

Original Article



Evaluation of antidepressant effects of hydroalcoholic extract of *Kelussia odoratissima* Mozaffarian in male mice

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Abstract

Background and aims: Depression is one of the most common psychiatric disorders with serious impacts on individuals, and is often associated with physiological symptoms. In this study, we investigated the antidepressant effects of *Kelussia odoratissima* Mozaffarian extract in male mice.

Methods: A total of 56 male mice (weight: 25-35 g; age: 6-8 weeks) were used. *K. odoratissima* Mozaffarian hydroalcoholic extract was prepared by maceration method. The forced swim test, open field test, and splash test were used to investigate the antidepressant effects. The mice were assigned into eight equal groups (n=7 each) as follows: receiving 25, 50, 75, and 100 mg/kg of *K. odoratissima* Mozaffarian extract; receiving 5 mg/kg reserpine; receiving 5 mg/kg reserpine along with 20 mg fluoxetine; and normal saline. All injections were done intraperitoneally for one week before the test. Malondialdehyde (MDA) levels and antioxidant capacity of serum and brain were also measured in all groups. Statistical analysis was performed by one-way ANOVA and Tukey's test.

Results: Extract of *K. odoratissima* Mozaffarian significantly decreased the immobility time in forced swim test ($P < 0.001$). The extract also significantly increased splash time and elapsed time in the open field test, which was statistically significant compared with reserpinated mice ($P < 0.001$). Reserpine increased MDA levels and decreased the antioxidant capacity of serum and brain, whereas hydroalcoholic extract of *K. odoratissima* decreased MDA dose-dependently and increased antioxidant capacity ($P < 0.001$).

Conclusion: The results of this study showed that hydroalcoholic extract of *K. odoratissima* has antidepressant effects, but further studies are necessary to investigate the involved mechanisms.

Keywords: *Kelussia odoratissima*, Depression, Flavonoids, Mice

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Introduction

Mood refers to inner feeling that influences behavior and understanding of the surrounding world environment. Mood can be low, high, or normal. Mood disorders include a wide range of mental illnesses, one of the most common of which is major depressive disorder (MDD) (1). The main features of MDD include low mood, pessimistic thinking, lack of pleasure, decreased strength, slowness, low self-esteem, loss of appetite and death or suicidal thoughts. Depression symptoms including feeling empty and hopeless or pessimistic, feeling guilty, irritability and restlessness, decreased interest in activities or enjoyable pastimes. Unknown depression is a common cause of discomfort and a factor for delaying recovery from physical illness; it is therefore essential for all physicians to have the ability to diagnose this disorder, treat mild cases,

and identify cases that require specialized care due to the risk of suicide (2).

Clinical experience has shown that patients with depression do not have appropriate adherence to using drugs due to side effects or fear of taking chemical drugs. In addition, some studies have shown that available drugs are effective in only half of the cases and occasionally have not provided a complete and appropriate response. Given that herbal medical plants are more economical than chemical treatments, this study aimed to investigate the effective herbal treatments for depression that have a higher effect than conventional drugs and fewer side effects.

In recent years, research to find herbal remedies as a complementary and even alternative drug for the treatment of depression has become particularly important. In this regard, the effectiveness of numerous plant extracts and

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fragrances against depression in animal models have been demonstrated in double-blind clinical trials (3-11). In 2003, Mozaffarian introduced the mountain celery belonging to the Apiaceae family, scientifically referred to as *K. odoratissima* Mozaffarian, as a species of the *Kelussia* genus (12, 13). *Kelussia odoratissima* is a plant with vertical, slightly thick roots and a hollow stem that reaches a height of one meter. Its growth period is from early March to late May (12). The plant is native to certain areas of Iran such as Chaharmahal and Bakhtiari, Isfahan, and Kohgiluyeh and Boyer-Ahmad. Its occurrence has not been reported in other parts of the world yet. In Persian, *K. odoratissima* is called *Kelows* (14). Research on the pharmacological properties of this plant has shown that it has analgesic, anti-inflammatory, sedative, hypotensive, hypoglycemic, fibrinolytic, acid- and stomach pepsin-lowering, and memory boosting effects.

According to studies, this plant has flavonoids, phthalide compounds, fatty acids, and phenolic substances to which cancer preventing and hepatoprotective effects have been attributed.

