کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت های کاربردی در تدوین و چاپ مقاله
Anti-depressant Activity of Hydroalcoholic Extract of *Asperula odorata* L. in Mice

Running title: Anti-depression effect of *Asperula odorata* L

Mohammad Amin Hatami Nemati¹, Kimia Vatani¹*, Zahra Abbasy², Mahsa Hadipour Jahromi³, Poorya Davoodi⁴

¹- Young Researchers and Elite Club, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
²- Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran
³- Associate professor, Department of Pharmacology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
⁴- Department of Molecular Medicine, University of Padua, Padua, Italy

**Correspondence to:** Kimia Vatani, Young Researchers and Elite Club, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

**Abstract**

**Background:** The relationship between the treatment of depression and plant-derived substances (e.g., flavonoids, coumarin, and Scopoletin) has been demonstrated through interference with the monoamine system. The present study was planned to evaluate the anti-depressant effects and active ingredients of *Asperula odorata* plant through behavioral tests in mice. **Material and methods:** In this experimental study, 35 male Syrian mice weighing 30-40 g were examined in five groups (n=7) as follow: received oral distilled water gavage (control), 10 mg/kg of fluoxetine solution gavage (reference standard), 10, 5, and 2.5 mg/kg of *A. odorata* L. extract gavage (treatment groups). After one week, all behavioral tests, including tail suspension test (TST), forced swimming test (FST), open field test (OFT), elevated plus maze test (EPMT), and fractionation tests were performed each morning for 4-6 h within five days. **Results:** The hydroalcoholic extract of *A. odorata* contained phenolic and flavonoid substances (Shinoda test confirmed flavonoid family). Administration of extract (10 and 5 mg/kg doses) versus fluoxetine (10 mg/kg dose) reduced the immobility of animals in both FST and TST (P<0.05). At the OFT, the administered extract increased the number of central square entries of animals with higher mobility (P<0.05). At a 10 mg/kg dose, the active flavonoid ingredients increased the mice's incline to enter and spent more time within no wall parts of EPMT (P<0.05). **Conclusion:** Our study suggests that the hydroalcoholic extract of *A. odorata* L. could have significant anti-depressant activity.

**Keywords:** Anti-Depressant; Flavonoid; *Asperula Odorata* L.; Monoamine Oxidase; Mice
Introduction

Depression is one of the most common and recurrent psychological disorders across the world. Depression-induced disorders are the typical cause of suicide and hospitalization in the neurology departments of hospitals [1]. Today, depression-induced suicide is known to be one of the leading causes of death in the world. According to the research, major depression risk factors are stress, vegetarian diets, and nutritional supplements. By altering neurotransmitters, stress changes behavior and habits and can increase depression [2].

According to conducted studies in the United States, depression is more prevalent among white people than African-American people [3]. Studies showed that depression is more common among females [4]. The prevalence of depression is directly associated with race, culture, and lifestyle [5]. Depression is highly prevalent due to its high risk of recurrence in human societies [6]. Studies on Iranian adults from 2001 to 2015 indicated that 43% of Iranian people suffer from varying degrees of depression [7].

Among different treatments proposed for depression, the most common ones are tricyclic drugs such as imipramine and amitriptyline, with side effects like drowsiness, agitation, tachycardia, and weight gain [8]. Also, selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine and citalopram with nausea and headache as side effects were used [9].

Numerous studies have been conducted on the anti-depressant effects of medicinal plants whose effects are received by consuming them in the forms of extract, herbal tea or the plant itself. Hypericum perforatum [10], Polygala sabulosa [2], and Asperula orodata L. [3] are examples of such plants.

A. orodata L. is from the Rubiaceae Juss family, with more than 200 species across the world. It is native to Europe, Northern America, Ukraine, and Russia [11]. It has been demonstrated by 40 species in Ukraine, the most common one being A. orodata L. It was anciently used as a diuretic, pain treatment, and wound healing drug [12]. A. Orodata L. contains phenol carboxylic iridoids, tanning agents, flavonoids, coumarins, and steroidal sapogenin. Also, the anti-depressant properties of some of these species have been proved [12]. Indeed, these substances influence the genes of neurotransmitter receptors with anti-depressant effects, such as noradrenaline (NE), serotonin (5-HT), dopamine (DA), and 5-Hydroxyindoleacetic acid (5-HIAA) and regulate their effects. This family also covers the reversible weaknesses of monoamine oxidases [13].

