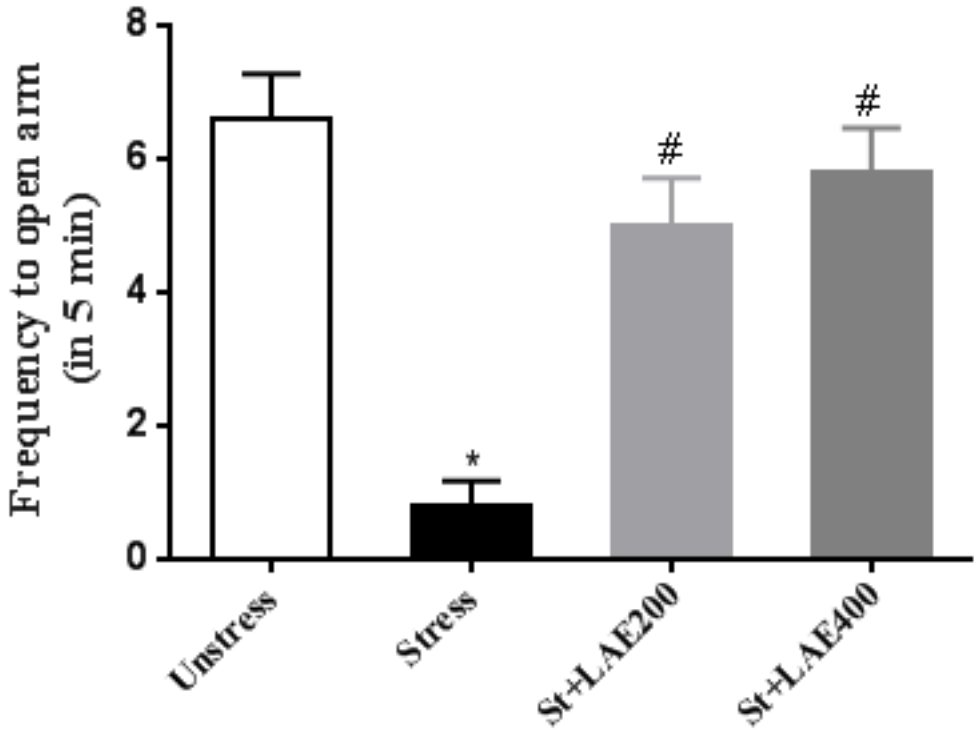

Figure 2A. The Frequency of central squares entry in OFT presented as mean ± SEM and P<0.05 is considered as significant (one-way ANOVA, Tukey post hoc tests). The Asterisk (*) indicates the significant differences compared to the control group. The Hash (#) shows the meaningful differences in comparison to STC rats and remarkable differences between two doses of LAE in ST +LAE groups depicted by delta (δ).

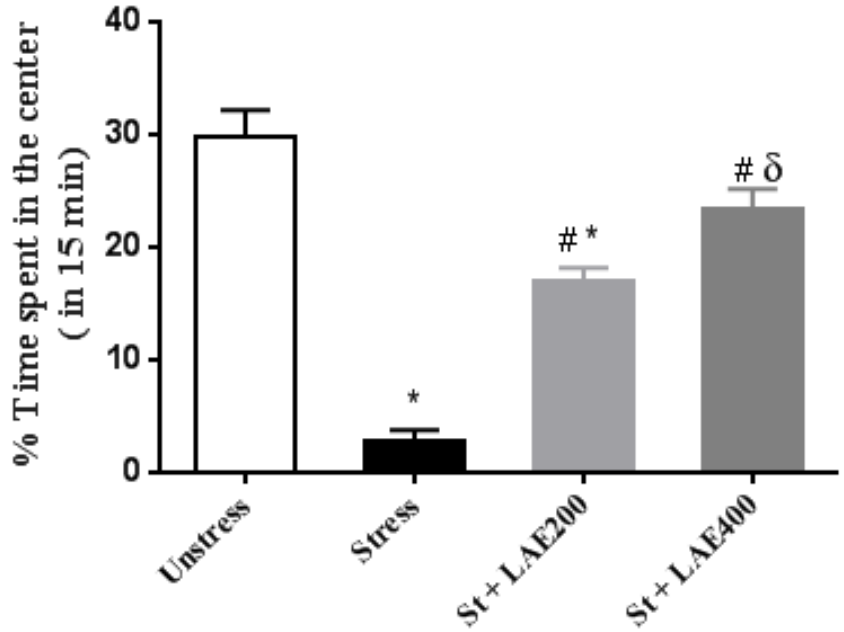

Figure 2B. Percentage of the time spent in the center square by male rats in 15 min. * P< 0.05 is a comparison against the control group, # P< 0.05 is against the Stress group, and **δ** P<0.05 shows comparison between two different doses of LAE (one-way ANOVA, Tukey post hoc tests). Data presented as mean ± SEM of 8 animals per group.

