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Anti-depressant Activity of Hydroalcoholic Extract of *Asperula odorata* L. in Mice

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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 of *Asperula odorata* L. 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.

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Introduction

Depression is one of the most common and recurrent psychological disorders worldwide. 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. Depression is

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usually caused by a combination of genetic, physical, environmental, and psychological factors. In addition to genetic disorders and inherited habits, some biological factors such as physical illnesses, hormonal disorders, neurotransmitter disorders, and the use of certain medications can contribute to depression [2].

According to the research, major depression risk factors are stress, vegetarian diets, nutritional supplements, socio-demographic characteristics, medical conditions, lifestyle behaviors, social support networks, and life events [3]. By altering neurotransmitters, stress changes behavior and habits can increase depression [4]. According to studies in the United States, depression is more prevalent among white people than African-American people [5]. Studies showed that depression is more common among females [6].

The prevalence of depression is directly associated with race, culture, and lifestyle [7]. Depression is highly prevalent due to its high risk of recurrence in human societies [8]. Studies on Iranian adults from 2001 to 2015 indicated that 43% of Iranian people suffer from varying degrees of depression [9].

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 [10]. Also, selective serotonin reuptake inhibitors such as fluoxetine and citalopram with nausea and headache as side effects are used [11].

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 and/or the plant itself. *Hypericum perforatum* [12], *Polygala sabulosa* [4], and *Asperula odorata* L. [5] are examples of such plants.

A. odorata 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 [13]. It has been demonstrated by 40 species in Ukraine, the most common one being *A. odorata* L. It was anciently used as a diuretic, pain killer, and wound healing drug [14]. *A. odorata* L. contains phenol carboxylic iridoids, tanning

agents, flavonoids, coumarins, and steroidal saponin. Also, the anti-depressant properties of some of these species have been proved [14].

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). This family also covers the reversible weaknesses of monoamine oxidases [15].

The present study aimed to explore the anti-depressant effects of *A. odorata* 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 of Iran). It is about 1 m high with white flowers. The authenticity of the plant was approved by Dr. Mahsa Hadipour Jahromi (pharmacologist, Tehran) 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. Then, 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 6-month-old male 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 (n=7 per group). 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: IR.IAU.TMU.REC.1398.379).

3. Study Design

In this experimental study, solutions were prepared from the HAE of *A. odorata* L. and fluoxetine (Tehran Darou Co., Iran) for behavioral tests. 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, so there was 2.5 mg of the drug in each cc of solution [16]. After the preparation of these solutions, different doses were adjusted for each experimental group and orally gavaged daily at 9-12 A.M. for one week [17-24]. Animals 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 fluoxetine 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

4. Behavioral tests

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

4.1. 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 [25].

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 minutes. The immobility time was measured separately for individual mice.

4. 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 to avoid getting their hands to the junction and not suffering from pain and irritation [26].

4. 3. Open Field Test (OFT)

This test is used to determine excitement, anxiety, and depression in rodents [27]. 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 minutes 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.

4. 4. Elevated Plus Maze (EPM)

This test is used to examine anxiety and depression levels in rodents [27]. 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.

5. Fractionation Test

The fractionation tests were used to investigate the presence of flavonoids, phenolics, and terpenoids in *A. odorata* L. [28]. Flavonoids are compounds with proven anti-depressant effects [15]. This study investigated the contents of flavonoids, phenolics, and terpenoids 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 1 h. The resultant extract was dried, and the aqueous layer was extracted with 500 ml of ethyl acetate for 1 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). 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). 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.

6. Statistical Analysis

Data were analyzed using SPSS software version 22 (version 20, IBM. Corporation, Armonk, NY, USA) using one-way analysis of variance (ANOVA) and Tukey's Post hoc tests.

Significant levels were considered at $P \leq 0.05$.

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 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 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 HES groups (both at the dose of 10 mg/kg) and the control group indicated higher efficacy of the HES than the drug solution in shortening the immobility time in the FST ($P < 0.01$).

OFT

Figure-3 represents the OFT results. The number of crossed lines by mice was compared between HES (10 mg/kg) group

Figure 1. TST assessment. Each group contains seven mice exposed to oral gavage of fluoxetine and plant HES for one week. All mice were tested one day after gavage. ** Specified groups in the figure were compared with the control group at $P < 0.01$.

Figure 2. Result of FST. 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. **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 were compared. All animals were tested after seven 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 5 and 10 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 5 and 10 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 ($P<0.01$).

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 mice entry to the open arm.

The first criterion (Figure-4) showed that the increased percentage of time in the open arm in all HES groups doses 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 indicated that 2.5 mg/kg dose of HES had less effect on the elapsed time within the open arm ($P<0.01$).