Due to its phenolic and flavonoid compounds, this plant has also free radical-inhibiting and antioxidant effects, which can be compared with those of alpha-tocopherol and prevent the formation of fat plaques in the arteries (15-17). In recent years, medicinal plants have attracted the attention of researchers as natural, safe, and beneficial compounds for patients.

According to the evidence, no studies have yet been performed on the antidepressant effects of hydroalcoholic *K. odoratissima* extract, and given the presence of flavonoid compounds for the neuroprotective activity in plant extracts, it appears to be effective in treating depression. In the present study, we investigated the antidepressant effects of hydroalcoholic *K. odoratissima* extract.

Materials and Methods

In this experimental study, extraction was performed by maceration. For this purpose, the stems and leaves of *K. odoratissima* collected from Chaharmahal and Bakhtiari province, Iran were purchased from a local grocery in 2019. The quality and originality of the samples were confirmed in the Medical Plants Research Center of Shahrekord University of Medical Sciences, Iran. After pulverizing the samples with electric mill, we added the resulting powder in an appropriate amount to water (30%) and methanol 97% (70%). The resulting liquid was filtered 72 hours later by a Buchner funnel and extraction was done using a rotary evaporator. After the extract was dried in the incubator, the extract was shaved. Then, by dissolving an appropriate amount of extract in normal saline, the extract was prepared at concentrations of 25, 50, 75, and 100 mg/kg (13).

In this experimental study, 56 male mice weighing 25-35 g and aged 6-8 weeks were used in the Medicinal Plants

Center and Animal House of Shahrekord University of Medical Sciences between March and September, 2019. The injection of normal saline, drug, or extract for all mice was done intraperitoneally at 1 ml/kg body weight.

Measurement of serum/brain tissue antioxidant capacity

Three solutions were used to measure serum antioxidant capacity, including buffer (1.55 mL of acetate sodium and 8 mL of concentrated acetic acid to a final volume of 500 ml by addition of distilled water), 20 mL of chloride iron (III) to a final volume of 500 ml by addition of distilled water, and a solution of triazine (47 mg of triazine dissolved in 40 ml of hydrochloric acid). Then, 25 µL of sample was added to 1.5 mL of the stock solution and placed at 37°C for 10 minutes. Then, optical absorbance was read at 593 nm wavelength.

Measurement of serum/brain malondialdehyde levels

Briefly, 0.5 g of thiobarbituric acid was mixed with 80 ml of 20% acetic acid; its pH was adjusted to 3.5 by adding sodium hydroxide, and its volume was increased to 100 ml by addition of 20% acetic acid. Next, 100 µL of serum or homogeneous brain tissue sample was mixed with 100 µL of SDS 1.8% solution and 2.5 mL of the sample solution. The samples were immersed in boiling water for one hour, then cooled and centrifuged at 4000 rpm. Optical absorbance of zinc solution was recorded at 523 nm (18).

Investigations of depression

Forced swim test

Forced swim test is one of the most common standard pharmacological animal models for estimating the antidepressant effects of various chemical and plant compounds in rodents. This method is sensitive to the effects of all antidepressants (19).

In this method, after injecting extracts or drugs, the mice were placed separately in glass cylinders 25 cm in height, 12 cm in diameter, containing water at 25°C; conventionally, the time when hands and feet discontinue moving is regarded as the time of immobility. The entire test lasted 6 minutes, the first 2 minutes of which was specified for animal adaptation to the environment, and during the next 4 minutes, the duration of immobility and swimming was recorded in seconds by a stopwatch. Swimming was considered to be the movement of the animal's arms and legs and rotating around the cylinder (20).

Open field test

Open field test was used to investigate the exploring or motor activity (21,22). In this test, the animal is placed in an unknown environment, the surrounding walls of which prevent the animal from escaping. Several versions of this test are currently available that differ in terms of

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shape, ambient lighting, or the presence of objects in the environment and other characteristics. The procedure is usually performed by placing a mouse or rat in the center or near the surrounding walls, and a variety of animal behaviors are recorded over 2-20 minutes.

To perform open field test, a square box made of fiberglass was used. At a distance of 8 cm from the bottom of the box, a green screen of the same material with dimensions of 100 × 100 cm was installed. This page was divided into 25 equal squares, with 5 squares on each side of the box. In the center of each of the squares, as well as in the corners and in the middle of the sides of the middle squares, there were holes with a diameter of 3 cm. After the last injection, the mice were separately placed in a box from one corner of the box, and behavioral factors were measured for 5 minutes. After each test, the box was cleaned with a cotton swab dipped in alcohol for the next mouse. The entrance to the box and the location and time were the same for all mice. The test was performed in a quiet environment illuminated by a fluorescent lamp located just above the box.