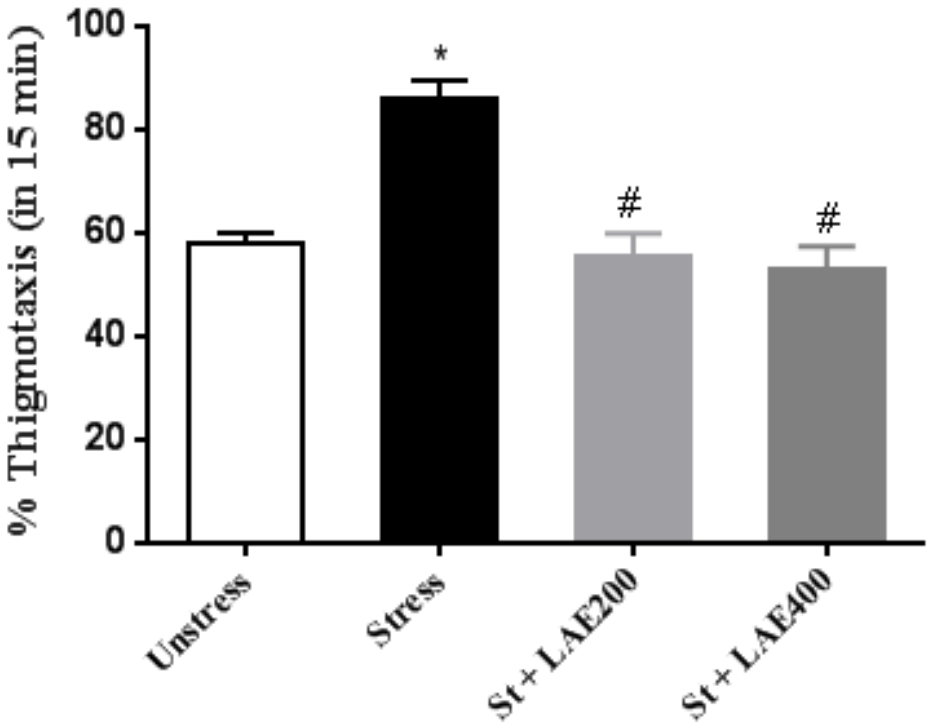

Figure 2C. The percentage of the time spent in the corners or near the walls (Thigmotaxis) in 15 min of OFT. Data presented as mean ± SEM. P < 0.05 is considered as statistically significant differences. * P < 0.05 is a comparison against the control group, # P < 0.05 is against the stress group (one way ANOVA, Tukey post hoc tests).