As the second measured parameter (Figure-5), the results of either fluoxetine or HES groups at doses of 5 and 10 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 5 and 10 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 [29]. Depression is defined as a prevalent condition that has plagued human society during the recent decades [30]. Since the standard anti-depressant drugs have not been very effective and their administration has caused side effects in patients, a novel therapeutic approach is required to promote existing treatments [31]. In this regard, some types of research have concentrated on using herbal medicine to improve depression. Our behavioral tests revealed that the HAE of *A. odorata* L. possesses anti-depressant and anxiolytic effects. In the research of Hellión-Ibarrola *et al.*, the fractionation and Shinoda tests confirmed flavonoids in *A. odorata* L. HAE [28]. Similarly, the effects of flavonoids on the monoaminergic system intervening in the depression treatment were reported by the FST analysis [28]. The current study proved the effect as mentioned earlier by taking advantage of four tests. Another research demonstrated different amounts of phenol, caffeic acid, and other substances in *A. odorata* L. extract [14].

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.* [14] reported that the dried extract of *A. odorata* L. had sedative and anti-hypoxic effects. The phenolic compounds of *A. odorata* L. were identified and quantified according to high-performance liquid chromatography: chlorogenic, o-coumaric, p-coumaric, 4,5-caffeoylquinic acid, quercetin, rutin, kaempferol-3-O-glucoside, diosmetin-7-O-glucoside, and luteolin-7-O-glucoside [14]. Chlorogenic acid and cynarozide were dominant in the dry extract. Also, they demonstrated that the dry extract at a dose of 100 mg/kg showed a high anti-hypoxic effect, which was almost two times higher than the reference drug [14]. The dry extract showed a dose-dependent sedative ability on the central nervous system, but it doesn't have any side effects on skeletal coordination and muscle tone [14]. In line with our evaluation, several studies support the anti-depressant features of herbal medicine. Capra *et al.* [4] showed 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 TST compared to fluoxetine as the positive control [4].

The results indicated that scopoletin could decrease immobility time in the TST but not affect the FST [4]. Nonetheless, scopoletin exerts its anti-depressant-like effects probably through the involvement of noradrenergic ($\alpha 1$ - and $\alpha 2$ -adrenoceptors), dopaminergic (D1 and D2 receptors), and serotonergic (5-HT_{2A} receptors) systems [4].

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 [32]. Increased oxidative stress, apoptosis, inflammation, decreased neurogenesis (especially in the hippocampus), and brain-derived neurotrophic factor (BDNF) are all involved in inducing depressive-like behavior [33]. Neuroinflammation and decreased levels of BDNF play a major role in depression. Abnormal activity of astrocytes in depression

has also been demonstrated [33-35].

Neuroprotection by flavonoids (*A. odorata* L.) is by modulating intracellular signaling pathways [36].

On the one hand, flavonoids activate neuronal survival pathways (ERK, PKC, and Akt) and inhibit neuronal death pathways (JNK and p38). These effects ultimately led to increased expression of transcription factors responsible for neuronal survival and decreased transcription factors responsible for neuronal death [36].

BDNF plays a very important role in mood regulation. Many environmental factors and drugs affect BDNF activity [37].

By modulating BDNF activity, these factors alter neurogenesis in important brain areas, such as the hippocampus, affect neuronal survival and plasticity, and affect neuronal communication and activity [37].

Ultimately, these changes lead to mood swings [37]. Stress reduces and/or impairs BDNF function, which in turn reduces neuronal flexibility. Subsequently, due to a lack of flexibility and decreased glutamate levels, neurotransmission decreases, and neuronal degeneration increases [38]. This vicious cycle leads to depression [38].

Among the other reports related to the anti-depressant activity of herbal extracts, Zhang *et al.* [39] 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 two 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 [39]. Furthermore, GJ could stimulate upregulation of BDNF expression in the hippocampus, which is associated with anti-depressant responses [39]. 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 [40].

It has been reported that the duration of immobility in TST and FST were decreased considerably, albeit this effect was not dose-

dependent [40].

Modulation of the monoaminergic system was assumed as the probable mechanism for the anti-depressant function of KSX [40].

In agreement with the data as mentioned earlier to approve the hypothesis anti-depressant effect of plant-derived extracts, our 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*. However, it is recommended that further studies are needed especially to clarify the involved mechanisms of its action.

A high dose of *A. odorata* L. can cause headaches, and very high doses can even have psychoactive properties as well as cause dizziness, drowsiness, even paralysis, and

coma [4].

It may even have physical side effects; hence, excessive consumption is not recommended, and its use during pregnancy and lactation is prohibited [41].

Limitations

Our study did not evaluate the antioxidant and anti-inflammatory properties of *A. odorata* L. Therefore, future studies are necessary to investigate these properties and find the mechanisms of *A. odorata* L.

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* L.

Conflict of Interest

The authors declare that they have no competing interests.