The present study aims to explore the anti-depressant effects of A. orodata L. on the adult male laboratory mice using the behavior and fractionation tests.

Materials and Methods

1. Plant Materials and Extraction Preparation

A. odorata L. was cultivated and obtained from medicinal plant farmlands of Malard (Central Iran). It is about 1 m high with white flowers. The authenticity of the plant was approved by Dr. Hadiipur based on the Herbarium book. To prepare the plant extract, the aerial parts (leaves and petals) of the plant were separated and air-dried at 25-30 °C. From the dried aerial parts (95 g), 93 g of the powder was mixed with 300 ml of distilled water and 50% methanol at 70-80 °C to obtain the hydroalcoholic extract (HAE), and incubated in a water bath at 60 °C. The mixture was then passed through Whatman filter paper No. 3 to obtain the extract resulting in 6.104 g of HAE from the 93g
of dry powder, and the remainder of the powder was used for fractionation test to prove the presence of flavonoids, phenolics, and terpenoids.

2. Animals
Thirty-five male 6-month-old Syrian mice weighing 30-40 g standard fed meals from birth that had grown at 12 h light/12 h dark cycle, and ambient temperature of 20-30 °C was used. Mice were randomly divided into five groups. Each group involved seven animals for different examinations. They were provided with identical diet and environmental conditions throughout the experimental period. Each mouse was only included in the assessments once. All mice were bred and maintained in the animal house of the Pharmacology Research Center at the Faculty of Medical Sciences, Islamic Azad University, Tehran. All laws and ethics were followed by the Ethics Research Code of the Islamic Azad University of Iran and approved by the faculty (code: ).

3. Chemicals
The fluoxetine was prescribed routinely for the treatment of depression. Fluoxetine capsule (10 capsules, Tehran Darou Co., Iran) was used to make a therapeutic solution.

4. Study Design
In this experimental study, for behavioral tests, solutions were prepared from the HAE of *A. odorata* L. and fluoxetine. To prepare the herbal solution, 100 mg of the HAE was mixed with distilled water (40 ml) to make a stock solution. Fluoxetine (100 mg) was combined with 40 ml of distilled water to prepare the drug solution. After preparation of these solutions, different doses were adjusted for each experimental group and orally gavaged daily at 9-12 am for one week. The groups received oral gavage of distilled water, herbal solution, and drug solution as follows:
- Group 1: distilled water gavage (the same volume as other groups)
- Group 2: 10 mg/kg of drug solution gavage
- Group 3: 10 mg/kg of herbal solution gavage
- Group 4: 5 mg/kg of herbal solution gavage
- Group 5: 2.5 mg/kg of herbal solution gavage

5. Behavioral tests
After one week of oral gavage, all behavioral tests were performed each morning for 4-6 h within 5 days.

5.1. Forced Swimming Test (FST)
This test is one of the most validated tests for analyzing rodent depression. It indicates that mice become frustrated after exposure to continuous stress and stop their activity and motility [14]. To perform this test, a cylindrical acrylic bucket with a depth of 80 cm was filled with water (23-24 °C) up to 30 cm. The mice were then gently inserted into the water, and their activity was monitored for 5 min. The immobility time was measured separately for individual mice.

5.2. Tail Suspension Test (TST)
This test is similar to the FST for assessing rodent depression. In this test, mice in each group were hung from the middle 2/3 of their tails at 60 cm height. They freely left, and immobility as an index was then measured individually. Normal mice get frustrated after one minute and stop their
activity. The mice must be hung from the middle 2/3 of their tails not to get their hands to the junction and do not suffer from pain and irritation [15].

5. 3. Open Field Test (OFT)
This test is used to determine excitement, anxiety, and depression in rodents [16]. The test device was a 60 cm cube with the open upper side for examination, a floor with 16 squares with specified colored sides, and a clearly distinguishable central square. Mice in each group were placed in the corner of the cube, and a camera monitored their activity for 5 min in terms of the following parameter:
Line crossing (the number of crossed lines): Number of entries into small side squares.
Mice with a depression background usually have less mobility in this test and rarely stand on their paws.

