Research in Pharmaceutical Sciences, December 2019; 14(6): 544-553
Received: April 2019
Accepted: October 2019

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Evaluation and comparison of the antidepressant-like activity of *Artemisia dracunculus* and *Stachys lavandulifolia* ethanolic extracts: an *in vivo* study

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Abstract

Several studies have supported the preventive and therapeutic values of phenolic compounds including chlorogenic acid, syringic acid, vanillic acid, ferulic acid, caffeic acid, luteolin, rutin, catechin, kaempferol, and quercetin in mental disorders. Since these secondary metabolites are reported as the phenolic compounds of *Artemisia dracunculus (A. dracunculus)* and *Stachys lavandulifolia (S. lavandulifolia)*, the main aim of this study was the evaluation and comparison of the phenolic contents, flavonoids, and antidepressant-like activity of *Artemisia dracunculus* with *Stachys lavandulifolia*. Antidepressant-like activity of the extracts was evaluated in the forced swimming test (FST) and the tail suspension test (TST). Moreover, the open field test was conducted to evaluate the general locomotor activity of mice following treatment with the extracts. Since phenolic compounds and flavonoids play main roles in pharmacological effects, the phenolic and flavonoid contents of the extracts were measured. Though significant difference between the phenolic contents of the extracts was not observed, but *S. lavandulifolia* exhibited higher flavonoid contents. Animal treatment with extracts decreased the immobility times in both FST and TST compared to the vehicle group without any significant effect on the locomotor activity of animals. Also, *S. lavandulifolia* at 400 mg/kg showed higher potency in both tests compared to *A. dracunculus.* Our results provided promising evidence on the antidepressant-like activity of both extracts which could be related to flavonoids as the main components of the extracts, but more studies need to be conducted to specify the main compounds and the mechanisms involved in the observed effects.

Keywords: Flavonoid; Forced swimming; Open field; Phenolic content; Tail suspension.

INTRODUCTION

Depression is an important psychiatric disorder that affects the life quality of most depressed patients. Depression causes symptoms such as low mood, sadness, irritability, energy loss, loss of interest in activities, loss of concentration, tiring easily, change in sleep patterns and appetite, loss of confidence, change in libido, and thoughts of self-harm or suicide (1). Nowadays, more than 120 million people are affected by depression across the world. Based on the World Health Organization (WHO) reports, 10 to 15 percent of people have experienced a period of depression in their lives (2). A notable statistic reveals that 20 to 80 percent of depressed patients experience a recurrence, even when their main symptoms are fully treated (3). Although the impaired transmission pathways like gamma-aminobutyric acid, dopamine, and serotonin pathways can cause depression (4,5), the etiology of depression is still unclear.

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Therefore, the impairment of a special pathway in the brain cannot be pinpointed as the main cause of depression. Selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, and tricyclic antidepressants are the main groups of pharmacological agents used in the treatment of depression. On the other hand, deep brain stimulation, electroconvulsive therapy, and transcranial magnetic stimulation are some of the non-pharmacological treatments of depression (6). However, these remedies have their own side effects and some patients are resistant to the routine treatments (7). Only 70 to 80 percent of patients will positively respond to common treatments and it takes 5 to 8 weeks until all intended effects appear (8).

Nowadays, there is a growing desire to specify plant species with considerable antidepressant activity, similar efficacy to chemical medicines, and fewer side effects to be used as preventive agents (9-14). Previous studies have introduced a number of plant species with antidepressant-like properties, such as *Hypericum perforatum, Rosmarinus officinalis, and Valeriana officinalis* (15-17). Chlorogenic acid, syringic acid, vanillic acid, ferulic acid, caffeic acid, luteolin, and quercetin are reported as the phenolic compounds of *Artemisia dracunculus (A. dracunculus)* (18). Also, there are some reports about quercetin, rutin, catechin, kaempferol, and luteolin as the phenolic compounds of *Stachys lavandulifolia (S. lavandulifolia)* (19). Various findings in recent preclinical studies have supported the therapeutic value of these phenolic compounds in mental disorders (20,21). The anxiolytic effect of *S. lavandulifolia* and the significant role of *A. dracunculus* in the treatment of stress-induced depression are well reported by scientists (22-24). The imbalance between neurotransmitters and receptors in the central nervous system, hyperactivity of immune-inflammatory responses, and disruption in the normal synaptic plasticity are three major aspects of depression (25-27). Conventional antidepressant therapies mainly target neurotransmitters. *A. dracunculus* and *S. lavandulifolia* extracts may probably show antidepressant-like effects in animal models by targeting neurotransmitters and receptors, inflammation, and brain synaptic plasticity. Therfore, this study will focus on the evaluation of phenolic and flavonoid contents and antidepressant-like activity of *A. dracunculus* and *S. lavandulifolia*, in experimental animal models.

