http://jbcp.shahed.ac.ir 10.22070/JBCP.2024.19834.1188

Open Access Research Article

Journal of Basic and Clinical Pathophysiology
Print ISSN: 2322-1895, **Online ISSN:** 2345-4334
Vol. 13, No. 1, February 2025

A Semiannual Publication of
Shahed University Press

Evaluation of the anti-depressant effect of *Melissa officinalis* along with electroshock therapy in male rat

Hakimeh-Sadat Karimi[1], Hasti Jabbari[2*], Mohsen Khalili[3]

1. Department of Physiology, School of Medicine, Shahed University, Tehran, Iran
2. Attending Gynecologist and Obstetrician at Isfahan University of Medical Science, Isfahan, Iran
3. Neurophysiology Research Center, Shahed University, Tehran, Iran

Abstract

Background and Objective: Depression is a chronic disease with a high prevalence in the world and electroconvulsive therapy (ECT) as well as *M. officinalis* treatment are effective and less complicated methods in its treatment. Considering the side effects of electroshock therapy, in this study, the antidepressant effect of *M. officinalis* plant along with electroshock therapy on the behavioral and biochemical parameters of depressed animals were investigated.

Materials and Methods: For this purpose, 56 male rats were divided into seven groups: 1- control, 2- depressed, 3- ECT, 4 and 5- treatment with *M. officinalis* at doses of 600 and 1000 mg/kg and 6 and 7- ECT+ *M. officinalis* at doses of 600 and 1000 mg/kg. Chronic unpredictable mild stress (CUMS) method was used to induce depression and finally the behavioral parameters of depression and oxidative stress biomarkers of the brain were evaluated.

Results: In the ECT+*M. officinalis* treatment groups, the parameters of sucrose consumption, swimming and moving time in the open box and settling in the open arm of the elevated maze significantly increased ($P<0.05$). Also, MDA and NO were considerably decreased ($P<0.05$) and GSH, CAT and SOD were markedly increased ($P<0.05$).

Conclusion: In general, our study showed that the consumption of the *M. officinalis* combined with ECT is quite effective in modulating the behavioral parameters and oxidative stress factors in depressive rats.

Keywords: Depression, Electroshock therapy, *Melissa officinalis*

1. Introduction

Depression is a dangerous, debilitating and common disease among different levels of society. It is also a chronic, multifactorial and potentially life-threatening mental disorder that affects more than 20% of the world's population (1). Currently, chemical drugs are the main part of depression treatments (2); however, due to the risk of recurrence of the disease and numerous side effects of these drugs, researchers are looking for more specific drugs with safety and lower cost. Also, one of the other ways to treat depression is the use of electroconvulsive (ECT), which cures depression in most patients and works faster than antidepressants. This method can be a treatment choice for cases that do not recover through currently available treatments and is an effective and safe treatment for depression disorder; and it causes almost high recovery even in patients with drug resistance. Unfortunately, the most common side effect of ECT is disorders of memory function and perturbation related to mind attention (3). On the other hand, medicinal plants have attracted the attention of researchers in this field, because they have been used for the treatment of various diseases, including psychological diseases, and have fewer side effects than synthetic and chemical drugs (4). In addition, the past evidences indicate the sedative and anticonvulsant effects of various plants, especially M. officinalis, so that based on the past evidences, it probably exerts its effects through the GABAergic

Received: 27 November 2024**, Revised:** 04 December 2024, **Accepted:** 24 December 2024
***Corresponding Author:** Hasti Jabbari **E-mail:** hastijabbari22@gmail.com

system. Also, the hydroalcoholic extract of M. officinalis probably has an anti-depressant effect by inhibiting the reabsorption of serotonin, which can be an adjunctive treatment and possibly strengthen the ECT treatment method (5). According to the contents mentioned in this research, the laboratory study of the effect of electroshock along with the use of *M. officinalis* plant extract on rats is carried out; this method may be more effective in reducing the symptoms of depression and the *M. officinalis* plant may reduce the side effect of ECT on destruction of learning and memory.

2. Materials and Methods

2.1. Animals

In this study, 56 male rats weighing 250-300 g were used. The animals were randomly divided into 7 groups, including: 1- control, 2-depressed 3-depressed+ECT, 4 and 5- depressed+*M. officinalis* (600 and 1000 mg/kg) and 6 and 7-depressed+ECT+*M. officinalis* (600 and 1000 mg/kg).