5. 4. Elevated Plus Maze (EPM)
This test is used to examine anxiety and depression levels in rodents [16]. It uses a cross-like (+) background, 60 cm from the floor and a line were enclosed by a wall, and the other was open. In this test, mice from each group were placed in the field, and their motility was measured in terms of the number of entries into the enclosed line and the open arm. Depressed mice have less tendency to exit the enclosed line and enter the open arm.

6. Fractionation test
The fractionation tests were used to investigate the presence of flavonoids, phenolics, and terpenoids in *A. odorata* L. [17].
Flavonoids are compounds with proven anti-depressant effects [13]. In this study, the contents of flavonoids, phenolics, and terpenoids were investigated through content analysis tests. To investigate the constituents of *A. odorata* L., the dried extract (1.1 g) of the aerial parts was combined with 500 ml of distilled water and then extracted with 500 ml of hexane three times each for 1 h. The obtained extract was dried, and the aqueous layer was extracted with 500 ml of dichloromethane three times each for one h. The resultant extract was dried, and the aqueous layer was extracted with 500 ml of ethyl acetate for one h. The extract was dried for the Shinoda, phenolics, and terpenoids tests.
For phenolic assessment, the plant ethyl acetate extract was mixed with a solution containing ethyl acetate, formic acid, acetic acid, and water (26, 11, 11, and 100 ratios), and the resultant solution was examined for the presence of blue-violet droplets. For terpenoid testing, the plant ethyl acetate extract was combined with a solution of toluene, dichloromethane, and ethanol (10, 40, and 40 ratios) and the solution was examined for the presence of blue, red or yellow clots.
The Shinoda test was used to evaluate the plant for flavonoids. For this test, the plant ethyl acetate extract was combined with magnesium, a few drops of hydrochloric acid were added, and then the solution was examined in terms of pink or cherry color, indicating the presence of flavonoids in the plant.
Statistical Analysis

Results

Fractionation

The tests' results indicated that the HES of *A. odorata* L. contains some amounts of phenolics, terpenoids, and flavonoids. The presence of flavonoids was confirmed through the Shinoda test.

**TST**

The TST results confirmed the effects of all HES doses on decreasing the immobility time of male Syrian mice (Figure-1). A comparison between either fluoxetine (10 mg/kg) or *A. odorata* HES (10 mg/kg) groups and the control group (P<0.01) indicated the higher effect of fluoxetine in reducing the immobility time.

**FST**

According to Figure 2, the FST results indicated the effects of all plant HES doses on shortening the immobility time. A comparison between the results of fluoxetine and plant HES received groups (both at the dose of 10 mg/kg) and the control group (P< 0.01) indicated higher efficacy of the HES than the drug solution in shortening the immobility time in the FST.

**OFT**

Figure 3 represents the OFT results. The number of crossed lines by mice was compared between HES (10 mg/kg) group and either fluoxetine (P < 0.05) or the control groups (P < 0.05). The results indicated more reduction in the number of crossed lines in the plant HES (10 mg/kg) group than the control and fluoxetine solution received groups.

**EPM**

Two parameters were measured in the EPM test; 1) the percentage of time when the mice were elapsed within the open arm, and 2) the frequency of male mice entry to the open arm. The first criterion (Figure-4) showed that the percentage of spent time in the open arm increased in all doses of the HES groups compared to the control group. Figure-4 also compared the results of both fluoxetine or plant HES groups (at doses of 10 and 5 mg/kg) and the control group (P< 0.01). It was observed that the dose of the drug had the highest effect on this parameter, as the dose of 5 mg/kg showed the uppermost impact. Comparison between fluoxetine and plant HES group (at the dose of 2.5 mg/kg, P< 0.01) indicated that 2.5 mg/kg dose of HES had less effect on the elapsed time within the open arm.

As the second measured parameter (Figure-5), the results of either fluoxetine or HES groups at doses of 10 and 5 mg/kg were compared with the control group (P<0.05). Also, the results of the HES group at the dose of 2.5 mg/kg compared with either the fluoxetine group (P< 0.01) or control (P< 0.01). The results showed that HES at doses of 10 and 5 mg/kg increased the number of mice entry to the open arm. Contrary, at the dose of 2.5 mg/kg, it reduced the number of mice entry to the open arm as compared with either the control or fluoxetine groups.
Discussion

