Antidepressant effects of oleuropein in male mice by forced swim test and tail suspension test

Zahra Rabiei
Saeid Jahanbazi
Zahra Alibabaei
Mahmoud Rafieian-kopaei

Medical Plants Research Center, Basic Health Sciences Institute,
Shahrekord University of Medical Sciences, Shahrekord, Iran

Corresponding author:
Mahmoud Rafieian-kopaei
Medical Plants Research Center, Basic Health Sciences Institute,
Shahrekord University of Medical Sciences,
Shahrekord, Iran
Email address: rafieian@yahoo.com

Received: January, 20, 2018; Accepted: February 28, 2018; Published: April 1, 2018
Citation: Rabiei Z. et al. Antidepressant effects of oleuropein in male mice by forced swim test and tail suspension test. World Family Medicine. 2018; 16(4):132-144. DOI: 10.5742/MEWFM.2018.93366

Abstract

Objectives: With increasing prevalence of depression in communities and public concern regarding the side effects of synthetic drugs, special attention has recently been paid to identifying natural compounds with antidepressant effects. Oleuropein is an antioxidant polyphenol that is present in the leaves and fruits of different variants of olive. The aim of the present study was to investigate the antidepressant effects of oleuropein in mice by forced swim test (FST) and tail suspension test (TST).

Methods: In this experimental study, 50 mice were randomly divided into 5 groups of 10. Group 1 received normal saline; group 2 intraperitoneally received reserpine at 5 mg/kg 18 hours before behavioral testing; group 3, in addition to reserpine, intraperitoneally received oleuropein at 10 mg/kg 15 minutes before behavioral testing; group 4, in addition to reserpine, received fluoxetine at 20 mg/kg; and group 5 received oleuropein at 10 mg/kg for 3 days and then reserpine 18 hours before behavioral testing.

Behavioral tests were FST and TST. Finally, the antioxidant capacity and malondialdehyde (MDA) and nitric oxide (NO) levels in the serum and brain of the mice were measured.

Results: Reserpine significantly increased the duration of immobility in FST and TST and significantly decreased serum and brain antioxidant capacity, significantly increased MDA levels in the brain and serum, and significantly increased serum NO level ($P < 0.05$). Oleuropein treatment for 3 days caused a significant decrease of immobility in FST, a significant increase in brain and serum antioxidant capacity, and a significant decrease of brain and serum MDA and NO levels ($P < 0.05$).

Conclusion: Oleuropein was found to exhibit significant antidepressant effects in mice, probably due to its antioxidant activity.

Key words: Oleuropein, depression, reserpine

Introduction

Depression is one of the common psychiatric disorders that can decline function in all areas of life, including occupation, social relationships, and family crises. Two thirds of depressed patients develop suicidal thoughts and the suicide rate ranges from 10% to 15%. Fifty percent of depressed patients are 25-26 years old, and an increase in the prevalence of depression has been observed in the age group of under 20 years [1].

Studies have shown that most symptoms of depression are due to decreased function of certain transmitters such as norepinephrine, serotonin or 5-hydroxytryptamine, dopamine, glutamate, and GABA. Therefore, drugs that increase these neurotransmitters in the brain exert antidepressant effects [2].

Antidepressant treatments can be generally divided into two categories of pharmacological and non-pharmacological treatments. The available antidepressant drugs include tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors, and several new drugs such as nefazodone and bupropion. In severe cases, the use of antidepressants has been found to be the best choice. These drugs have quantitatively and qualitatively grown remarkably in recent decades, but due to their long-term use, many adverse side effects may occur [3].

The research on olive has shown that the main reason for bitter taste of olives is oleuropein. Oleuropein is one of the active compounds in the olive tree (*Olea europaea*). Oleuropein is found in high concentrations in unprocessed fruit and leaf of *O. europaea*. During the ripening of *O. europaea* fruit or processing of olive (e.g. oil production), certain chemical and enzymatic reactions occur, which decreases the concentration of oleuropein and increases the concentration of hydroxytyrosol, which is the main cause of the decomposition of oleuropein. The oleuropin molecule has three structural subunits consisting of a polyphenol called hydroxytyrosol (4- (2-hydroxyethyl) benzene-1,2-diol), a secoiridoid called oleic acid, and a glucose molecule [4].

The use of oleuropein plays a significant role in health. It has been reported that oleuropein and its antioxidant compounds, such as tyrosole, verbascoid, and dimethyl oleuropein, reduce the risk of coronary heart disease (arteriosclerosis) [5].

Studies have shown that oleuropein lowers the growth of colon cancer cells. Oleuropein may result in tumor reversal and prevention of proliferation of intestinal cancer cells by causing cell-cycle arrest at the G2-M phase. Oleuropein also increases apoptosis in intestinal cancer cells [6].

The antioxidant and hypotensive effects due to oleuropein have been shown in rabbits with alloxan-induced diabetes [7]. The analgesic and anti-inflammatory effects of oleuropein have also been shown to affect the pain and carrageenan-induced foot edema [8].

Therefore, our aim was to investigate the effects of one of the natural benefits, oleuropein, isolated from leaf and fruit of *O. europaea,* on depression, a chronic, costly, and complicated disease.

Materials and methods

Measuring oleuropein antioxidant effect

Oleuropein antioxidant effect was measured by the 2, 2-diphenyl-1-picryl Hydrazyl (DPPH) assay and the trolox equivalent antioxidant capacity (TEAC) assay.

The DPPH assay

Oleuropein (3.5, 6.25, 12.5, 25, 50 and 100µg/ml) was first prepared and equal amount of the DPPH solution (1mg/ml) was added to oleuropein at all concentrations. The resulting solution was kept in the dark at room temperature for 15 minutes, the absorbance values measured at 517nm using a spectrophotometer and then the activity of the DPPH radical inhibition calculated[9] .

Ic50 (%) = (Acontrol-Asample)/Acontrol×100

IC50 is the concentration of the solution in which 50% of the DPPH radical was scavenged.

The TEAC assay

To prepare azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), an aqueous solution of ABTS (7mM) was prepared. Potassium persulfate was added to this ABTS solution to a final concentration of 2.45mM and the resulting solution left in the dark at room temperature for 16 hours. Meanwhile, ABTS was converted to its radical cation by addition of potassium persulfate. Then, oleuropein at 75, 125, 250 and 500µg/ml was prepared and 20µg/ml of each concentration of the sample mixed with 2ml of ABTS•+ and the absorbance read at 734nm. The results were expressed as TEAC value (the ability to inhibit ABTS radical by the Trolox standard)[10] .

Metal chelating assay

Briefly, oleuropein (30, 50 and 100mg/ml) was mixed with FeCl2 (0.5mM, 2mM) and ferrozine (0.2 mL, 5 mM) and shaken. After 10 minutes, the absorbance was read using a spectrophotometer at 562nm. EDTA was used to plot the standard curve. The percentage of the ferrous ion-chelating capacity was measured by the equation below:

(Absorbance of control - Ab of sample)/Ab of control x 100 [11].

Reducing power assay

Reducing power of a compound represents its electron-donating ability. Oleuropein (1mM) at 25, 50, 100, 200 and 300µg/ml was mixed with phosphate buffer (0.2M, pH=6.6) and 1% potassium ferricyanide (K3Fe(CN)6) and the resulting solution left to incubate at 50°C for 2 minutes. Chloro-acetic acid was added to stop the reaction. The mixture was centrifuged at 3000rpm for 10 minutes, the supernatant mixed with H2O2 and ferric chloride 1% and the absorbance measured at 700nm[12] .

Hydroxyl radical scavenging assay
First, 1,10-phenanthroline (1ml, 1.865mM) was mixed with 2ml of oleuropein (25, 50, and 100µg/ml), and FeSO4 (1ml, 1.865mM) then added to the resulting mixture. The reaction was started by the addition of H2O2 (0.03%). The resulting solution was incubated in water bath at 37°C for 60 minutes and the absorbance read at 536nm. The scavenging activity of hydroxyl radical was measured by the equation below:

HRSA (%) = [(Absorbance of sample _Ab of A negative control)/(Ab of blank _ Ab of negative control)] ×100 [13] .