MATERIALS AND METHODS

Plants and extraction

Plant samples were collected from the Taleghan region of the Alborz province located in the central mountains of I.R. Iran. *A. dracunculus* and *S. lavandulifolia* were authenticated by a botanist at the herbarium department of Shahid Beheshti University of Medical Sciences (Tehran, I.R. Iran) where the voucher specimens (SBMU-8101 and SBMU-8102, respectively) were deposited. Usually, plants that originate in warm (low altitudes) or dry (west and south hillsides) regions carry more active compounds. Collected plants were originated from different altitudes between 500 to 1500 m and in four geographical hillsides. All the gathered plant samples were mixed together. The aerial parts of plants were crushed into a very fine powder. The extracts of the plant powder were prepared by maceration method in 900 mL ethanol 96% during 5 days, using a shaker (Stuart SSL1 shaker, UK). The products were filtered by paper filters. Finally, the filtrates were dried in a rotary evaporator (Heidolph, Germany). Both solid extracts were refrigerated until the experiment day.

Chemicals and treatment

All reagents used in the determination of the total phenolic and flavonoid contents were purchased from Sigma-Aldrich Chemical Co. (USA). Spectroscopy measurements were performed on a UV-Vis Shimadzu Multispect-1501 spectrophotometer (Kyoto, Japan). In animal experiments, ethanolic extract of each plant was suspended in distilled water using Tween® 80 (1%). Fluoxetine HCL (Sigma-Aldrich, USA)

and imipramine HCL (Sigma-Aldrich, USA) were both dissolved in normal saline. The plant extracts, fluoxetine, imipramine, and vehicle were injected intraperitoneally (10 mL/kg, i.p.) 30 min before each experiment.

Total phenolic content

The Folin-Ciocalteu reagent was used for spectrophotometrically (765 nm) measurement of total phenolic contents (28,29). A linear calibration curve was prepared with 1 mL of the rutin solution at different concentrations (25, 50, 75, 100, 150, and 200 µg/mL), 5 mL of Folin-Ciocalteu reagent (diluted 1/10) and 4 mL of the sodium carbonate solution (75 mg/mL). The absorbance was measured following 30 min. The plant extract samples were prepared at 400 µg/mL, and the same procedure was carried out.

Total flavonoid content

The aluminium chloride reagent was used for colorimetrically (415 nm) measurement of the total flavonoid contents (28,29). A linear plot was developed by mixture of rutin solution at different concentrations (2.5 mL; 25, 50, 75, 100, and 150 µg/mL) and aluminium chloride reagent (2.5 mL; 20 mg/mL). The absorbance was measured following 40 min. The same procedure was carried out on the plant extract samples (400 µg/mL).

Animals

Male Swiss mice and male NMRI mice were used in the forced swimming test (FST) and tail suspension test (TST), respectively. The open field test (OFT) was conducted on both strains of mice. Animals (8-12 weeks; weighed 18-25 g) were obtained from the Animal House of Shahid Beheshti University of Medical Sciences, Tehran, I.R. Iran. Mice were caged in groups of ten in plexiglass cages inside the animal room with a temperature of 22 ± 2 °C and 12/12-h light/dark cycle. They had free access to water and food and were handled for 3 days before each experiment to get acclimatized to the laboratory conditions. All experiments were carried out according to the Animal Experimentation Committee of Shahid Beheshti University of Medical Sciences guidelines, and the study was approved by the ethics committee (Code number: IR.SBMU.PHNM. 1394.355). Possible efforts were made to decrease animal number and distress.