Plant-derived extracts are owing to rendering unique therapeutic properties and their natural origin, leading to minimal side effects that have attracted a wide range of attention to treating various diseases such as depression [18]. Depression is defined as a prevalent condition that has plagued human society during the recent decades [19]. Since the standard antidepressant drugs have not been reported to be very effective and their administration has caused side effects in patients, a novel therapeutic approach is required to promote existing treatments [20]. In this regard, some types of research have concentrated on using herbal medicine to improve depression. This study aimed to investigate the anti-depressant effects of HAE of *A. odorata* L. in comparison with fluoxetine in the experimental animals. Our behavioral tests revealed that the HAE of *A. odorata* L. possesses anti-depressant and anxiolytic effects. In this research, the fractionation and Shinoda tests confirmed flavonoids in *A. odorata* L. HAE. Similarly, the effects of flavonoids on the monoaminergic system intervening in the depression treatment were reported by the FST analysis [17]. The current study proved the above-mentioned effect by taking advantage of four tests. Another research demonstrated different amounts of phenol, caffeic acid, and other substances in *A. odorata* L. extract [12]. In addition to confirming the presence of phenolics, this investigation specifically proved that *A. odorata* L. contains flavonoids according to the Shinoda test. Besides, Sergeevna et al. [12] reported that the dried extract of *A. odorata* L. had sedative and anti-hypoxic effects. In line with our evaluation, there are several studies to support the anti-depressant features of herbal medicine. Capra et al. [2] showed in their study that scopoletin, a coumarin extract from *P. sabulosa*, played the anti-depressant role. They induced depression-like behavior in mice via immobility stress then evaluated the impact of scopoletin as the herbal extract on immobility time in the FST and tail suspension test in comparison to fluoxetine as the positive control. The results indicated that scopoletin can decrease immobility time in the TST but does not affect the FST [2]. Nonetheless, scopoletin exerts its anti-depressant-like effects probably through the involvement of noradrenergic (*α*1- and *α*2-adrenoceptors) dopaminergic (dopamine D1 and D2 receptors) and serotonergic (5-HT2A receptors) systems [2]. *H. perforatum* is the well-known herbal medicine for curing depression disorder, which so far has been characterized as the only traditional alternative medicine to the common synthetic anti-depressant drugs [21]. The assessment done by Ernst et al. has revealed that *H. perforatum* is well tolerated and seems to be a safe herbal extract for the treatment of mild to moderate depression [22]. Also, this evidence is supported by another analysis which indicated that *H. perforatum* is more effective than placebo in treating depression [23]. Among the other reports related to the anti-depressant activity of herbal extracts, Zhang et al. [24] designed the experimental study to investigate the rapid anti-depressant effect of ethanol extract of *Gardenia jasminoides* Ellis (GJ) in Kunming mice. They observed that about 2 hours after GJ administration, the number of escape failures in the learned helplessness test and the latency of food consumption in the novelty suppressed-feeding test reduced significantly. Furthermore, GJ could stimulate upregulation of *BDNF* expression in the hippocampus which is associated with anti-depressant responses [24]. In the research conducted in 2012, the mouse models of depression were treated intragastrically with Kai Xin San (KXS) at 175, 350, 700, and 1400 mg/kg/day for three days and were tested for the depression-related test [25]. It has been reported that the duration of immobility in TST and FST were decreased considerably, albeit this
effect was not dose-dependent. Modulation of the monoaminergic system was assumed as the probable mechanism for the anti-depressant function of KSX [25]. *Rosmarinus officinalis* is the other plant extract that was tested to exhibit an anti-depressant-like effect via adjusting the monoaminergic system [26]. Machado et al. [26] investigated the effect of a hydroalcoholic extract isolated from the stems and leaves of this plant in the experimental model of depression. Their study revealed that the extract of *R. officinalis* significantly diminished the immobility time in the FST and TST at 100 mg/kg and 10–100 mg/kg, respectively in comparison with the control group; hence, it could act as the anti-depressant agent [26]. Turmeric is the other plant that has been shown to rendering anti-depressant activity [27]. Oral administration of its aqueous extract in mice caused a significant decrease in immobility time in the TST under the dose-dependent manner [27]. In agreement with the data as mentioned earlier to approving the hypothesis anti-depressant effect of plant-derived extracts, our present research demonstrated that the HAE of *A. odorata* L. contains flavonoids, a substance with effective depression treatment. One-week oral gavage of *A. odorata* at 10 mg/kg compared with the same dose of fluoxetine solution increased immobility of mice in the TST and FST analyses. Compared to the same dose of fluoxetine, HAEs increased the mice entry into the central square in the OFT as a measure to show the anti-depressant effects of *A. odorata*. In the EPM test, it was found that male mice in the HAE-treated groups had more entry into the open arm than the fluoxetine group, suggesting the anxiolytic and anti-depressant effects of the *A. odorata*. Although, it is recommended that further studies are needed especially to clarifying the involved mechanisms of its action.