2.2. Development of the chronic unpredictable mild stress (CUMS) model

In this model, the animals were subjected to a series of mild and unpredictable stressors on a daily basis, which include swimming in cold water (4°C) for 5 min, tail pinching for 5 min, food deprivation for 24 h, water deprivation for 24 h, increased animal socialization in cages (24 rat per cage) with a 30° incline for 24 h, shaking for 20 min (one shake per second) , lighting conditions for 24 hours, placing in a dirty cage for 24 hours, heat stress (45°C) for 5 min were performed. Stress stimuli were applied three times during four weeks (6).

2.3. Electroshock therapy method

Electroshock is a method that was performed by causing a mild brain seizure by passing a mild electric current in the head area. The shock was applied bitemporal through the earlobe; current 60 mA, duration 1 s, frequency 100 pulses/s and width 0.5 ms (3).

2.4. Extraction method of *M. officinalis* plant extract

M. officinalis was crushed with a mill. The obtained powder was mixed with 70% alcohol to the extent of 4 times the weight of the plant powder. For this purpose, 800 g of plant powder was mixed with 3200 ml of 70% ethanol, and kept in a dark place with a closed lid for 48 hours, and the contents of the container were mixed once every 12 hours. After 48 h, the obtained plant extract was passed through filter paper. The containers containing the extract were placed in a bain-marie until their alcohol evaporated. After the complete evaporation of the alcohol and the concentration of the plant extract based on two doses of 600 and 1000 mg/kg and proportional to the average weight of the animals, it was dissolved in physiological serum. After standardization, the plant extract was kept in the refrigerator and was injected intraperitoneally (i.p.) to the animals daily for one week (7).

2.5. Behavioral tests

2.5.1. Forced swimming test (FST)

This test is used to evaluate frustration. In this test, the animals were placed in a water cylinder and the immobility time of the animals was recorded. This immobility is interpreted as a strategy to cope with stress or depression. To measure depression, each animal was placed in a cylinder with a height of 45 cm and a diameter of 25 cm and a temperature of 25 oC for 15 min (training phase), 24 hours later they were tested for 5 min in the same cylinder and the total time and the number of immobility were recorded as indicators of depressive behaviors. Increase of the immobility time which was concluded from rise in immobility index calculated based on the following formula (7):

(Total time movement)/ (elapsed time of total duration) × 100

2.5.2. Sucrose preference test (SFT)

In the sucrose preference test, the animal's preference for fresh water is measured. Reluctance to drink water indicates depression. Animals were placed in separate cages. On the first day, two bottles containing sucrose were placed instead of tab water. On the second day, water bottles replaced sucrose ones. After that the animals were deprived of water and sucrose for 24 hours, and finally, the sucrose preference test was performed on the fourth day by using both water and sucrose base battles. More consumption of sucrose base battle rather than tab water shows the lower level of depression (8).

2.5.3. Open field test (OFT)

In the open box test, a 40 × 40 × 40 cm cubic box was used. The basis of this test is based on the animal's inherent fear of being in the central area of this device. To perform this test, each animal was placed individually in the open field center for 5 min. The number of entering the center was calculated, and then the percentage of the number of times entering the central area for the elapsed time (seconds) in this area was calculated based on the following formula (9).

(Center to entry time)/ (elapsed time period) × 100

2.5.4. Elevated plus maze test (EPMT)

In the elevated plus maze test, a maze device that has two open arms and two closed arms was used. The animals were placed individually in the middle square of this device and facing the open arm, and the

number of animals entering each arm was recorded in 5 min. Increasing the number and duration of time spent in the open arm is an index of the animal's anti-anxiety and anti-depressant behavior. In this way, the increase in the number of entries into the arms means a decrease in the animal's anxiety, and a decrease in the number of entries into the open arms indicates an increase in the animal's anxiety (10).

2.6. Biochemical tests

2.6.1. Preparation of tissue homogenate

At the end of the behavioral tests, after anesthetizing the animals, the rats' brain were removed, and subjected for biochemical tests. First, the brains were homogenized with 0.9% sodium chloride solution to the extent of 4 times the volume of the brain in a test tube for 1-2 minutes in a homogenizer at a speed of 5000 rpm. Then the homogenize solution centrifuged at a temperature of 4°C (to prevent protein degradation) for 5 min at a speed of 4000 rpm. After that, the clear upper solution (supernatant) was separated by a sampler and kept at -70°C for laboratory tests.

2.6.2. Findings of the level of reactive oxygen radicals

Estimation of ROS level was done using dichlorofluorescein-diacetate probe (DCF-DA). In this test, the fluorescence intensity has a direct relationship with the ROS level. At first, 10µl of 10µM non-fluorescent DCF-DA was added to 100µl of lysate and incubated for 40 min in a dark environment at 37°C. The fluorescence intensity was also measured based on the DCF value of the formed fluorescence at the excitation wavelength of 488 nm and the emission wavelength of 525 nm and reported as RFU.