Forced swimming test

This test was conducted according to the Porsolt method; a rodent screening test developed for evaluating the effectiveness of antidepressants. FST is based on the animal's willingness to escape from a stressful situation. In this research, each male Swiss mouse was placed in a plexiglass cylindrical container of water with a fixed temperature (22-25 °C) 30 min after i.p. injection of each extract in different doses (100, 200, and 400 mg/kg) or vehicle. The diameter of the cylindrical container and the depth of water were 14 and 30 cm respectively. Animals were observed for 6 min, and the immobility time was calculated in the last 4 min. The period of time that animals stopped swimming and floated on the surface of the water was considered as immobility time. Animals were allowed to dry in a warm environment after removal from the water (30).

Tail suspension test

TST is a screening test used to evaluate the effectiveness of antidepressants. In this study, male NMRI mice were used in TST. Plant extracts in different doses (100, 200, and 400 mg/kg) and the vehicle were administered (i.p.) to each mouse. Thirty min later, each mouse was dangled by the tail, using adhesive tape in a consistent position ¾ of the distance from the base of its tail, and its body dangled in the air. Mice were observed for 6 min and immobility time was calculated in the last 4 min. When animals stopped struggling, it was considered as immobility time (30).

Open field test

OFT assessed the general locomotor activity of mice. The open field box was constructed of plexiglass with dimensions of 40 × 40 × 40 cm, so mice could be observed inside the box. Thirty minutes after i.p. injection of different doses of plant extracts or vehicle, mice were placed individually in the center of the apparatus and allowed to

explore for 10 min. During this period, the animal locomotor activity was recorded using a digital camera placed above the open field apparatus which was connected to a computer. After each test, mice were moved to their home cages; the open field area was cleaned with 70% ethyl alcohol and permitted to dry between tests. All recorded videos were analyzed by Ethovision XT (Noldus, The Netherlands) software and total movement of animals was considered as their locomotor activity (31).

Statistical analysis

All data were statistically analyzed with the Graph Pad Prism 5 software. Immobility time in FST, TST, and total distance moved in OFT among different groups were compared by the one-way ANOVA and the Tukey post-test was used to locate the differences between groups. To assess statistical significance between the extracts at same doses in FST and TST, Student's t-test was performed. The *P value* < 0.05 was considered as significant difference.

RESULTS

Total phenolic and flavonoid contents

The obtained rutin calibration curves ($y = 0.0045x + 0.1058$; $r^2 = 0.994$ and $y = 0.0101x - 0.0106$; $r^2 = 0.996$) were used for calculation of total phenolic and flavonoid contents, respectively. Results (Table 1) are presented as µg of rutin equivalents in mg of the dry matter of the extract according to the following equation:

$$Total\ flavonoid\ contents\ (\mu g/mg) = \frac{C \times V}{M}$$

where C, V, and M stand for concentration of rutin (µg/mL), volume of the extract (mL), and weight of the extract (mg), respectively.

Immobility time in the forced swimming test

Animals were treated with different doses of extracts, fluoxetine (32 mg/kg), and imipramine (32 mg/kg). As shown in Fig. 1A, *A. dracunculus* at 100, 200, and 400 mg/kg decreased immobility time (162.30 ± 6.87, 161.60 ± 5.54, and 153.60 ± 6.87 sec respectively) compared to the vehicle group (202.30 ± 4.99 sec). *S. lavandulifolia* at 100, 200 and 400 mg/kg also showed antidepressant activity by decreasing immobility time (165.60 ± 5.14, 160.8 ± 9.93, and 94 ± 8.90 sec respectively) compared to the vehicle group (202.30 ± 4.99 sec) (Fig. 1B). Antidepressant-like activities of both extracts were similar to positive controls, including fluoxetine (132.40 ± 8.24 sec) and imipramine (146.70 ± 7.73sec) (Fig. 1).