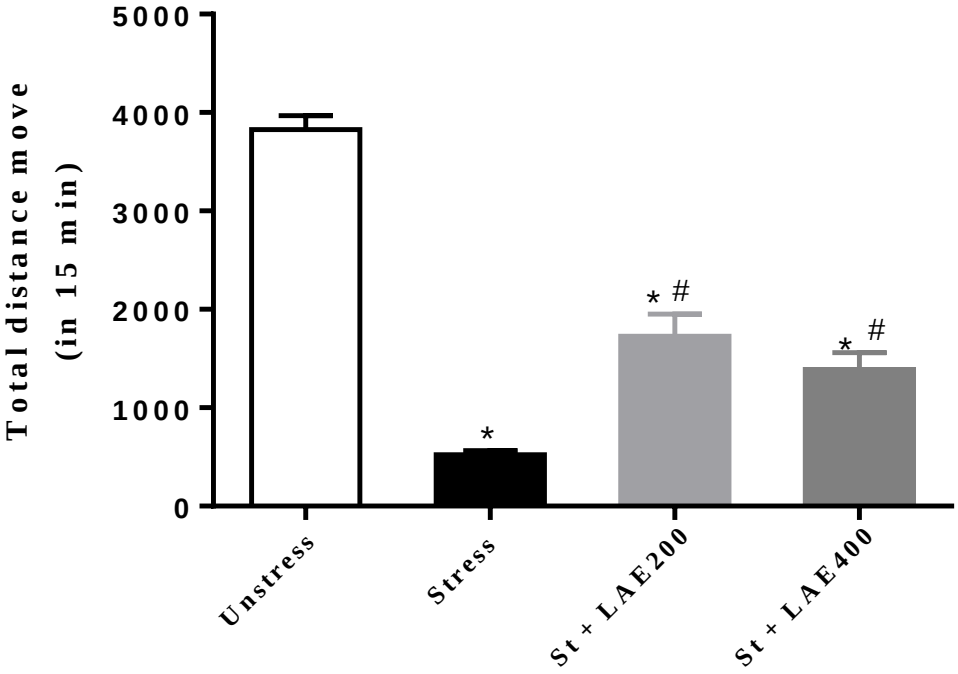

Figure 2D. The total distance traveled by rats in 15 min of OFT. Data presented as mean ± SEM. P < 0.05 is considered as statically significant differences. * P < 0.05 is a comparison against the control group, # P < 0.05 is against the stress group (one-way ANOVA, Tukey post hoc tests).

comparing the frequency to center for the ST + LAE 400 mg /kg animals (10 times) to the control rats (13.6 times). Moreover, Figure 2C shows that Thigmotaxis time in both ST + LAE 200 and ST + LAE groups decreased significantly compared to the STC group ($P<0.05$); however, there were no significant differences between 200 and 400 mg / kg doses. Figure 2D also indicates that administration of two doses of LAE (200 and 400 mg / kg) to the animals continuously exposed to CMS enhanced the total distance traveled by ST + LAE 200 and ST + LAE 400 rats by 3.30% and 2.65% than the STC rats, ($P<0.05$) respectively.

Effect of CMS and treatment of two doses of LAE on elevated plus maze

As depicted in Figure 3A. 5 week CMS exposure significantly reduced the number of entries in open arms during 5 min (0.8 times) as compared to unstressed rats (6.6 times). Chronic consumption of two doses of LAE (200 and 400 mg / kg) for 5 weeks coincided with CMS led to a sharp increase in the number of entries to open arms during 5 min (5 and 5.7 times respectively) compared to those related to CMS group ($P< 0.05$). Also, there was no significant difference between the effects of two doses. Besides, CMS resulted in 19.96% reduction in the time spent in open arms in stressed group when compared with unstressed groups ($P<0.05$), as presented in Figure 3B. We found that LAE (200 and 400 mg/kg) reversed CMS by increasing the total time spent (1.73 and 1.23% respectively) compared to those related to CMS group ($P< 0.05$). There were no significant differences in the time spent on the open arms between the administrations of two doses of LAE (200 and 400 mg/ kg) in stress groups when compared with normal rats.