Splash test

Splash test has been approved for studying depression in rodents. In this test, 10% sucrose solution was sprayed on the back of the animal, and the mouse's licking liquid was recorded in 5 minutes and statistically studied (23).

Statistical analysis

After collecting and entering the data in SPSS software version 17, Shapiro-Wilk test was done to test the normality of data. Statistical analyses were performed using one-way ANOVA and Tukey's test, and $P < 0.05$ was considered as significant level.

Results

The effect of intraperitoneal injection of four different concentrations of *Kelussia odoratissima* Mozaffarian extract (25, 50, 75, and 100 mg/kg) on immobility time in mice

Intraperitoneal injection of reserpine at a dose of 5 mg/kg resulted in an increase in immobility time compared with the normal saline-receiving group ($P < 0.001$).

Intraperitoneal injection of *K. odoratissima* extract at 25, 50, 75, and 100 mg/kg body weight resulted in a significant decrease in immobility time compared with the reserpinated group in the forced swim test, so that the difference was significant at 25 mg/kg ($P < 0.01$) as well as at 50, 75, and 100 mg/kg body weight ($P < 0.001$) (Figure 1).

Fluoxetine at 20 mg/kg body weight also significantly reduced the duration of immobility compared with the group receiving reserpine alone, which is a good indication of the antidepressant effects of fluoxetine ($P < 0.001$) (Figure 1).

The effect of intraperitoneal injection of four different concentrations of *Kelussia odoratissima* Mozaffarian extract (25, 50, 75, and 100 mg/kg) on the time spent by mice in the central part

Intraperitoneal injection of reserpine at 5 mg/kg resulted in a reduction in the time spent by the mice in the central part of the open field test compared with the group receiving normal saline ($P < 0.001$). Intraperitoneal injection of *K. odoratissima* extract at 25, 50, 75, and 100 mg/kg body weight resulted in an increase in the time spent by mice in the central part compared with the group receiving reserpine in the open field test with the difference being statistically significant, so that at the concentration of 50 mg/kg, the difference was significant ($P < 0.05$) as well as at 75 and 100 mg/kg body weight ($P < 0.001$). Moreover, fluoxetine at 20 mg/kg body weight resulted in a significant increase in the time spent in the central part compared with the group receiving reserpine alone ($P < 0.001$) (Figure 2).

The effect of intraperitoneal injection of four different concentrations of *Kelussia odoratissima* Mozaffarian extract (25, 50, 75, and 100 mg/kg) on the licking time of mice

Intraperitoneal injection of reserpine at 5 mg/kg resulted in a reduction in licking time in the splash test compared with the group receiving normal saline ($P < 0.001$). Intraperitoneal injection of *K. odoratissima* extract at 25, 50, 75, and 100 mg/kg body weight resulted in increased licking time compared with the reserpinated group in the open field test, and at only two doses of 75 and 100 mg/kg, the difference was statistically significant ($P < 0.001$ and $P < 0.05$, respectively).

Fluoxetine at 20 mg/kg body weight also resulted in a significant increase in licking time compared with the

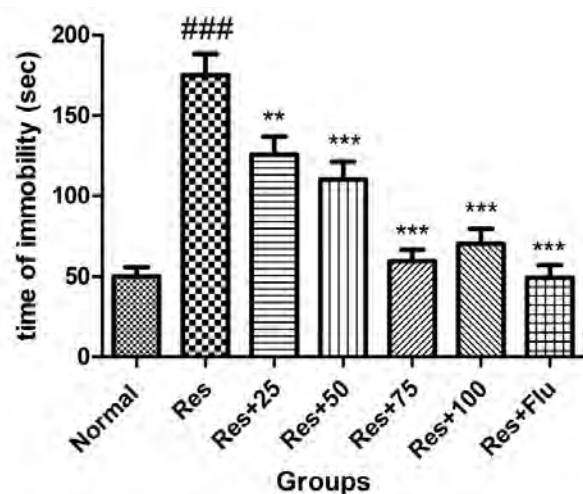


Figure 1. The effect of intraperitoneal injection of four different concentrations of *K. odoratissima* Mozaffarian extract (25, 50, 75, and 100 mg/kg) on immobility time in mice. Flu= Fluoxetine, Res=reserpine. #Vs. normal group (*** $P < 0.001$), *Vs. reserpine-receiving group (** $P < 0.01$, *** $P < 0.001$)