Table 1. Total phenolic and flavonoid contents of *Artemisia dracunculus* and *Stachys lavandulifolia* extracts. Values are expressed as mean ± SEM, n = 3. * Indicates significant difference in the same column, $P < 0.05$.

Plant species	Phenolic content (µg rutin/mg extract)	Flavonoid content (µg rutin/mg extract)
Artemisia dracunculus	167.20 ± 21.32	48.84 ± 2.04
Stachys lavandulifolia	166.70 ± 14.71	88.87 ± 3.67*

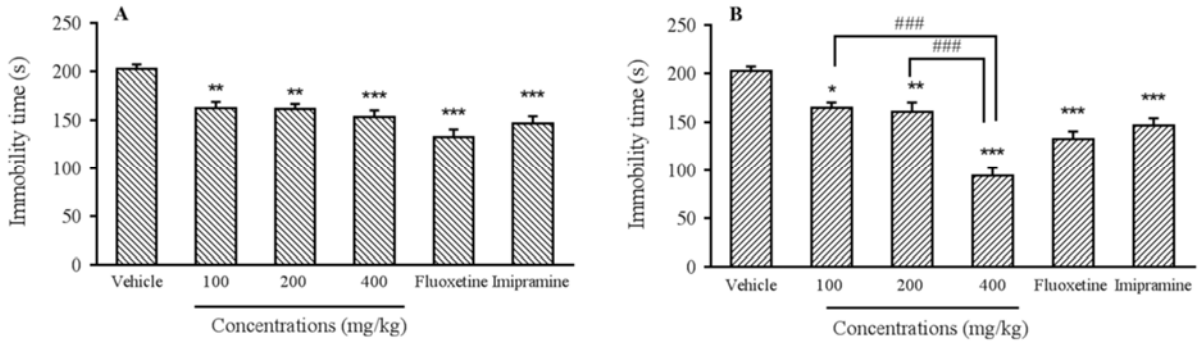

Fig. 1. Effects of (A) *Artemisia dracunculus* and (B) *Stachys lavandulifolia* extracts on the duration of immobility time in the forced swimming test. Fluoxetine and imipramine were used at 32 mg/kg. Values are presented as mean ± SEM; n = 10; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicate significant differences compared with the vehicle group. ### $P < 0.001$ shows significant difference between indicated groups.

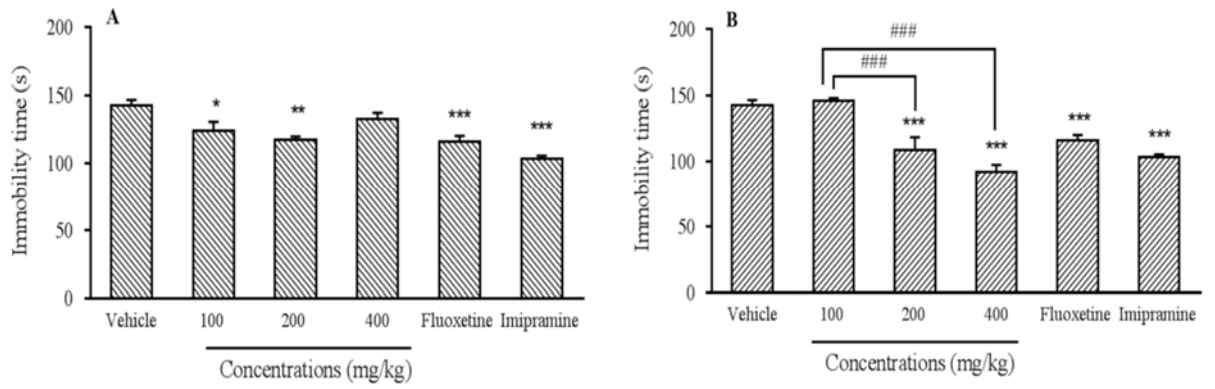

Fig. 2. Effects of (A) *Artemisia dracunculus* and (B) *Stachys lavandulifolia* extracts on the duration of immobility time in the tail suspension test. Fluoxetine and imipramine were used at 32 mg/kg. Values are presented as mean ± SEM; n = 10; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicate significant differences compared with the vehicle group. ### $P < 0.001$ shows significant difference between indicated groups. Values are presented as mean ± SEM; n = 10; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the vehicle group.