2.6.3. Malondialdehyde (MDA) level findings

Measuring the level of malondialdehyde is based on a method based on the thiobarbituric acid (TBA) reaction, which is carried out at boiling temperature. In this method, malondialdehyde reacts with thiobarbituric acid and creates a pink colour whose maximum optical absorption is at the wavelength of 532 nm. For this, standard solutions were prepared according to the following manner. After preparing the required solutions and reaching the ambient temperature, 50 µl of the sample or standard solution was added to each micro tube and then 1ml of chromogenic solution was added, finally, the micro tubes were placed in boiling water for one hour. The micro tubes were cooled in an ice container and centrifuged at 4000 rpm for 10 min. 200 µl of the pink supernatant solution was put into the micro plate well and its absorbance was measured at 535 nm with an ELISA reader. Analysis of the results was calculated based on the obtained standard curve.

2.6.4. Superoxide dismutase enzyme level

To measure the level of superoxide dismutase enzyme, which is considered a large molecule antioxidant enzyme, a solution containing xanthine-xanthine oxidase in Nitro blue tetrazolium (NBT) potassium phosphate buffer was used. The reason for using this solution was that the basis of this method is to measure the inhibition of NBT regeneration by the xanthine-xanthine oxidase system as a superoxide producer. After performing the above procedure, the optical absorption of the samples was read once every 30 s for 5 min at a wavelength of 550 nm. Then, by using the formula provided in the assay kit, the absorption percentage was calculated and the enzyme activity was obtained in the standard curve of the kit.

2.6.5. Nitric oxide level

To measure the level of nitric oxide, the brain tissue was homogenized with a ratio of 1:1 and then centrifuged at 14000 rpm. The supernatant was separated and 150 µl of the sample was mixed with 80 µl of buffer A included in the kit. After complete vortexing, 80 µl of buffer B was added and again mixed them. After complete mixing, the solution was centrifuged for 10 min at 14000 rpm, and then the supernatant was separated and used as a sample. To start the experiment, all the kit solutions were placed at room temperature for 30 min to reach temperature equilibrium. The standard solution in the kit was diluted at a ratio of 1 to 10 and then 50 ml diluted standard was added to sample in the well and was leave for 10 min away from the light at room temperature. Finally, 50 ml of 2R reagent was pour and mix to each well and after 10 min the plate was read at a wavelength of 570 nm using an ELISA reader. By using the obtained results, a standard curve was drawn and it was used to determine the concentration of nitric oxide in the target sample.

2.6.6. Assessment the amount of catalase (CAT)

Ten to 20 g of the brain tissue was lysed by homogenizer in PBS buffer containing protease inhibitor cocktail and then centrifuged at 12000 rpm for 15 min at 4 oC and its supernatant (as sample) was frozen in a new micro-tube at -70 oC. To perform the test, 20 µl of standard sample and blank were added to each well, then 30 µl of methanol catalase and 20 µl of Substrate Catalase were decanted them and mixed. After that, 30 µl Stop Catalase solution and 30 µl Chromogen Catalase were added to each well and incubated at room temperature for 10 min. Finally, solute absorbance was read at a wavelength of 520-560 nm.

2.6.7. Glutathione (GSH) measurement

The amount of 0.1 ml of the sample as well as 0.3 ml of 0.2 M Tris buffer and 0.02 ml of DTNB (2,2-dithiol -bis-nitrobenzoic acid) 0.1 M were supplied to a falcon tube. In the next step, 2 ml methanol was added to the container and was shaken every 5 min in a dark environment. Tubes were incubated for 30 min at room temperature and then centrifuged at 4000 rpm for 10 min. The clear liquid in the upper part of the tubes was transferred to the micro plate by using a sampler and their optical absorption was read at wavelength of 412 nm.

3. Results

3.1. Behavioral tests

3.1.1. Sucrose preference test (SPT)

According to figure 1 (A) and table 1, the consumption of sucrose in the depressed group showed a significant 58% decrease compared to the control group (P<0.05). It was also observed that the amount of sucrose consumption in the ECT groups treated with Mel 600 and Mel1000 increased compared to the depressed group (44% and 35%, respectively), which were marked, but not significant compared to the control group. In addition, in the ECT+Mel 600 and ECT+Mel1000 treatment groups, a significant increase in sucrose consumption was observed compared to the depressed group (100% and 52%) and a significant increase and decrease compared to the control group (119% and 10%). As a result, according to the observations, the ECT+Mel 600 treatment group showed a more significant result than the other groups (Fig. 1).