Laboratory animals and grouping
In this experimental study, 50 male mice (weighing 25 to 30 g) were divided into 5 groups of 10. Animals were kept in separate cages at 20-25°C with a 12:12-hour light: dark cycle. During this time, the mice were provided with sufficient water and food, and all experiments were carried out during lighting (9-1700 hours). The protocol of this study was in compliance with the Guide for the Care and Use of Laboratory Animals and the regulations of Shahrekord University of Medical Sciences.

Group 1 received normal saline; group 2 received reserpine at 5 mg/kg intraperitoneally 18 hours before behavioral testing; group 3 first received reserpine and then oleuropein at 10 mg/kg intraperitoneally 15 minutes before behavioral testing; group 4 first received reserpine and then fluoxetine at 20 mg/kg intraperitoneally; and group 5 (treatment group) first received oleuropein at 10 mg/kg intraperitoneally for 3 consecutive days and then reserpine at 5 mg/kg 18 h before the behavioral testing. Fifteen minutes after drug administration, behavioral testing consisting of forced swimming test (FST) and tail suspension test (TST) was performed. After behavioral testing, the rats had a deep anesthesia and their brain was removed. Blood samples were centrifuged at 3000 rpm and the serum was isolated. Serum and brain samples were stored at -30°C for biochemical tests.

TST
To carry out the TST, metal bases with a height of 70 cm were used, with a 50 cm string stretching between the two metal bases longitudinally. The tail of the mouse was fastened with a string so that it is suspended by the tail. At the beginning, the mouse starts to move, and then becomes totally motionless and inactive, which indicates immobility [14].

FST
The FST is one of the most reliable and most commonly used assays for the study of depression. In this test briefly, a glass container, 25 cm length, 12 cm width, and 15 cm height, is filled with water at 25°C and the animal is placed at a height of 20 cm from the water surface and then is gently immersed in water. Discontinuation of the movement of hind/forelimbs indicates immobility. The FST lasts 10 minutes with the first 2 minutes for acclimation. During the acclimation period, the duration of immobility is not recorded, but it is recorded for the following 8 minutes [15].

Measuring the antioxidant capacity of the serum and the brain
To measure antioxidant capacity, three solutions were used consisting of (1) buffer (1.55 ml of sodium acetate and 8 ml of concentrated acetic acid diluted to a final volume of 500 ml with distilled water), (2) iron chloride solution [270 mg of iron chloride (III) diluted to a final volume of 50 ml with distilled water], and (3) triazine (47 mg of triazine dissolved in 40 ml of 40 mM hydrochloric acid). To prepare the stock solution, 10 ml of solution 1 was dissolved in 1 ml of solution 2 and 1 ml of solution 3. Twenty five µl of serum sample or brain homogenate was added to 1.5 ml of the stock solution and the resulting solution was left at 37 °C for 10 minutes. Then optical absorbance was recorded at a wavelength of 593 nm [16].

Measuring serum malondialdehyde (MDA) levels
Briefly, 0.5 g of thiobarbituric acid was dissolved in 80 ml of acetic acid 20% and the pH of the resulting solution was adjusted to 5.3 by adding sodium hydroxide, and its final volume was increased to 100 ml by adding acetic acid 20%. One hundred µl of the serum sample was dissolved in 100 µl of SDS 1.8% solution and 2.5 ml of the stock solution. The samples were placed in boiling water for one hour, and then were cooled and centrifuged at 4000 rpm. Optical absorbance of the supernatant was measured at a wavelength of 523 nm [16].

Measuring the level of malondialdehyde (MDA) in the brain
One g of the brain tissue was homogenized in 2.5% potassium chloride (10% w/w) and incubated at (37±1) °C in a metabolic shaker for 60 minutes. After one hour of incubation, 1 ml of 5% tetrachloroacetic acid and 1 ml of 67% thiobarbituric acid were added to the hemogenate and the resulting mixture was finely mixed after each step. The content of each vial was transferred to a centrifuge tube and centrifuged at 2000 rpm for 15 minutes. Next, the supernatant was transferred to another tube and placed in a boiling water bath; after 10 minutes the test tubes were cooled and the absorbance was measured at a wavelength of 535 nm [16].

Measuring the levels of nitric oxide (NO)
NO levels were measured by measuring the levels of its products, i.e. nitrate and nitrite, in serum using a calorimetric kit. First, nitrate was converted to nitrite by nitrate reductase and then the NO levels were measured by measuring nitrite with the Griess reagent at a wavelength of 570 nm [15].

Analysis statistic:
Statistical analysis was performed using the SPSS version 16. First, distribution normality of the data was tested by using Kolmogorov-Smirnov test and then homogeneity of variances was investigated by using Levene's test. Then, one-way ANOVA was used to determine the significant difference between treatments and Tukey's test was used to compare the mean values. Data were expressed as mean (± standard error) and $P < 0.05$ was considered significance level.

Results

Oleuropein antioxidant property

DPPH levels

The results demonstrated that the anti-radical activity of oleuropein increased with increasing its concentration. In addition, the EC50 of oleuropein was derived at 11.7µg/ml. EC50 was directly correlated with oleuropein antioxidant activity.

TEAC assay

Metal chelating assay

The ferrous ion-chelating capacity of oleuropein increased with increasing its concentration. Ferrous ion-reducing power (%) of oleuropein at 50, 30 and 100µg/ml was derived at 17.75%, 21.07% and 22.20%, respectively.

Ferrous ion-reducing power

The antioxidants with high ferrous ion-reducing power can exert potent effects in terminating damaging oxidative chain reactions. We observed that the ferrous ion-reducing power of oleuropein increased with increasing its concentration such that the absorbance was derived 0.007 at 25µg/ml, with the highest absorbance (1.958) at 300µg/ml . The EC50 of oleuropein was derived 95.51µg/ml.