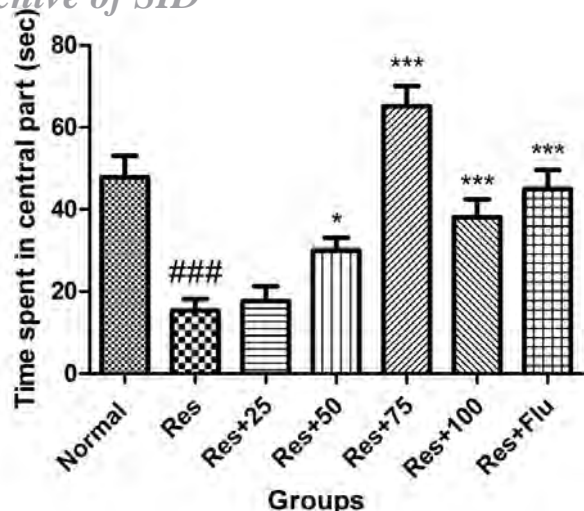


Figure 2. The effect of intraperitoneal injection of four different concentrations of *Kelussia odoratissima* Mozaffarian extract (25, 50, 75 and 100 mg/kg) on the time spent by mice in the central part. Flu= Fluoxetine, Res=reserpine. *Vs. normal group (*** $P < 0.001$), #Vs. reserpine-receiving group (* $P < 0.05$, *** $P < 0.001$).

group receiving reserpine alone ($P < 0.001$) (Figure 3).

The effect of intraperitoneal injection of four different concentrations of *Kelussia odoratissima* Mozaffarian extract (25, 50, 75, and 100 mg/kg) on the brain and serum malondialdehyde levels of mice

Intraperitoneal injection of reserpine at 5 mg/kg resulted in an increase in serum and brain malondialdehyde (MDA) levels in mice compared with the group receiving normal saline ($P < 0.001$).

Intraperitoneal injection of *K. odoratissima* extract at 25, 50, 75, and 100 mg/kg body weight resulted in a decrease in serum and brain MDA levels compared with the reserpine-receiving group, with the difference being statistically significant, so that at 25 mg/kg, the difference was significant at the significance level of $P < 0.01$, and at 50, 75, and 100 mg/kg body weight, the difference was statistically significant at the significance level of $P < 0.001$ level in serum and at 75 mg/kg at the significance level of $P < 0.001$ level and at 100 mg/kg at the significance level of $P < 0.05$ level in brain tissue, the difference was statistically significant.

Fluoxetine at 20 mg/kg body weight also significantly reduced MDA levels in brain tissue and serum in mice compared with the group receiving reserpine alone ($P < 0.001$) (Figure 4).

Effects of intraperitoneal injection of four different concentrations of *Kelussia odoratissima* Mozaffarian extract (25, 50, 75 and 100 mg/kg) on serum and brain antioxidant capacity in mice

Intraperitoneal injection of reserpine at 5 mg/kg resulted in an increase in serum and brain MDA levels in mice compared with the group receiving normal saline

($P < 0.001$).

Intraperitoneal injection of *K. odoratissima* extract at 25, 50, 75, and 100 mg/kg body weight resulted in a decrease in serum and brain MDA levels compared with the group receiving reserpine with the difference being statistically significant, so that at 75 mg/kg, the difference was statistically significant at the significance level of $P < 0.001$, and at 100 mg/kg body weight, the difference was statistically significant at the significance level of $P < 0.01$ level in serum and at 50 mg/kg at the significance level of $P < 0.05$ and at 75 and 100 mg/kg at the significance level of $P < 0.001$, the difference was statistically significant in brain tissue.

Fluoxetine at 20 mg/kg body weight also significantly reduced MDA levels in brain tissue and serum in mice compared with the group receiving reserpine alone ($P < 0.001$) (Figure 5).

Discussion

It has been observed that reserpine injection in mice is associated with a significant increase in immobility in the forced swim test, which is in line with the findings of Petit-Demouliere et al in 2016 (19).