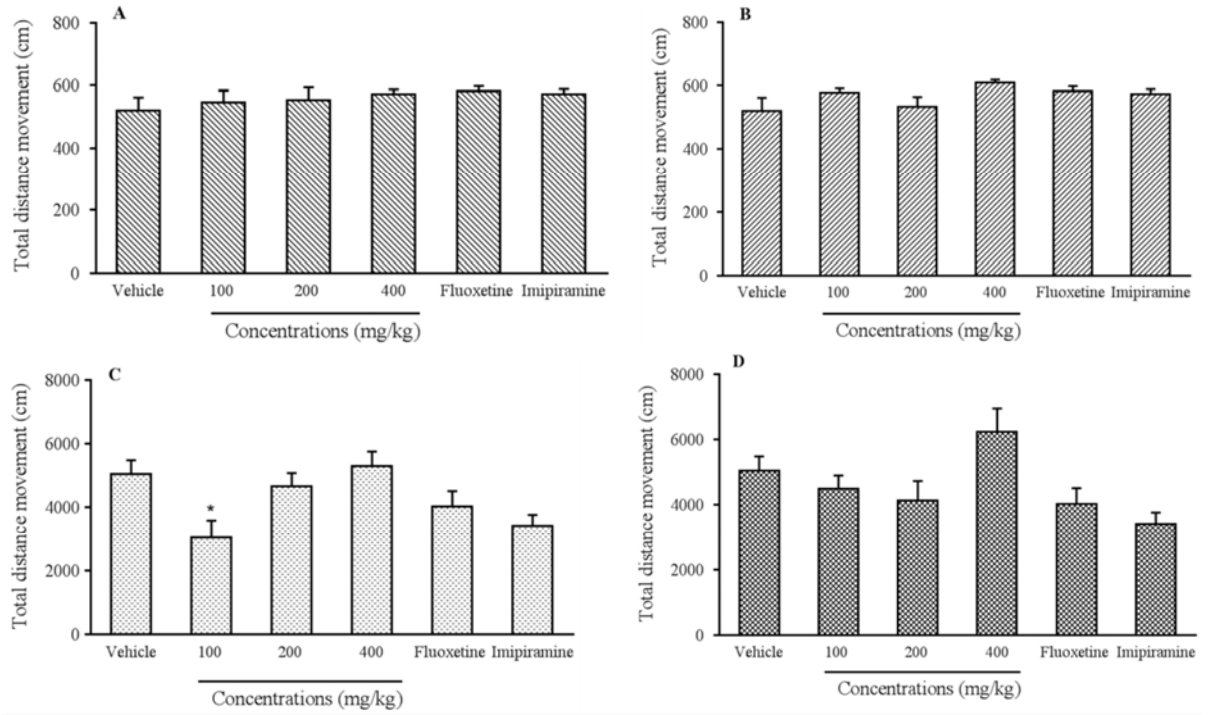

Fig. 3. Effects of *Artemisia dracunculus and Stachys lavandulifolia* on the locomotor activity of (A and B) NMRI mice and (C and D) Swiss mice in open field test. Fluoxetine and imipramine were used at 32 mg/kg. Values are presented as mean ± SEM; n = 10. * Indicates significant difference compared to the vehicle group, $P < 0.05$.