Hydroxyl radical scavenging assay

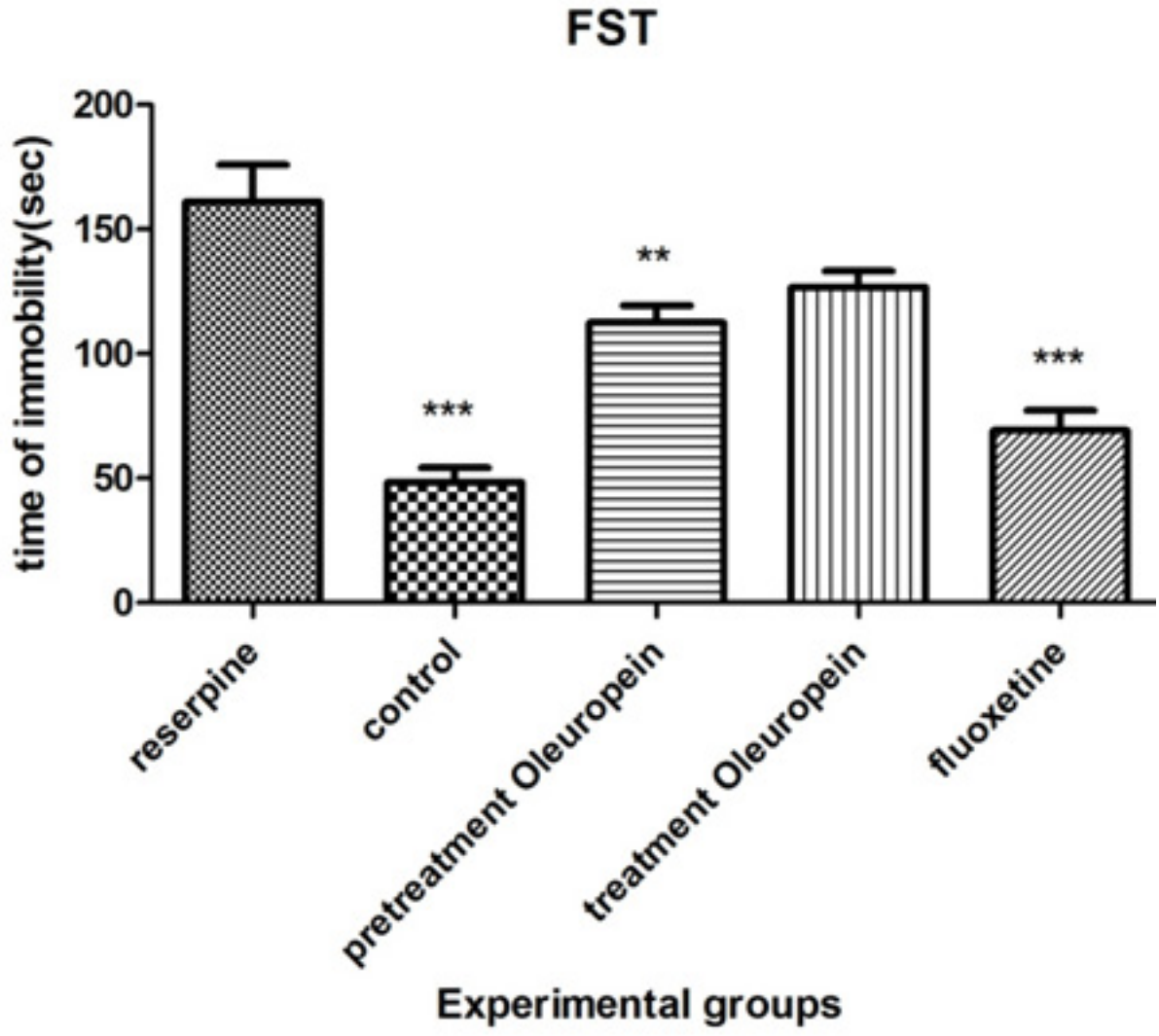
Hydroxyl scavenging activity (%) of oleuropein at 50, 25 and 100µg/ml was derived at 4.98%, 4.88% and 8.8%, respectively.

Behavioral Results:

The results of the effect of oleuropein and fluoxetine on the duration of immobility in the FST are illustrated in Figure 1. According to the results, the duration of immobility in the FST was significantly higher in the group receiving reserpine than in the control group ($P < 0.001$).

The oleuropein pretreatment for 3 days in mice receiving reserpine significantly reduced the duration of immobility when compared to reserpine group ($P < 0.05$). The fluoxetine treatment in mice receiving reserpine significantly reduced the duration of immobility when compared to the reserpine group ($P < 0.01$).

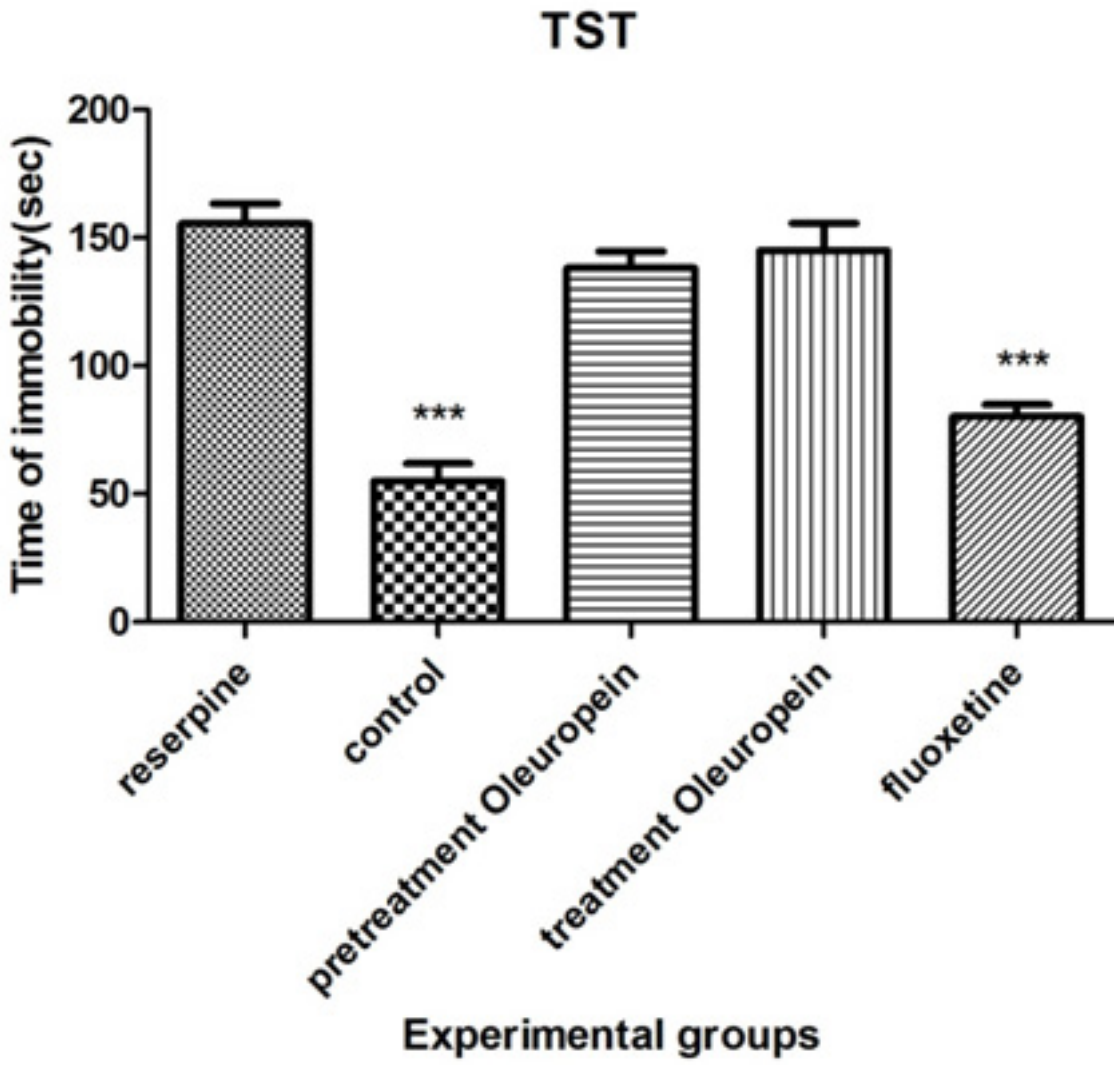
Figure 1: The effect of oleuropein and fluoxetine on the duration of immobility in the forced swimming test; *** =P < 0.001; ** =P < 0.01# significant difference from reserpine (P < 0.01).



The results of the effect of oleuropein and fluoxetine on the duration of immobility in the TST are illustrated in Figure 2. According to the results, the duration of immobility in this test was significantly higher in the group receiving the reserpine when compared to control group (P < 0.001).