In the present study, open field test was used to evaluate the exploring activity, depression, and anxiety in mice, and it was observed that reserpine injection in the mice was associated with significant reduction in the frequency of rearing and grooming behavior compared with control group mice. Angrini and colleagues' study on reserpinated mice showed a significant reduction in rearing and grooming behavior in the open field test (24). In a 2014 study by Ahmed et al, reserpinated mice showed a significant decrease in rearing and grooming behavior as well as motor activity (25). As can be seen, the findings of the above studies confirm our results.

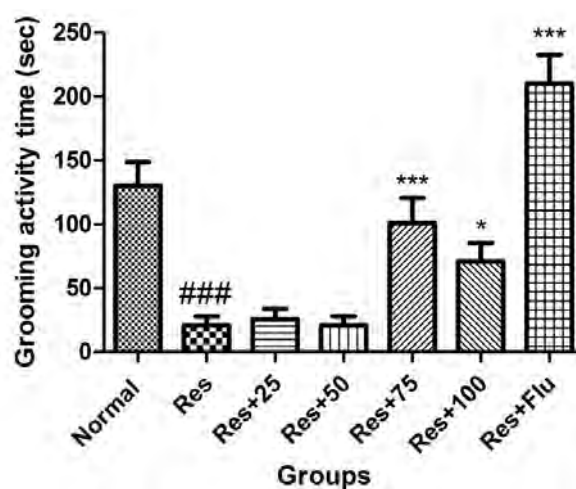


Figure 3. The effect of intraperitoneal injection of four different concentrations of *Kelussia odoratissima* Mozaffarian extract (25, 50, 75 and 100 mg/kg) on the licking time of mice. Flu= Fluoxetine, Res=reserpine. *Vs. normal group (*** $P < 0.001$), #Vs. reserpine-receiving group (* $P < 0.05$, *** $P < 0.001$).

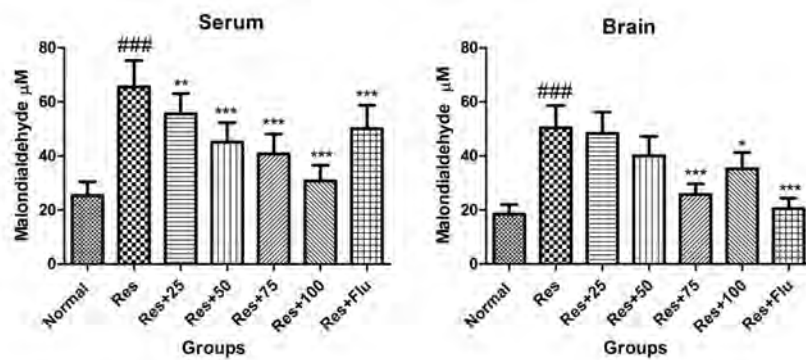


Figure 4. The effect of intraperitoneal injection of different concentrations of *Kelussia odoratissima* Mozaffarian extract (25, 50, 75, and 100 mg/kg) on the brain and serum MDA levels of mice. Flu= Fluoxetine, Res=reserpine. ^{###}Vs. normal group (^{###} $P < 0.001$), ^{***} $P < 0.001$ Vs. reserpine-receiving group (^{*} $P < 0.05$, ^{**} $P < 0.01$).

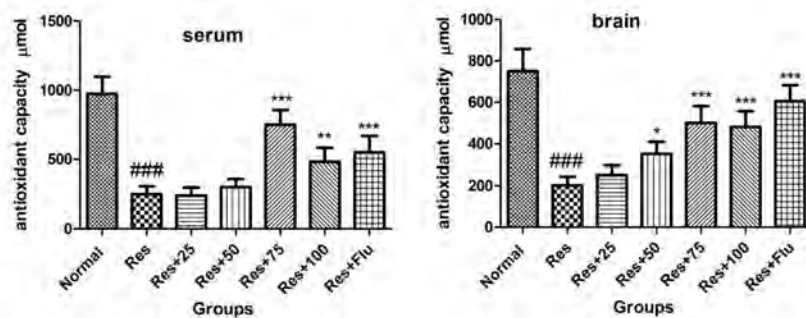


Figure 5. Effects of intraperitoneal injection of four different concentrations of *Kelussia odoratissima* Mozaffarian extract (25, 50, 75, and 100 mg/kg) on serum and brain antioxidant capacity in mice. Flu= Fluoxetine, Res=reserpine. ^{###}Vs. normal group (^{###} $P < 0.001$), ^{*}Vs. compared with the reserpine-receiving group (^{*} $P < 0.05$, ^{**} $P < 0.01$, ^{***} $P < 0.001$).