Table 1. Anxiety and depression behavioural tests in animal groups

Test/group	Cont	Dep	ECT	Mel 600	Mel 1000	ECT+Mel 600	ECT+Mel 1000
SPT	37.1±12.6	15.7±5.5 *	28±8.7	25.1±0.6	24.3±0.5	44.4±3.6$$	33.3±8.3$$
FST	26.8±5.1	55.4±3.1	45.34±3.5	48.9±4.8	20.5±4.3 $$	37.03±5.4	47.9±3.1
OFT	4.5±0.8	2.1±0.4 *	1.08±10	3.5±0.4	3.8±0.7	5.2±1.2 $$	4.3±1.1 $$
EPM (OAE)	56.4±6.5	32.3±11.7	41.4±4.7	58.3±5.5	44.4±8.2	69.4±9.1$$	83.3±5.3 $$
EPM (OAE/total arm time)	18.8±5.2	64±1.1	50±13.7 $$	28±5.5	27.08±9.9	64.2±9.6$$	77.9±8.3 $$

It shows the behavioral tests with sucrose preference, forced swimming, open box and elevated plus maze tests in the control, depressed and treatment groups. The numbers in each row of the table represent the mean value of the data ± the standard error of the mean. * indicates the significant difference of that group compared to the control group with P<0.05 and $$ indicates the significant difference of that group compared to the depressed group with P<0.01, n=8.

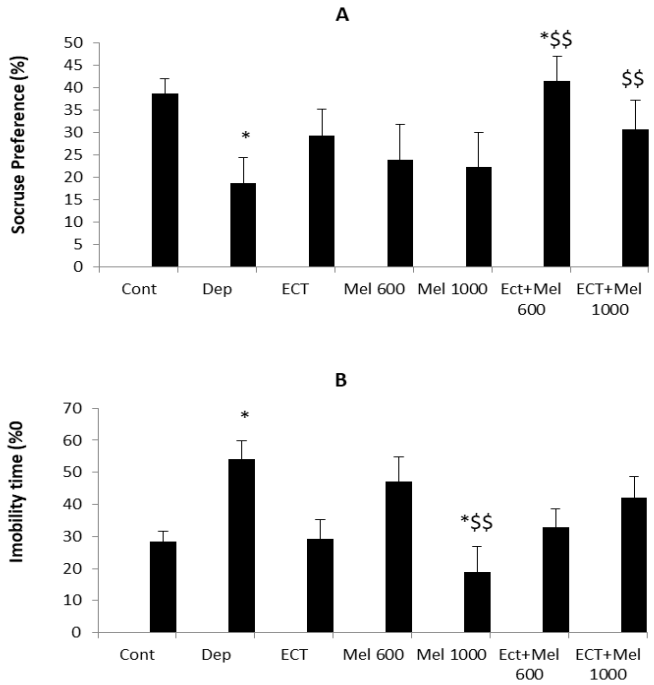

Fig. 1. In this figure, the percentage of fresh water consumption in the sucrose preference test (A) and the percentage of immobility time in the forced swimming test (B) can be seen in the control, depressed and treatment groups. The columns represent the mean value of the data ± the standard error of the mean. * indicates the significant difference of that group compared to the control group with P<0.05 and $$ indicates the significant difference of that group compared to the depressed group with P<0.01. (n=8)

3.1.2. Findings in the forced swimming test (FST)

According to Fig. 2 (B), the total immobility time to the total time in the animals of the depressed group showed twofold and significant increase compared to the control group ($p<0.05$). In the ECT group, immobility time decreased and increased compared to the depressed and control groups, respectively (10 and 18%), which were not significant. It was also observed that there was a significant decrease in the Mel1000 group compared to the depressed and control groups ($P<0.05$). According to the observations made, the Mel1000 treatment group has shown a more significant result than the other groups in this test.

3.1.3. Open field test (OFT) frequency index findings

According to Fig 2 (A), it has been observed that in the depressed group compared to the control group, entering the center of square has decreased by 60%, and this decrease was significant ($P<0.05$). While the ECT+Mel 600 and ECT+Mel1000 treatment groups showed a two fold increase compared to the depressed group, which was significant ($P<0.05$). Meanwhile, the ECT+Mel 600 treatment group had the highest mobility and the closest passing percentage compared to the control group.