Immobility time in the tail suspension test

The immobility time was measured for each mouse and considered as the antidepressant activity. *A. dracunculus* at the doses of 100 and 200 mg/kg decreased immobility time (124.00 ± 6.58, 117.20 ± 2.50 sec, respectively), but it could not decrease the immobility time at the dose of 400 mg/kg compared to the vehicle group (142.60 ± 4.08 sec) (Fig. 2A). It should be noted that by repeating the experiment at the dose of 400 mg/kg, the same result was obtained. *S. lavandulifolia* at the doses of 200 and 400 mg/kg decreased immobility time (109.10 ± 9.42 and 92.20 ± 5.42 sec, respectively) compared to the vehicle group (142.60 ± 4.08 s), but it was not effective at 100 mg/kg (Fig. 2B). Furthermore, positive controls (fluoxetine 116.00 ± 2.01 sec

and imipramine 103.00 ± 2.34 sec) showed antidepressant activity by decreasing immobility time in mice compared with the vehicle group (Fig. 2).

Evaluation of locomotor activity in open field test

Total distance movement was considered as the locomotor activity of mice in different groups, which were treated with different doses of plant extracts, fluoxetine, or imipramine. The locomotor activity did not change following different treatments except in Swiss mice treated with *A. dracunculus* extract at the dose of 100 mg/kg. This group revealed a significant decrease in the locomotor activity compared to the vehicle group (Fig. 3).

Antidepressant activity of two extracts

In the FST, the antidepressant-like activity of *A. dracunculus* extract at 100 and 200 mg/kg was equal to the antidepressant activity of *S. lavandulifolia* extract at the same doses, but 400 mg/kg of *S. lavandulifolia* showed significantly higher activity compared to 400 mg/kg of *A. dracunculus* extract (Table 2). In the TST, both extracts had the same activity at the dose of 200 mg/kg. Although 100 mg/kg of *A. dracunculus* extract decreased the immobility time in mice and revealed a higher activity compared to the *S. lavandulifolia* extract, it was not statistically significant. Finally, the potency of *S. lavandulifolia* extract (400 mg/kg) was significantly higher than that of *A. dracunculus* extract (Table 2).