DISCUSSION

To gain a further insight into the possible antidepressant properties of Lavender aqueous extract, we studied the effects of chronic treatment with 200 and 400 mg / kg doses of LAE on the animal model of depression. Taking into account previous studies, at first, the depression model rat was successfully created by using CMS as the current well-accepted method for this aim (Dang et al., 2009; Levshina and Shuĭkin, 2002; Willner, 2017; Yang et al., 2014). CMS caused an obvious anhedonia, tested by sucrose preferences test (Willner, 1997), as well as an anxiogenic effect and depression-like behaviors in the EPM and OFT box (Denenberg, 1969; Plaznik et al., 1989; Walsh and Cummins, 1976; Willner et al., 1987). Evaluating the effects of LAE revealed that it could be considered as a potent herbal antidepressant and anxiolytic compound. Higher explorative behaviors and elevated desire for sucrose 2% are reported to be acceptable indicators of the reduced levels of depression (Willner et al., 1987). Our data also revealed that under the antidepressant properties of the 200 and 400 mg / kg doses of LAE, concomitant with CMS, all the LAE treated rats got the courage to distance themselves more from the walls of the test box to investigate the new environment they faced. Also, the time they spent in the center arena was remarkably more than anxious and depressed animals (STC rats). Bondi et al., 2007 have also demonstrated that chronic antidepressant treatment was able to prevent depression and anxiety-related behaviors (Bondi et al., 2007). In the same vein, Czéh et al. (2005) reported that concomitant fluoxetine treatment with stress prevented the stress-induced numerical decrease of the hippocampal astrocytes (Czéh et al., 2005). In contrast with our observation, Kageyama et al. (2012) stated that acute consumption of 3428 mg / kg of LAE did not change the locomotor activity in the OFT. This difference is probably explained by the fact that the duration of any treatment plays a crucial role in the effectiveness of the drug, so that there is a number of antidepressant drugs, namely fluoxetine, desipramine, and imipramine which have shown to exert completely different effects when taken in an acute form compared to when taken chronically (Kageyama et al., 2012; Porsolt et al., 1978; Paul Willner et al., 1987). Several reports have shown that the Thigmotaxis in the OFT box and the frequency of entries into, plus the time spent on, the open arms in the EPM test box are the major anxiety-related factors that provide a measurement of drug potency to attenuate the fear-induced inhibited activity (Almeida et al., 1991; Pellow et al., 1985; Pellow and File, 1986; Simon et al., 1994; Treit and Fundytus, 1988). The rats' behavioral analysis illustrated considerable anxiolytic properties of lavender aqueous extract. LAE at two doses (200 and 400 mg / kg) has remarkably reversed the effect of CMS in stressed groups; however, there were no significant differences between two doses of LAE in normal group. These results suggest that LAE have powerful anxiolytic properties which makes lavender a possible herbal option to manage anxiety. To find out whether the effect of LAE is completely dose-dependent, and recognize the most effective dose, we used two different doses of LAE based on the literature. To find out whether the effect of LAE is completely dose-dependent, and recognize the most effective dose, we used two different doses of LAE based on the literature (Rahmati et al., 2017). The rats' behavioral examination has shown that the LAE effects on the depression-related behaviors – frequency of entry to the open field center arena and the time spent into the center along with sucrose preference – have totally been dose-dependent. However, it was not significantly different in the anxiety levels indicators including Thigmotaxis, and EPM. The accuracy of this observation is confirmed by the fact that the relationship between two doses in the unstressed

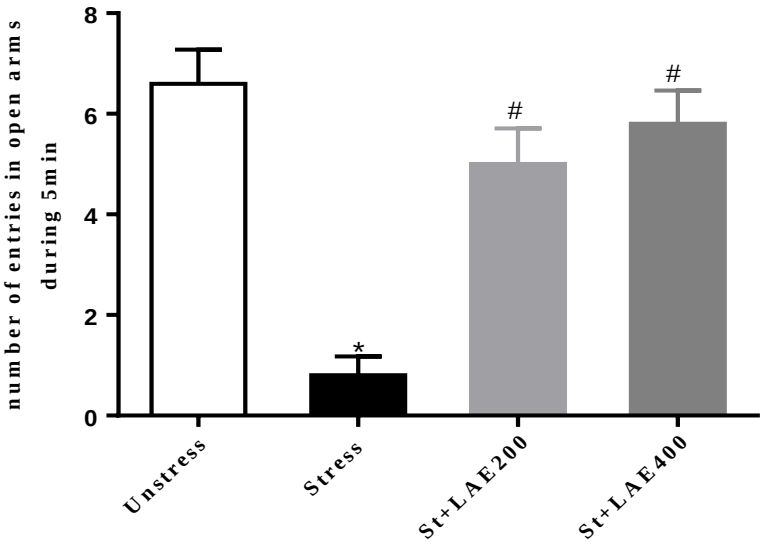

Figure 3. (A) The frequency of open arms entire in EPM test presented as mean ± SEM and P < 0.05 is considered as significant (one way ANOVA, Tukey post hoc tests). The Asterisk (*) indicates the significant differences compared to the control group. The Hash (#) shows the meaningful differences in comparison to STC rats. There was no significant difference between the effects of two doses.