References

1. Yi LT, Li CF, Zhan X, Cui CC, Xiao F, Zhou LP, Xie Y. Involvement of monoaminergic system in the antidepressant-like effect of the flavonoid naringenin in mice. *Prog Neuropsychopharmacol Biol Psychiatry*. 2010; 34(7): 1223-8.
2. Zavvari F, Karimzadeh F. A methodological review of development and assessment of behavioral models of depression in rats. *Neurosci J Shefaye Khatam*. 2015;3(4):151-60.
3. Wu CS, Yu SH, Lee CY, Tseng HY, Chiu YF, Hsiung CA. Prevalence of and risk factors for minor and major depression among community-dwelling older adults in Taiwan. *Int Psychogeriatr*. 2017;29(7):1113-21.
4. Capra JC, Cunha MP, Machado DG, Zomkowski AD, Mendes BG, Santos AR, Pizzolatti MG, Rodrigues AL. Antidepressant-like effect of scopoletin, a coumarin isolated from *Polygala sabulosa* (Polygalaceae) in mice: evidence for the involvement of monoaminergic systems. *Eur J Pharmacol*. 2010;643(2-3): 232-8.
5. Riolo SA, Nguyen TA, Greden JF, King CA. Prevalence of depression by race/ethnicity: findings from the National Health and Nutrition Examination Survey III. *Am J Public Health*. 2005; 95(6): 998-1000.
6. Lehtinen V, Joukamaa M. 1994. Epidemiology of depression: prevalence, risk factors and treatment situation. *Acta Psychiatr Scand*. 1994; 89:7-10.
7. Simon GE, Goldberg DP, Von Korff M, Ustün TB. Understanding cross-national differences in depression prevalence.

- Psychol Med. 2002;32(4):585-94.
8. Pérez JH, Furlow JD, Wingfield JC, Ramenofsky M. Regulation of vernal migration in Gambel's white-crowned sparrows: role of thyroxine and triiodothyronine. *Horm Behav.* 2016; 84:50-6.
 9. Sarokhani D, Parvareh M, Hasanpour Dehkordi A, Sayehmiri K, Moghimbeigi A. Prevalence of Depression among Iranian Elderly: Systematic Review and Meta-Analysis. *Iran J Psychiatry.* 2018;13(1):55-64.
 10. Mahmoudi F, Gollo KH. Influences of Serotonin Hydrochloride on Adiponectin, Ghrelin and KiSS1 Genes Expression. *GMJ.* 2020;9:e1767.
 11. Adalat M, Khalili M, Ayromlou H, Haririan S, Fazljou SM, Rezaeizadeh H, Safari AA, Zargaran A. Antidepressant Effects of a Persian Medicine Remedy on Multiple Sclerosis Patients: A Double-Blinded Randomized Clinical Trial. *GMJ.* 2019;8:e1212.
 12. Ernst E, Rand JJ, Barnes J, Stevinson C. Adverse effects profile of the herbal antidepressant St. John's wort (*Hypericum perforatum* L.). *Eur J Clin Pharmacol.* 1998;54(8):589-94.
 13. Yurchenko NS, Il TV, Kovaleva AM. Amino-Acid Composition of *Asperula odorata* Herb. *Chem Nat Compd.* 2013;49(2):401-2.
 14. Sergeevna IN, Mihaylovna KA, Leonidivna TE, Aleksandrovna KI. The Antihypoxic and Sedative Activity of the Dry extract from *Asperula odorata* L. *Pharmacogn. Commun.* 2015;5(4):233-6.
 15. Guan LP, Liu BY. Antidepressant-like effects and mechanisms of flavonoids and related analogues. *Eur J Med Chem.* 2016; 121:47-57.
 16. Mattioli L, Funari C, Perfumi M. Effects of *Rhodiola rosea* L. extract on behavioural and physiological alterations induced by chronic mild stress in female rats. *J Psychopharmacol.* 2009;23(2):130-42.
 17. Vazquez-Palacios G, Bonilla-Jaime H, Velazquez-Moctezuma J. Antidepressant-like effects of the acute and chronic administration of nicotine in the rat forced swimming test and its interaction with fluoxetine. *Pharmacol Biochem Behav.* 2004;78(1):165-9.
 18. Costa AP, Vieira C, Böhner LO, Silva CF, da Silva Santos EC, De Lima TC, et al. A proposal for refining the forced swim test in Swiss mice. *Prog Neuropsychopharmacol Biol Psychiatry.* 2013;45:150-5.
 19. Page ME, Detke MJ, Dalvi A, Kirby LG, Lucki I. Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. *Psychopharmacology.* 1999;147(2):162-7.
 20. Chen PJ, Hsieh CL, Su KP, Hou YC, Chiang HM, Lin IH, et al. The antidepressant effect of *Gastrodia elata* Bl. on the forced-swimming test in rats. *Am J Chinese Med.* 2008;36(01):95-106.
 21. Kudryashov NV, Kalinina TS, Shimshir AA, Korolev AO, Volkova AV, Voronina TA. Antidepressant-like effect of fluoxetine may depend on translocator protein activity and pretest session duration in forced swimming test in mice. *Behav Pharmacol.* 2018;29(4):375-8.
 22. Osanloo N, Najafi-Abedi A, Jafari F, Javid F, Pirpiran M, Jafari MR, et al. *Papaver rhoeas* L. hydroalcoholic extract exacerbates forced swimming test-induced depression in mice. *Basic Clin Neurosci.* 2016;7(3):195.
 23. Szymczyk G, Zebrowska-Lupina I. Influence of antiepileptics on efficacy of antidepressant drugs in forced swimming test. *Pol J Pharmacol.* 2000;52(5):337-44.
 24. Haj-Mirzaian A, Kordjazy N, Ostadhadhi S, Amiri S, Haj-Mirzaian A, Dehpour A. Fluoxetine reverses the behavioral despair induced by neurogenic stress in mice: role of N-methyl-D-aspartate and opioid receptors. *Can J Physiol Pharmacol.* 2016;94(6):599-612.
 25. Petit-Demouliere B, Chenu F, Bourin M. Forced swimming test in mice: a review of anti-depressant activity. *Psychopharmacol.* 2005;177(3):245-55.
 26. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method