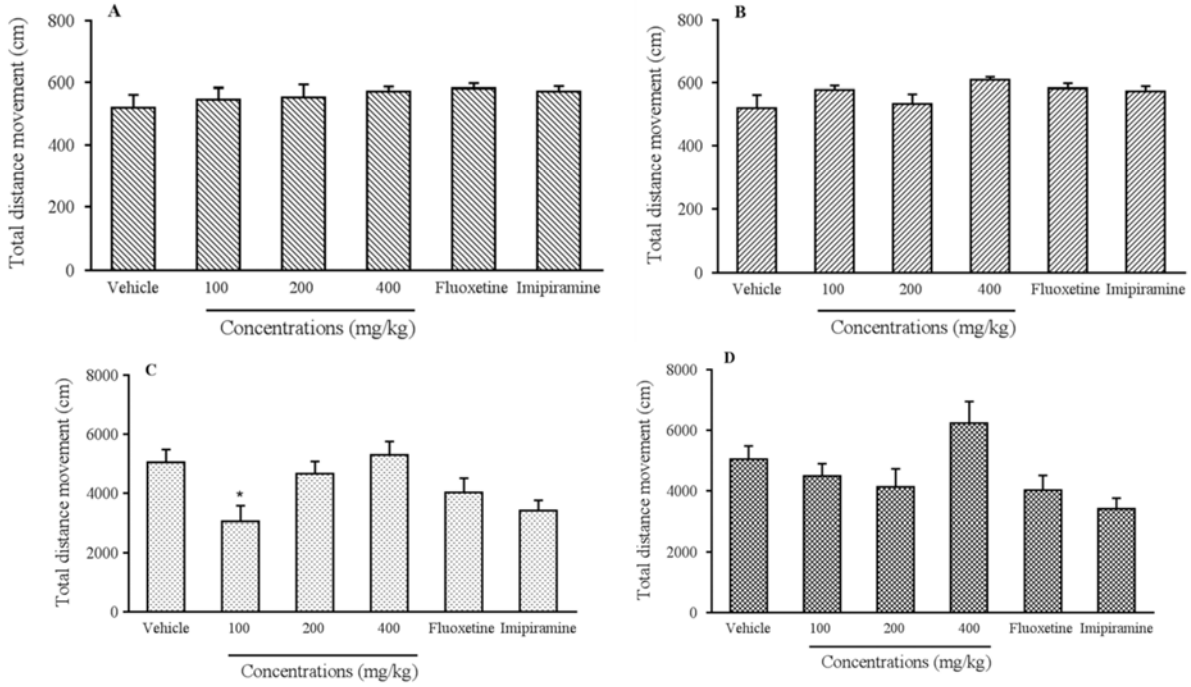

Fig. 3. Effects of *Artemisia dracunculus and Stachys lavandulifolia* on the locomotor activity of (A and B) NMRI mice and (C and D) Swiss mice in open field test. Fluoxetine and imipramine were used at 32 mg/kg. Values are presented as mean ± SEM; n = 10. * Indicates significant difference compared to the vehicle group, $P < 0.05$.

Table 2. Comparison of antidepressant activity of *Artemisia dracunculus and Stachys lavandulifolia* extracts in the FST and TST models based on the immobility time. # Indicates significant differences between two extracts at 400 mg/kg in FST and TST, respectively ($P < 0.05$). Values are expressed as mean ± SEM, (n = 10).

Tests	Plant species	Immobility time (sec) at different doses		
		100 (mg/kg)	200 (mg/kg)	400 (mg/kg)
FST	*Artemisia dracunculus*	162.33 ± 6.87	161.66 ± 5.54	153.62 ± 6.87
	Stachys lavandulifolia	165.65 ± 5.14	160.86 ± 9.93	94.00 ± 8.90#
TST	*Artemisia dracunculus*	124.01 ± 6.58	117.26 ± 2.50	132.83 ± 4.37
	Stachys lavandulifolia	146.15 ± 2.13	109.17 ± 9.42	92.20 ± 5.42#

FST, forced swimming test; TST, tail suspension test.

DISCUSSION

Nowadays, more people suffer from psychiatric diseases like depression. Sometimes these mental diseases are associated with suicide attempts. Although a variety of drug categories are prepared for the treatment of mental disorders, according to the WHO report the global burden of mental diseases is increasing every year (32). Since chemical antidepressants in different categories show effective results only in 70 to 80 percent of patients and have different side effects, the interest in discovering herbs with antidepressant-like activity and evaluation of their potency has been increased (33,34).

TST and FST are accepted as rapid, easy, and cheap tests with high potency for the prediction of antidepressant activity of chemicals and plant extracts. These tests are based on the immobility time of mice in a stressful situation (35).

The results of this study demonstrated that *A. dracunculus* and *S. lavandulifolia* extracts have antidepressant-like activity in both FST and TST. The results indicated (Figs. 1A and 2A) that the immobility time in FST and TST was decreased after i.p. injection of *A. dracunculus* extract at different doses compared to the vehicle group. This reduction seems to be dose-dependent in FST, while 400 mg/kg of *A. dracunculus* extract did not decrease the immobility time in TST. This difference between the FST and TST results could be due to the different sensitivity of FST and TST (36), or different neurochemical pathways which have a significant role in these tests (35). In addition to the sensitivity, the patterns of dose-response to a distinct treatment are different between the two models. For example, a U-shape dose-response function is reported by administration of imipramine in FST while it has a linear pattern in TST (36).

S. lavandulifolia caused a reduction in immobility time in both FST and TST, at all tested doses. This reduction in immobility time was statistically significant and dose-related compared to the vehicle (Figs. 1B and 2B). OFT was conducted after i.p. injection of each extract at different doses in order to rule out the hypothesis that the reduction in immobility time is the result of psycho-stimulant effects of the extracts which can provide a false-positive result in FST and TST. Treatment with *A. dracunculus* extract did not change the locomotor activity of NMRI and Swiss mice treated with different doses compared to the vehicle, but 100 mg/kg in Swiss mice was an exception. This finding is in agreement with the results of a recent study conducted by Khosravi *et al*. on male rats. In their study, oral treatment of rats with *A. dracunculus* extract for 21 days caused no significant change in the locomotor activity of animals. Moreover, the anti-oxidant activity of the extract was reported as the possible mechanism for anxiolytic and antidepressant effects (37). Contrary to the findings of Rabbani *et al*. (23) treatment with *S. lavandulifolia* extract did not change the exploratory activity of any group compared with the vehicle group. It should be noted that in the study conducted by Rabbani *et al*. reduction in locomotor activity was observed only during the first 5 min of activity measurement.