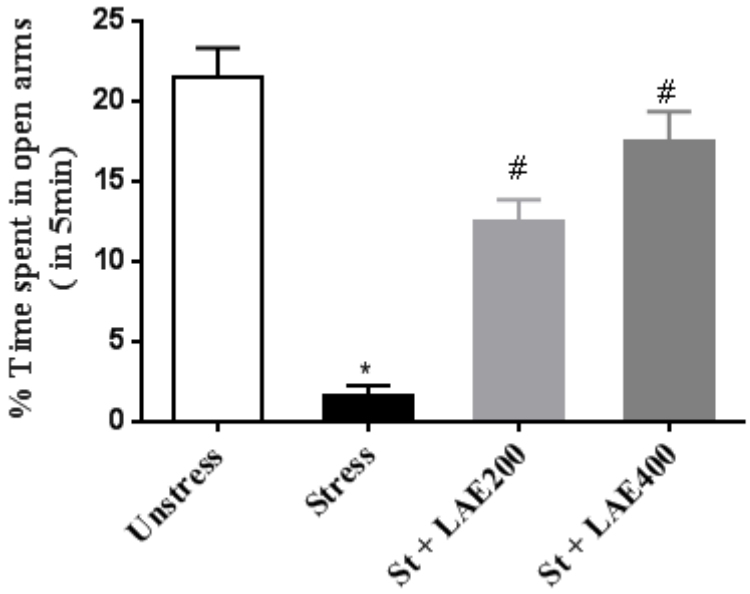

Figure 3. (B) The percentage of time spent in open arms in Elevated plus Maze box. Data presented as mean ± SEM and P < 0.05 is considered as significant (one way ANOVA, Tukey post hoc tests). There was no significant difference between the effects of two doses. The Asterisk (*) indicates the significant differences compared to the control group. The Hash (#) shows the meaningful differences in comparison to STC rats.

group rats treated with 200 and 400 mg / kg (data not given) LAE was exactly the same as the ST + LAE 200 and ST + LAE 400 groups, so the probability of any random result has been eliminated. Treit and Fundus in

1988 reported that Thigmotaxis might provide pharmacological criteria of a drug such as dose-dependent sensitivity and relative potency. Hence, LAE anxiolytic effect could not decisively be considered as dose-dependent. Furthermore, all dosages of lavender have been previously examined and reported to have an anxiolytic nature (Alnamer et al., 2012; Cline et al., 2008; Shaw et al., 2007). Thus, it could conceivably be hypothesized that LAE anxiolytic properties are not dose-dependent, contrary to the antidepressant properties which occur at the highest doses. Previous studies have shown that the stimulus or sedation effect of a drug can be partially determined by examining the distance traveled in the test box. In these studies, clinically effectual antidepressant components decreased the locomotor activity and total distance, but on the contrary, psychostimulants such as caffeine significantly increased it (Bodnoff et al., 1988; Porsolt et al., 1978; Viola et al., 1995; Yadav et al., 2008). Considering this, a comparison of the distance traveled by the LAE consumer groups of ST + LAE 200, ST + LAE 400 to both STC and control groups raises the possibility that lavender exerts sedative effect. The effect is dose-dependent and broadly supports the work of other studies in this area (Chen and Chen, 2015; Koulivand et al., 2013).

In the search for active substances of LAE, many compounds have been isolated and identified from lavender oil, but it is still uncertain which one is responsible for the observed actions in the aqueous extract. The effect of the lavender ethanolic extract or oil is usually attributed to linalool (Akhondzadeh et al., 2003; Cline et al., 2008; López et al., 2017; Souto-Maior et al., 2011), while linalool as a volatile compound is eliminated by the evaporation- lyophilization process for LAE preparation (Kageyama et al., 2012). As a result, the LAE effects could be related to its non-volatile parts such as flavone glycosides and hydroxycinnamic acids (Harborne and Williams, 2002). Among these, rosmarinic acid has been reported to have an antidepressant-like activity (Takeda et al., 2002; Torras-Claveria et al., 2007) which is related to its potency to restore the hippocampal brain-derived neurotrophic factor (BDNF) (Jin et al., 2013; Kondo et al., 2015). Recent studies have strongly emphasized the role of hippocampal BDNF elevation and the following interaction of this protein with others molecules, in the treatment of depression (Chen et al., 2001; Shimizu et al., 2003). However, the data from this study are not enough to confirm or reject any of these assumptions and it would be interesting to assess the effects of the lavender aqueous extract on some of the neurotransmitter pathways involved in the etiology of depression and anxiety.

Conclusion

This research revealed that LAE, regardless of the dose, could strikingly exhibit anxiolytic property and it could decrease the depression-like behaviors in the rats in a completely dose-dependent manner. Besides, it seems that the dose of 400 mg/kg might be more effective in this regard; however, further research must be undertaken to assess the possible toxicity of this dose.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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