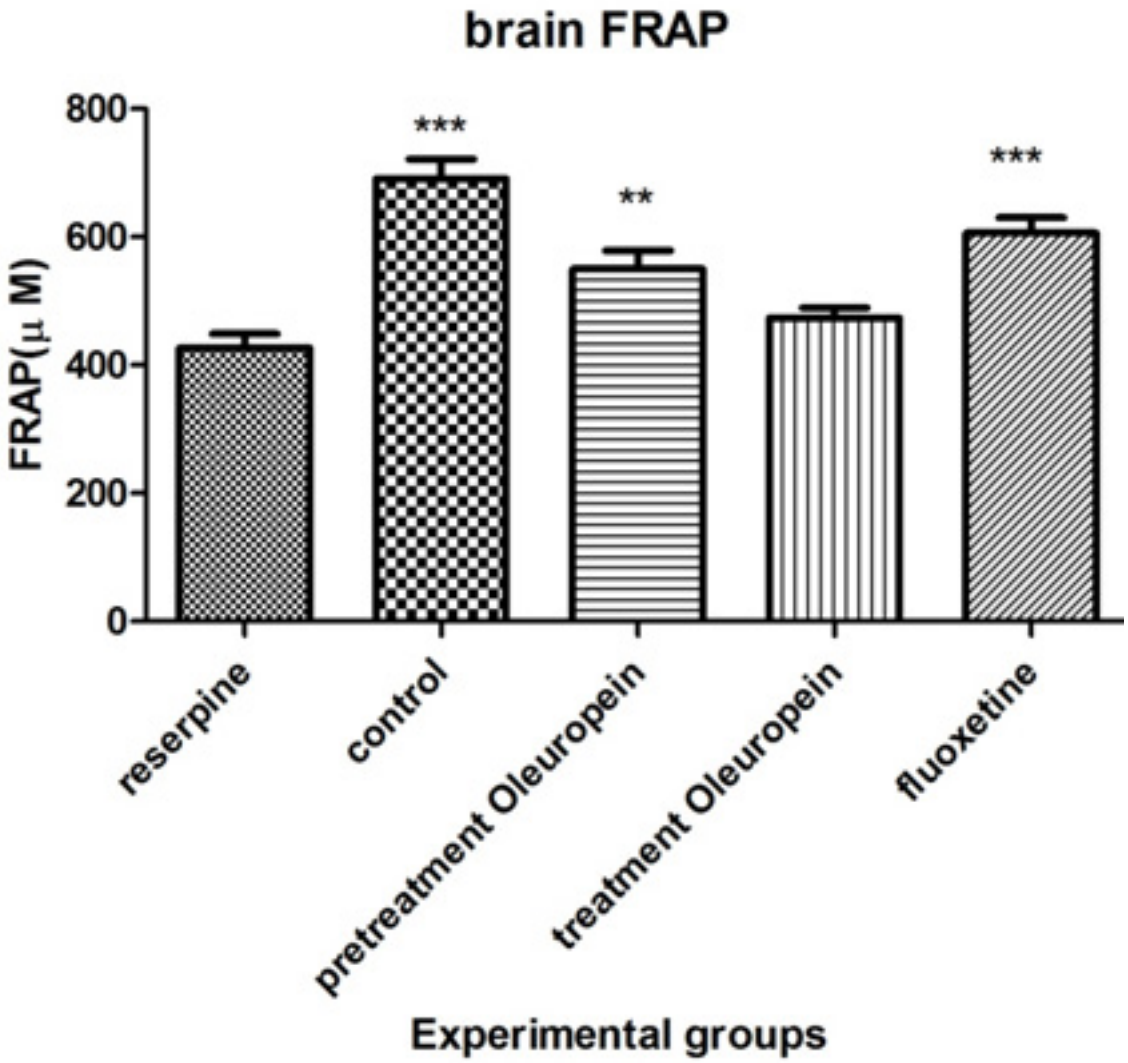
The oleuropein treatment or pretreatment in mice receiving reserpine did not significantly change the duration of immobility compared to the reserpine group. The fluoxetine treatment in mice receiving reserpine significantly reduced the duration of immobility (P < 0.001).

Figure 2: The effect of oleuropein and fluoxetine on the duration of immobility in the tail suspension test; *** =P < 0.001



The results of oleuropine and fluoxetine effects on brain antioxidant capacity are illustrated in Figure 3. According to the results, brain antioxidant capacity was significantly lower in he reserpin group than in the control group ($P < 0.001$). The fluoxetine treatment in mice receiving reserpine significantly increased brain antioxidant capacity ($P < 0.001$). The oleuropein pretreatment in rats receiving reserpine for 3 days resulted in a significant increase in brain antioxidant capacity ($P < 0.01$). Single-dose administration of oleuropein 18 hours after reserpine injection did not significantly change brain antioxidant capacity.

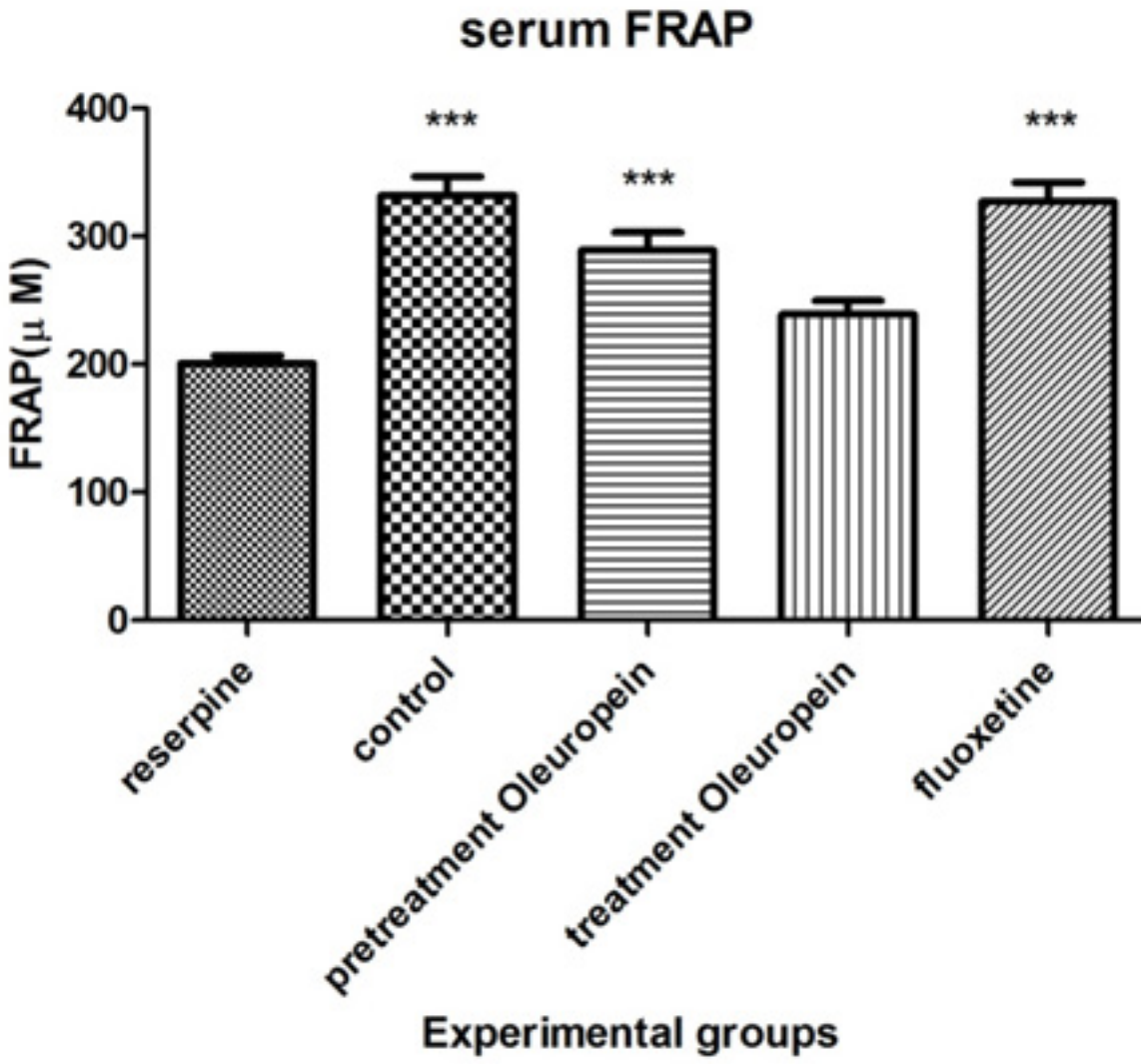
Figure 3: The effect of oleuropein and fluoxetine on the antioxidant capacity of the brain; *** =P < 0.001; **=P<0.01



The results of the effect of oleuropine and fluoxetine on serum antioxidant capacity are illustrated in Figure 4. According to the results, serum antioxidant capacity was significantly lower in the reserpine group than in the control group ($P < 0.001$).