The results of OFT demonstrate that these extracts do not change the locomotor activity of mice, and the anti-depressant like activity of the plants are most likely specific and not related to the stimulation of general motor activity.

Chlorogenic acid, syringic acid, vanillic acid, ferulic acid, caffeic acid, luteolin, quercetin, rutin, catechin, and kaempferol have been reported as the main phenolic compounds and flavonoids of *A. dracunculus* and *S. lavandulifolia* extracts (18,19). A recent study revealed that chlorogenic acid can regulate hippocampal astrocytes monoamine oxidase-B activity and has antidepressant-like effects in mice (38). In another study, *Eucommia ulmoides* extract, which is rich in chlorogenic acid, showed an antidepressant activity by promoting serotonin release through enhancing synapsin I expression (39). Administration of syringic acid exerted an antidepressant-like property in the behavioral models by counteracting the induced-glutamate death in

the hippocampal and cortical slices (40). Ferulic acid as a glutamate antagonist and an antioxidant compound reverses depression-like behavior and oxidative stress in mice (41). It has been also reported that combination therapy with ferulic acid and piperine increases the level of monoaminergic intermediates in the brain (42). Caffeic acid and caffeic acid phenethyl ester also produce an antidepressive-like effect in the FST and TST, which are well-accepted models of depression (43). Anti-inflammatory, anti-allergic, neuroprotective, the increase in spatial memory, and reduction of cognitive decline are some of the proved effects of luteolin (44). Luteolin also inhibits neuronal cell death and endoplasmic reticulum stress. These two mechanisms are involved in the pathogenesis of depression (45). Quercetin reverses anxiety and depression-like effects induced by a corticotrophin-releasing factor in mice (46). Increasing the availability of serotonin and noradrenaline in the synaptic cleft is a defined mechanism of the antidepressant-like effect of rutin (47). Chronic treatments with catechin can decrease depression and show anxiety-like behaviors in animal models (43). According to the literature, kaempferol and quercetin isolated from *Apocynum venetum* have antidepressant-like activity, and this effect is probably due to increased norepinephrine, dopamine, and serotonin and reduced 5-HT metabolism (48).

Based on the measurement of total phenolic and flavonoid contents, there was no significant difference between the phenolic contents of *A. dracunculus* and *S. lavandulifolia*, but *S. lavandulifolia* was found to have higher flavonoid content. This finding is in agreement with the higher potency of *S. lavandulifolia* in revealing antidepressant-like activity. Therefore, it seems that phenolic compounds and flavonoids as the main components of *A. dracunculus* and *S. lavandulifolia* have significant roles in the antidepressant-like effects of the extracts, but flavonoids have a higher impact on the observed effects. However, more studies need to be carried out to specify the exact components and mechanisms related to the observed activity.

CONCLUSION

The antidepressant-like activity of *A. dracunculus* and *S. lavandulifolia* extracts were investigated through FST and TST. Positive evidence was provided by the results, but *S. lavandulifolia* extract had a higher potency compared to the *A. dracunculus* extract. It seems that some flavonoids such as luteolin, quercetin, rutin, catechin, and kaempferol play a significant role in the explained effect. Also, the antidepressant-like effects of the extracts may be related to the enhancement of serotonin and norepinephrin release in the central nervous system. However, investigation into the potential mechanism of the antidepressant-like effect of the extracts such as metabolism analysis of monoamine neurotransmitters in the brain tissue and the main isolated compounds which are responsible for this effect is highly recommended.

ACKNOWLEDGMENTS

This work was financially supported by the Research Council of Shahid Beheshti University of Medical Sciences, Tehran, I.R. Iran through the Grant No. 8098.

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