- for screening anti-depressants in mice. *Psychopharmacol.* 1985; 85(3):367-70.
27. Carola V, D'Olimpio F, Brunamonti E, Mangia F, Renzi P. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. *Behav Brain Res.* 2002;134(1-2):49-57.
 28. Helli6n-Ibarrola MC, Ibarrola DA, Montalbetti Y, Kennedy ML, Heinichen O, Campuzano M, et al. The antidepressant-like effects of *Aloysia polystachya* (Griseb.) Moldenke (Verbenaceae) in mice. *Phytomedicine.* 2008;15(6-7):478-83.
 29. Rabiei, Z, Rabiei S, A review on antidepressant effect of medicinal plants. *Bangladesh Journal of Pharmacology.* 2017. 12(1): 1-11.
 30. Friedrich MJ, Depression is the leading cause of disability around the world. *Jama.* 2017;317(15):1517.
 31. Eloziia N, Kumar N, Kothiyal P, Deka P, Nayak BK. A Review on Antidepressant Plants. *Journal of Pharmacy Research.* 2017;11(5):382-96.
 32. Gamasae NA, Radmansouri M, Ghiasvand S, Shahriari F, Marzouni HZ, et al. Hypericin induces apoptosis in MDA-MB-175-VII cells in lower dose compared to MDA-MB-231. *Archives of Iranian medicine.* 2018;21(9):387-92.
 33. Duman RS, Heninger GR, Nestler EJ. A molecular and cellular theory of depression. *Arch Gen Psychiatry.* 1997;54(7):597-606.
 34. Alcocer-Gomez E, Cordero MD. NLRP3 inflammasome: a new target in major depressive disorder. *CNS Neurosci Ther.* 2014;20(3):294-5.
 35. Verkhatsky A, Rodriguez JJ, Steardo L. Astroglipathology: a central element of neuropsychiatric diseases? *Neuroscientist.* 2014;20(6):576-88.
 36. Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov.* 2009;8(8):627-44.
 37. Yi LT, Liu BB, Li J, Luo L, Liu Q, Geng D, et al. BDNF signaling is necessary for the antidepressant-like effect of naringenin. *Prog Neuropsychopharmacol Biol Psychiatry.* 2014;48:135-41.
 38. Yi LT, Li J, Li HC, Su DX, Quan XB, He XC, et al. Antidepressant-like behavioral, neurochemical and neuroendocrine effects of naringenin in the mouse repeated tail suspension test. *Prog Neuropsychopharmacol Biol Psychiatry.* 2012;39(1):175-81.
 39. Zhang H, Xue W, Wu R, Gong T, Tao W, Zhou X, et al. Rapid antidepressant activity of ethanol extract of *Gardenia jasminoides* Ellis is associated with upregulation of BDNF expression in the hippocampus. *Evid Based Complement Alternat Med.* 2015.
 40. Zhou XJ, Liu M, Yan JJ, Cao Y, Liu P. Antidepressant-like effect of the extracted of Kai Xin San, a traditional Chinese herbal prescription, is explained by modulation of the central monoaminergic neurotransmitter system in mouse. *J Ethnopharmacol.* 2012;139(2):422-8.
 41. Bowen IH, Corrigan D, Cubbin IJ, de Smet PA, H6nsel R, Sonnenborn U, et al. *Adverse Effects of Herbal Drugs 2.* Springer Science & Business Media. 2012.