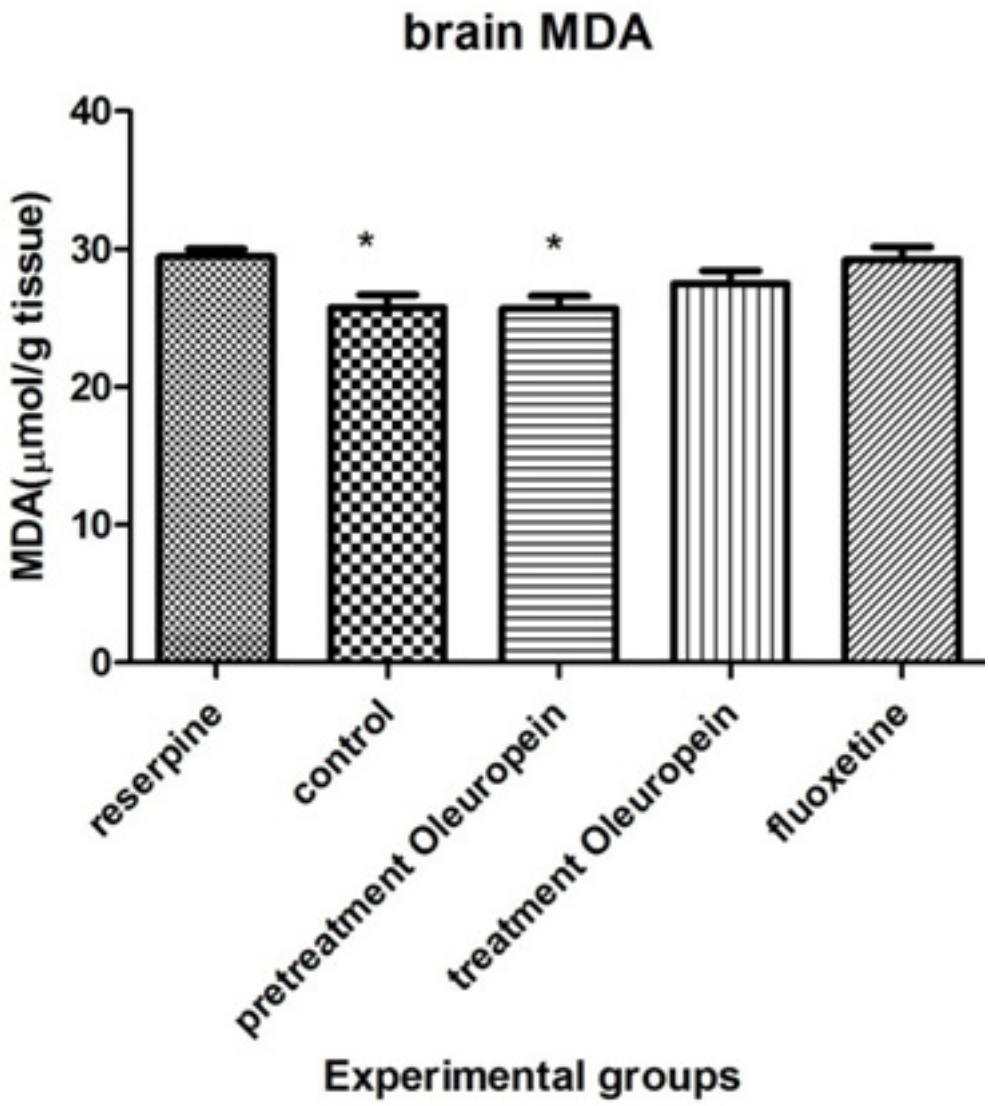
The fluoxetine treatment in rats receiving reserpine significantly increased serum antioxidant capacity ($P < 0.001$). Oleuropein pretreatment for 3 days in rats receiving reserpine significantly increased serum antioxidant capacity ($P < 0.001$). Single-dose administration of oleuropein 18 hours after reserpine injection did not have a significant effect on serum antioxidant capacity.

Figure 4: The effect of oleuropein and fluoxetine on serum antioxidant capacity; *** =P < 0.001;



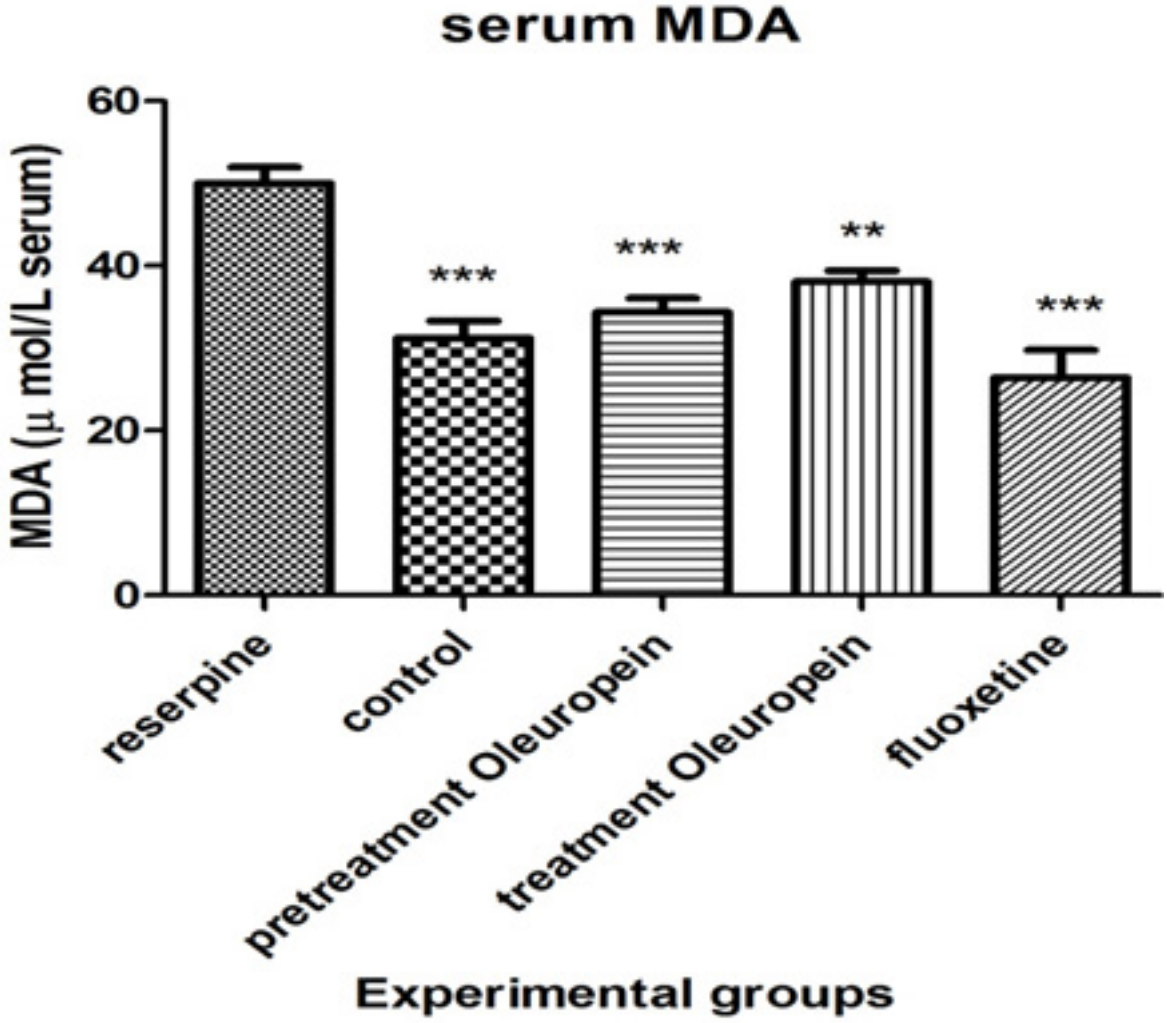
The results of the effect of oleuropein and fluoxetine on brain MDA levels are illustrated in Figure 5. According to the results, brain MDA levels were significantly higher in the group receiving reserpine than in the control group ($P < 0.001$). The oleuropein pretreatment for 3 days in rats receiving reserpine caused a significant decrease of brain MDA level ($P < 0.05$). Single-dose administration of oleuropein 18 hours after reserpine injection did not have a significant effect on the MDA levels in the brain.