In the present study, treatment of reserpine-receiving mice with 25, 50, 75, and 100 mg/kg of *K. odoratissima* extract was significantly associated with improvement of quasi-depressive behaviors in the forced swim test, represented by reduced duration of immobility.

The *K. odoratissima* extract at doses of 50, 75, and 100 mg/kg caused a significant increase in rearing behavior, and at 75 and 100 mg/kg caused a significant increase in grooming behavior, which indicates the antidepressant effects of plant extract. As far as the researchers investigated, no study has yet been conducted on the antidepressant effects of hydroalcoholic extract of *K. odoratissima*.

However, the anxiolytic and sedative effects of *K. odoratissima* extract were investigated by Rabbani et al. They observed that 75 and 100 mg/kg of the extract had no significant effect on animal behavior in the elevated plus maze, but it showed sedative effects in the rotarod test (15).

In a 2011 study by Arora et al, it was reported that the induction of depression by reserpine in rats significantly increased the oxidative and nitrosative stress markers (26). In other animal models of depression such as depression induced by unpredictable chronic stress (27), depression induced by separation from the mother (28), depression induced by corticosteroid injections (29), and depression induced by chronic immobilized stress (30), oxidative

stress parameters have been reported to increase, which confirm our findings.

Studies have also reported some degree of oxidative stress in people with depressive disorders. For example, in a study in Tehran, Iran on women with postpartum depression, the serum antioxidant capacity was significantly lower in depressed women than in healthy controls (31). The study conducted by Sarandol et al also showed that in patients with MDD, serum MDA levels increased compared with healthy individuals and total serum antioxidant capacity decreased (32). In the present study, doses of 75 and 100 mg/kg of mountain celery extract enhanced antioxidant capacity and reduced MDA in the brain and serum of reserpine mice. So far, the antioxidant effects of celery extract have been proven in a number of studies.

In a 2017 study by Safaeian et al, the effects of hydroalcoholic extract of *K. odoratissima* aerial parts on blood pressure and dexamethasone-induced oxidative stress in male Wistar rats were investigated and it was observed that treatment of dexamethasone-receiving mice with *K. odoratissima* extract (100, 200, and 400 mg/kg) reduces plasma concentrations of hydroperoxides (33). *In vitro*, the antioxidant activity of the plant's methanolic extract has been studied using beta-carotene bleaching, reducing activity, accelerated oxidation of sunflower oil by thiocyanate and inhibition of DPPH radicals, and has

been reported to be effective in some studies (17,34).

In general, in the present study, *K. odoratissima* extract exhibited antidepressant effects in reserpinated mice by boosting the antioxidant defense system and reducing oxidative stress marker. However, other mechanisms such as modulation of the hypothalamic–pituitary–adrenal axis, reduction of neural inflammation, and altered levels of monoamines may also be involved in the antidepressant effects of *K. odoratissima* extract, which are suggested to be considered in future studies.

In the present study, it was observed that fluoxetine treatment in mice receiving reserpine significantly reduced MDA and significantly increased serum and brain antioxidant capacity. In a 2009 study by Zafir et al, treatment of mice under chronic stress with fluoxetine, imipramine, and venlafaxine significantly improved behaviors associated with depression, associated with significant increases in the activity of antioxidant enzymes catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase (35).

Conclusion

According to the results of the present study, the treatment of reserpinated mice with *K. odoratissima* was associated with a reduction in quasi-depressive behaviors in forced swimming and open-plate tests. *K. odoratissima* extract also boosted antioxidant capacity and reduced MDA in the brain and serum in resinous mice. Dose 75 mg/kg of *K. odoratissima* was the best response in this study. The antidepressant activity of mountain celery extract seems to be due to its antioxidant activity of this plant.

Conflict of Interests

The authors declare that there is no conflict of interests.

Ethical Approval

The study followed the Helsinki Protocol, and was approved by the Ethics Committee of the Shahrekord University of Medical Sciences (approval number: IR.SKUMS.REC.1395.319).

Authors Contribution

In this study, NA, ZL, MM and FH participated in the design and implementation of the project and HAK and MM helped me in writing the article.

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