**Conclusions**

The present study showed that the HAE of *A. odorata* L. contains flavonoids. It decreased the immobility time of male mice at FST and TST analyses. The entry of mice into the central square increased in the OFT analysis. Also, the plant HAE increased the number of male mice entry into the open arm of the EPM test. These results indicate the anti-depressant effects of *A. odorata*. Future researchers are recommended to examine the presence of coumarins in the HAE of *A. odorata* L.

**Conflict of Interest**

The authors declare that they have no competing interests.
References


of Aloysia polystachya (Griseb.) Moldenke (Verbenaceae) in mice. Phytomedicine, 15(6-7):478-83.


**Figure 1.** TST assessment. Each group contains seven mice exposed to oral gavage of fluoxetine and A. odorata HES for one week. All mice were tested one day after gavage. **Specified groups vs. the control group at (P<0.01).**

**Figure 2.** Each group contained seven mice. After seven days of orally gavaged distilled water, fluoxetine solution, and plant HES, all animals were tested on the same day. **Fluoxetine group and A. odorata groups at a dose of 10 mg/kg vs. the control group at P<0.01.**
Figure 3. The OFT assessment. The number of crossed lines in the HES, fluoxetine, and control groups were compared. All animals within each group were tested after seven days of oral solution gavage. *A. odorata group at a dose of 10 mg/kg vs. control group (P<0.05). #A. odorata HES group at a dose of 10 mg/kg vs. fluoxetine group at a dose of 10 mg/kg (P<0.05).

Figure 4. EPM test for the percentage of elapsed time in the open arm. Each group contained seven mice subjected to oral gavage for one week. All the groups were assessed on the same day. **Fluoxetine and A. odorata groups at doses of 10 and 5 mg/kg vs. the control group (P<0.01); ##A. odorata group at the dose of 2.5 mg/kg vs. fluoxetine group (P<0.01).

Figure 5. Mice entry to the open arm in the EPM test. Comparison between the control, drug solution, and HES groups. *Fluoxetine and A. odorata groups at doses of 10 and 5 mg/kg vs. the control group (P<0.05). **A. odorata group at the dose of 2.5 mg/kg vs. the control group (P<0.01). ###A. odorata group at the dose of 2.5 mg/kg vs. fluoxetine group (P<0.01).
Figure 2. FST. Each group contained seven mice. After 7 days of orally gavaged distilled water, fluoxetine solution, and plant HES, all animals were tested in a same day. **Comparison of fluoxetine group and plant HES results at a dose of 10 mg/kg with the control group at p < 0.01

Figure 3. The OFT assessment. The number of crossed lines in the HES, fluoxetine, and control groups was compared. All animals within each group were tested after 7 days of oral solution gavage. *Comparison of plant HES group at a dose of 10 mg/kg with the control group (p < 0.05). #Comparison of plant HES group at a dose of 10 mg/kg with the fluoxetine group at a dose of 10 mg/kg (p < 0.05).
Figure 4. EPM test for the percentage of elapsed time in the open arm. Each group contained seven mice subjected to oral gavage for one week. All the groups were assessed on the same day. ** Comparison of both fluoxetine or plant HES groups at doses of 10 and 5 mg/kg and the control group (p < 0.01); ## Comparison between HES group at the dose of 2.5 mg/kg and fluoxetine group (p < 0.01).

Figure 5. Mice entry to the open arm in the EPM test. Comparison amongst the control, drug solution, and HES groups; * comparison of both fluoxetine or HES groups at doses of 10 and 5 mg/kg and the control group (p < 0.05). ** Comparison of HES group at the dose of 2.5 mg/kg and the control group (p < 0.01). ## Comparison of HES group at the dose of 2.5 mg/kg and fluoxetine group (p < 0.01).
کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت‌های کاربردی در تدوین و چاپ مقاله