Figure 5: The effect of oleuropein and fluoxetine on malondialdehyde levels in brain; *=p<0.05



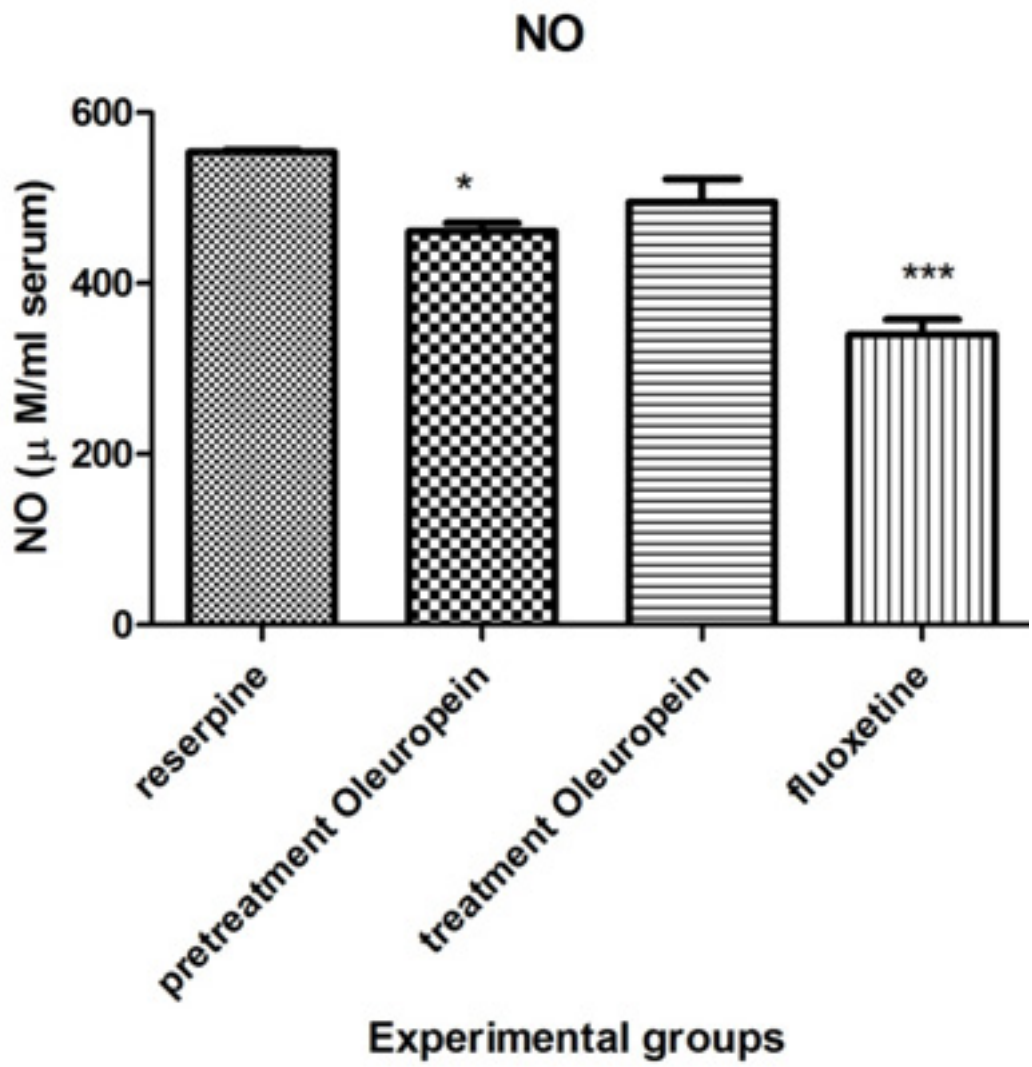
The results of the effect of oleuropein and fluoxetine on serum MDA levels are illustrated in Figure 6. Serum MDA level was significantly higher in the group receiving reserpine than in the control group (P < 0.001). Fluoxetine treatment in mice receiving reserpine resulted in a significant decrease of serum MDA level (P < 0.001). Oleuropein pretreatment for 3 days in rats receiving reserpine significantly reduced serum MDA levels (P < 0.001). Single-dose administration of oleuropein 18 hours after reserpine injection had a significant effect on serum MDA level (P < 0.001).

Figure 6: The effect of oleuropein and fluoxetine on serum malondialdehyde levels; *** =P < 0.001, **=p<0.01



Results of the effect of oleuropein and fluoxetine on serum NO levels are illustrated in Figure 7. Fluoxetine treatment in rats receiving reserpine significantly decreased serum NO level ($P < 0.001$). Oleuropein pretreatment for 3 days in mice receiving reserpine significantly reduced serum NO level ($P < 0.05$). Single-dose administration of oleuropein 18 hours after reserpine injection did not have a significant effect on serum NO level.

Figure 7: The effect of oleuropein and fluoxetine on serum nitric oxide levels; *** =P < 0.001; *=P < 0.05.



Discussion

The aim of this study was to investigate the effect of oleuropein on reserpine-induced depression in mice. Treatment of reserpine at 5 mg/kg 18 hours before behavioral testing significantly increased the duration of immobility in the FST and TST. Reserpine treatment also significantly reduced serum and brain antioxidant capacity and significantly increased serum MDA and NO levels. Reserpine is a drug that is mainly used to treat hypertension. Reserpine is also used as an antipsychotic for management and treatment of delirium (such as delusions and hallucinations), especially in diseases such as schizophrenia. Over the last few decades, it has been acknowledged that reserpine depletes the reserves of the catecholamines and serotonin of the tissues and the central nervous system (CNS), and thus leads to depressive-like behaviors by affecting the postnodular sympathetic nerve endings [17].

In the present study, reserpine injection, as expected, significantly increased the duration of immobility in the FST and TST.

In previous studies, it has been observed that reserpine has depression-inducing effects and increases the duration of immobility in the FST and TST [18, 19]. Reserpine interferes with the release, storage, and reuptake of monoamine neurotransmitters by inhibiting the vesicular monoamine transporter. Reducing the levels of serotonin, norepinephrine, and dopamine in the brain is one of the main causes of reserpine-induced depression. Reserpine also increases the autoxidation of dopamine and catalytic activity of monoamine oxidase. The monoamine oxidase binds to the mitochondrial membrane and causes the oxidation of neurotransmitters norepinephrine, dopamine, and serotonin, and while exerting its activity, produces free radicals and reactive oxygen species. Dopamine autoxidation produces dopamine-quinone, which in turn

degrades glutathione and produces free radicals. When the production of free radicals exceeds the antioxidant defense system, oxidative stress begins [20].

In the present study, a significant decrease of brain and serum antioxidant capacity and a significant increase in serum and brain MDA levels as well as in serum NO levels were observed in mice receiving reserpine, indicating reserpine-induced oxidative stress.

In agreement with these results, Bilska et al. (2007) reported that single-dose injection of reserpine was associated with significant reduction in the levels of non-enzymatic antioxidant enzymes glutathione, glutathione disulfide, and s-nitroxytol, a significant decrease in the levels of antioxidant enzymes such as glutathione peroxidase, glutathione-s-transferase, and L-γ-glutamyltranspeptidase, as well as in NO levels in striatum and prefrontal cortex in rat [21].

The study by Angélica et al. in 2009 also showed a significant increase in serum MDA level, which is the index of lipid peroxidation, in the brain of mice receiving reserpine [22].

In the present study, treatment with fluoxetine at 20 mg/kg 15 minutes before behavioral testing in mice receiving reserpine, significantly reduced the duration of immobility in the FST and TST. Fluoxetine also significantly increased the antioxidant capacity of the brain and serum, and significantly reduced the MDA levels in the brain and serum. In addition, fluoxetine treatment in mice receiving reserpine significantly reduced serum NO levels. In a study by Ahmed et al. in 2014, treatment with fluoxetine at 20 mg/kg in mice receiving reserpine significantly reduced the duration of immobility in the FST and TST [20], which is in agreement with our results.

Fluoxetine is an antidepressant drug that is widely used to treat a variety of depressive disorders. Fluoxetine is an SSRI; however, it, at high doses, can inhibit reuptake of dopamine and norepinephrine. It is also the agonist of σ1 (sigma 1) and inhibitor of the calcium-dependent chloride channel. In the study of Ahmed et al. in 2014, fluoxetine treatment in mice receiving reserpine significantly increased the levels of neurotransmitters serotonin, dopamine, and norapinephrine in the brain of mice receiving reserpine. Fluoxetine also significantly increased glutathione levels and antioxidant capacity in the brain and significantly decreased lipid peroxidation [20]. In the study of Gałecki et al. in 2009, fluoxetine treatment in patients with depressive disorders was associated with a significant increase in serum antioxidant capacity [23], which is consistent with the current study.

Fluoxetine may lead to certain side effects such as unusual dreams, abnormal ejaculation and other sexual complications, anxiety, dry mouth, cold-like symptoms, insomnia, tremor, nausea, anger, sweating, sleepiness, and skin complications in people with depression that can lead to discontinuation of drugs by some patients. Besides that, a number of patients do not respond appropriately to treatment. Therefore, the search for compounds with adequate efficacy and few side effects, especially nature-based ones, has begun [23]. In the present study, oleuropein (20 mg/kg) pretreatment for 3 days in rats receiving reserpine for 3 days caused a significant decrease in the duration of immobility in the FST, a significant reduction in brain and serum MDA levels and also serum NO levels, as well as a significant increase in brain and serum antioxidant capacity.

Oxidative stress is associated with disorders of the CNS including neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, and certain mental-psychological disorders such as schizophrenia, depression, and anxiety [24].

Increasing oxidative and nitrative stress parameters following induction of depression in animal models has been reported to be due to reserpine treatment [22], exposure to chronic stress [25], and corticosterone.

The increased production of reactive oxygen and nitrogen species, followed by oxidative and nitrative stress, causes peroxidation of membrane lipid and damage to DNA, proteins, and mitochondria of the nerve cells. The oxidative and nitrative stress following depression in different models has been found to lead to the destruction of key antioxidants such as coenzyme Q10, zinc, vitamin E, glutathione, and glutathione peroxidase. These events accelerate neurodegeneration and apoptosis and reduce neurogenesis and brain plasticity [26].

Antioxidant compounds can prevent depressive behaviors by reducing oxidative and nitrative stress parameters; for example, N-acetylcysteine and curcumin have shown significant antidepressant activity in various animal models of depression as well as in double-blind clinical trials. The mechanism of their action occurs via reducing the oxidative and nitrative stress parameters and inhibiting monoamine oxidases [27].

In the present study, oleuropein was observed to exhibit antidepressant effect in the FST, which was associated with reduction in lipid peroxidation in the brain and serum, decrease of serum NO levels, and increase in antioxidant capacity in the brain and serum. The anti-oxidative stress effects of oleuropein have been shown in a number of previous studies. The study of Al-Azzawie et al. in 2006 showed that in the rabbits with alloxan-induced diabetes orally given oleuropine at 20 mg/kg for 12 months, lipid peroxidation significantly decreased lipid peroxidation, and enzymatic and non-enzymatic antioxidants significantly increased [7]. In the study of Sarbishegi et al. in 2014, oral treatment of older mice with oleuropein at 50 mg/kg for 6 months reduced lipid peroxidation and increased the activity of antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase in the brain and serum [28].

In the study of Pourkhodadad et al. in 2016, oleuropein (10, 15, and 20 mg/kg) administration for 10 days in mice receiving significantly reduced the oxidative stress

parameters and significantly increased the activity of antioxidant enzymes in the brain [29].

In the study of Karabag-Coban et al. in 2016, intraperitoneal injection of oleuropein at 20 mg/kg, showed a protective effect against melatonin-induced oxidative stress. Administration of oleuropein reduced MDA, NO, and 8-hydroxy-2'-deoxyguanosine and prevented the oxidative DNA damage. In addition, the total antioxidant capacity and activity of superoxide dismutase and catalase increased in the blood, liver, and kidneys of the mice given oleuropein [30].

One of the most important problems that causes depression is impaired immune system. Studies have shown that depression, on the one hand, suppresses appropriate responses to infectious agents by inducing responses of type II T helper cells (responses involved in causing allergies and certain antibody-dependent autoimmune diseases), and, on the other hand, causes chronic and malignant inflammation by the immune system such that the inflammation per se leads to exacerbation of depression [31].

Chronic inflammation is one of the physical problems caused by depression. On the other hand, researchers argue that chronic inflammation can be one of the causes of depression [32]. Studies have shown that various molecules are involved in inflammation in patients with depression, the most important of which are inflammatory cytokines [33].

In the study of Liu et al. in 2015, the injection of cytokines IL-6, IL-1β, TNF-α, and lipopolysaccharides induced depression and anxiety-related behaviors in mice [32]. In patients with depression, high levels of IL-6, IL-1β, and TNF-α have been observed [31].

In a study conducted by Arora et al. in 2015, induction of depression in rats by reserpine significantly increased levels of inflammatory factors such as IL-6, TNF-α, and IL-1β [33].

Antidepressant drugs, such as fluoxetine and paroxetine, have also been reported to reduce levels of inflammatory cytokines in the brain and serum of mice with depression [34]. With regards to the evidence of anti-inflammatory effects of oloperopin in previous studies [34, 35], the antidepressant effects of oleuropein could be attributed to inhibition of inflammatory mediators. In an experimental study, oleuropin- and hydroxytyrosol-rich olive extracts were found to significantly decrease the pain and carrageenan-induced foot edema in rat [8].

In another study, oleuropein has been shown to have anti-inflammatory effects in mouse model of spinal cord injury. In rats with spinal cord injury, oleuropein significantly decreased levels of TNF-α, IL-1β, nitrotyrosine, nitric oxide synthase, cyclooxygenase-2, and PARP [8].

In the present study, oleuropein was found to exhibit significant antidepressant effects against depression induced by reserpine injection. Regarding the results of this study, it seems that the antidepressant effects observed for oleuropein are related to its antioxidant and anti-inflammatory effects. However, other mechanisms, such as the synaptic regulation of neurotransmitters such as serotonin, dopamine, and norapinephrine, and the regulation of the function of the hypothalamus-pituitary-adrenal axis, have also been reported to be related to the antidepressant effects of natural compounds and, particularly medicinal plants, culminating in extensive studies to identify the precise mechanism of the antidepressant action of oleuropein. Additionally, it is recommended that the antidepressant effects of oleuropein in other animal models of depression, such as depression induced by repeated corticosterone injections, depression induced by chronic unexpected stress, and depression due to mother-child separation, be studied.

Conclusion

It can be argued that reducing the parameters of oxidative and nitrative stress reduces the symptoms of depression in depressed mice receiving reserpine; however, further studies are needed to investigate the antidepressant effects of oleuropein in other models of depression, as well as to precisely identify its mechanism of action.

References

1. Miller K, Massie MJ. Depression and anxiety. The Cancer Journal. 2006;12(5):388-97.
2. Kalueff AV, Nutt DJ. Role of GABA in anxiety and depression. Depression and anxiety. 2007;24(7):495-517.
3. Seligman ME. Helplessness: On depression, development, and death: WH Freeman/Times Books/ Henry Holt & Co; 1975.
4. Amiot M-J, Fleuriet A, Macheix J-J. Accumulation of oleuropein derivatives during olive maturation. Phytochemistry. 1989;28(1):67-9.
5. Manna C, Migliardi V, Golino P, Scognamiglio A, Galletti P, Chiariello M, et al. Oleuropein prevents oxidative myocardial injury induced by ischemia and reperfusion. The journal of nutritional biochemistry. 2004;15(8):461-6.
6. Fabiani R, De Bartolomeo A, Rosignoli P, Servili M, Selvaggini R, Montedoro GF, et al. Virgin olive oil phenols inhibit proliferation of human promyelocytic leukemia cells (HL60) by inducing apoptosis and differentiation. The Journal of nutrition. 2006;136(3):614-9.
7. Al-Azzawie HF, Alhamdani M-SS. Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. Life sciences. 2006;78(12):1371-7.
8. Haloui E, Marzouk B, Marzouk Z, Bouraoui A, Fenina N. Hydroxytyrosol and oleuropein from olive leaves: Potent anti-inflammatory and analgesic activities. Journal of Food, Agriculture & Environment. 2011;9(3-4):128-33.
9. Asgharzade S, Rafieian-kopaei M, Mirzaeian A, Reiisi S, Salimzadeh L. Aloe vera toxic effects: expression of inducible nitric oxide synthase (iNOS) in testis of Wistar rat. Iranian journal of basic medical sciences. 2015;18(10):967.

10. Zulueta A, Esteve MJ, Frígola A. ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. Food Chemistry. 2009;114(1):310-6.
11. Wang J, Zhang Q, Zhang Z, Li Z. Antioxidant activity of sulfated polysaccharide fractions extracted from Laminaria japonica. International Journal of Biological Macromolecules. 2008;42(2):127-32.
12. Genskowsky E, Puente LA, Pérez-Álvarez JA, Fernández-López J, Muñoz LA, Viuda-Martos M. Determination of polyphenolic profile, antioxidant activity and antibacterial properties of maqui [Aristotelia chilensis (Molina) Stuntz] a Chilean blackberry. Journal of the Science of Food and Agriculture. 2016;96(12):4235-42.
13. Nikoo M, Benjakul S, Ehsani A, Li J, Wu F, Yang N, et al. Antioxidant and cryoprotective effects of a tetrapeptide isolated from Amur sturgeon skin gelatin. Journal of Functional Foods. 2014;7:609-20.
14. Rabiei Z, Naderi S, Rafieian-Kopaei M. Study of antidepressant effects of grape seed oil in male mice using tail suspension and forced swim tests. Bangladesh Journal of Pharmacology. 2017;12(4):397-402, Nov 5, 2017.
15. Rabiei Z, Gholami M, Rafieian-Kopaei M. Antidepressant effects of Mentha pulegium in mice. Bangladesh Journal of Pharmacology. 2016;11(3):711-5.
16. Rabiei Z, Movahedi E, Rafieian-Kopaei M, Lorigooini Z. Antidepressant effects of Trifolium pratense hydroalcholic extract in mice.
17. Ferguson JT. Treatment Of Reserpine-Induced Depression With A New Analeptic: Phenidylate. Annals of the New York Academy of Sciences. 1955;61(1):101-7.
18. Jahani Hashemi H, Jafari H, Abbasi E, Momeni A, Esmaeily H, Naserpour T. The effects of hydro alcoholic-extracts garlic on depression induced by reserpine in mice. Iranian Journal of Neurology. 2009;7(26):527-34.
19. Rabiei Z, Mokhtari S, Babaei F, Rafieian Km. Effect Of Kombucha Tea On Depression And Motor Activity In Mice. 2017.
20. Burger ME, Alves A, Callegari L, Athayde FR, Nogueira CW, Zeni G, et al. Ebselen attenuates reserpine-induced orofacial dyskinesia and oxidative stress in rat striatum. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2003;27(1):135-40.
21. Bilska A, Dubiel M, Sokołowska-Jez M, Lorenc-Koci E, Włodek L. Alpha-lipoic acid differently affects the reserpine-induced oxidative stress in the striatum and prefrontal cortex of rat brain. Neuroscience. 2007;146(4):1758-71.
22. Teixeira AM, Reckziegel P, Müller L, Pereira RP, Roos DH, Rocha JB, et al. Intense exercise potentiates oxidative stress in striatum of reserpine-treated animals. Pharmacology Biochemistry and Behavior. 2009;92(2):231-5.
23. Gałecki P, Szemraj J, Bieńkiewicz M, Zboralski K, Gałecka E. Oxidative stress parameters after combined fluoxetine and acetylsalicylic acid therapy in depressive patients. Human Psychopharmacology: Clinical and Experimental. 2009;24(4):277-86.
24. Praticò D, Clark CM, Liun F, Lee VY-M, Trojanowski JQ. Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease. Archives of neurology. 2002;59(6):972-6.
25. Zhao Z, Wang W, Guo H, Zhou D. Antidepressant-like effect of liquiritin from Glycyrrhiza uralensis in chronic variable stress induced depression model rats. Behavioural Brain Research. 2008;194(1):108-13.
26. Zafir A, Banu N. Modulation of in vivo oxidative status by exogenous corticosterone and restraint stress in rats. Stress. 2009;12(2):167-77.
27. Scapagnini G, Davinelli S, Drago F, De Lorenzo A, Oriani G. Antioxidants as antidepressants. CNS drugs. 2012;26(6):477-90.
28. Sarbishegi M, Mehraein F, Soleimani M. Antioxidant role of oleuropein on midbrain and dopaminergic neurons of substantia nigra in aged rats. Iranian biomedical journal. 2014;18(1):16.
29. Pourkhodadad S, Alirezaei M, Moghaddasi M, Ahmadvand H, Karami M, Delfan B, et al. Neuroprotective effects of oleuropein against cognitive dysfunction induced by colchicine in hippocampal CA1 area in rats. The Journal of Physiological Sciences. 2016;66(5):397-405.
30. Karabag-Coban F, Bulduk I, Liman R, Ince S, Cigerci I, Hazman O. Oleuropein alleviates malathion-induced oxidative stress and DNA damage in rats. Toxicological & Environmental Chemistry. 2016;98(1):101-8.
31. Liu B, Xu C, Wu X, Liu F, Du Y, Sun J, et al. Icariin exerts an antidepressant effect in an unpredictable chronic mild stress model of depression in rats and is associated with the regulation of hippocampal neuroinflammation. Neuroscience. 2015;294:193-205.
32. Rizzo SS, Neal S, Hughes Z, Beyna M, Rosenzweig-Lipson S, Moss S, et al. Evidence for sustained elevation of IL-6 in the CNS as a key contributor of depressive-like phenotypes. Translational psychiatry. 2012;2(12):e199.
33. Arora V, Kuhad A, Tiwari V, Chopra K. Curcumin ameliorates reserpine-induced pain–depression dyad: Behavioural, biochemical, neurochemical and molecular evidences. Psychoneuroendocrinology. 2011;36(10):1570-81.
34. Hwang J, Zheng LT, Ock J, Lee MG, Kim S-H, Lee H-W, et al. Inhibition of glial inflammatory activation and neurotoxicity by tricyclic antidepressants. Neuropharmacology. 2008;55(5):826-34.
35. Khalatbary A, Zarrinjoei GR. Anti-inflammatory effect of oleuropein in experimental rat spinal cord trauma. Iranian red crescent medical journal. 2012;14(4):229.