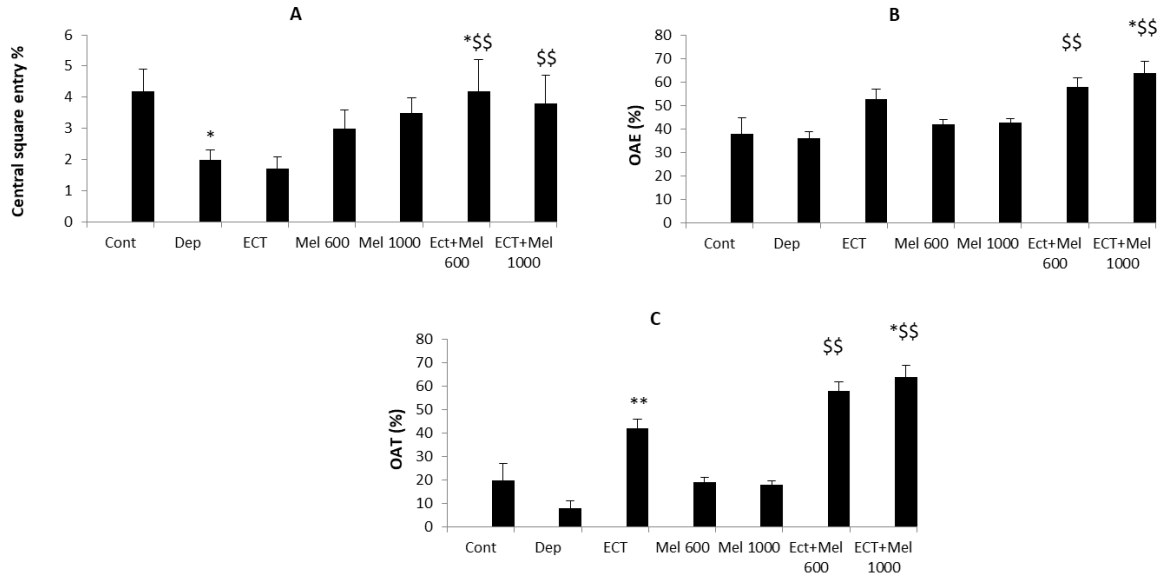

Fig. 2. The percentage of the number of entries into the central square to the total number of passes in the open box test (A), the percentage of the number of entries into the open arm to the total entries in the elevated plus maze test (B) and the percentage of time spent in the open arm to total time (C) can be seen in the control, depressed and treatment groups. The columns represent the mean value of the data ± the standard error of the mean. * indicates a significant difference compared to the control group with $P<0.05$ and $$ indicates a significant difference compared to the depressed group with $P<0.01$, n=8.

3.1.4. Elevated plus maze test (EPMT)

According to chart 1 (B and C), the percentage of OAT in the depressed group has decreased compared to the control group, which is significant ($P<0.05$). The percentage of OAE did not decrease significantly compared to the control group. It has also been observed that in the ECT treatment group, ECT+Mel 600 and ECT+Mel1000 compared to the depressed group, the percentage of OAT showed a significant increase ($P<0.05$), but the percentage of OAE only in the two groups ECT+Mel600 and ECT+ Mel1000 did not increase significantly compared to the depressed group ($P<0.05$). As a result, among the treatment groups, the ECT+Mel1000 group has the highest mobility in the open arm, and its OAE and OAT percentages have increased by two times compared to the depressed group, which is significant at the $P<0.05$ level.

3.2. Biochemical evaluation

3.2.1. Malondialdehyde assessment in the brain

According to table 2 the level of MDA in the homogenous tissue of the brain in the depressed group had a significant increase of compared to the control

group. Also, in the treatment groups, the level of MDA has decreased significantly compared to the depressed group. According to the observations made among these groups, the level of MDA in the ECT and ECT+Mel 1000 treatment groups showed a marked decrease in compare to the depressed ones (P<0.05).

3.2.2. Superoxide dismutase in the brain

According to table 2 the amount of SOD in the depressed group showed a significant decrease (85%) compared to the control group (P<0.05). Also, this parameter reach to a significant increase compared to the depressed group. According to the observations made among these groups, the level of SOD in the ECT and ECT+Mel 1000 treated groups increased by 78 and 84% respectively in compare to the depressed group (P<0.05).

3.2.3. Assessment of glutathione concentration in the brain

According to table 2, the amount of glutathione in the depressed group has decreased by 88% compared to the control group, and this decrease was significant at the P<0.05 level. In the treatment group, the level of GSH showed a significant increase compared to the depressed group. According to the observations made among these groups, the level of GSH in the ECT treatment group increased by 88% compared to the depressed group, and in the ECT+Mel1000 treatment group, it increased by 81% compared to the depressed ones, which was also significant (P<0.05).

3.2.4. Assessment of the concentration of nitrite in the brain

According to table 2 a marked increase in nitrite concentration was observed in the depressed group compared to the control group (P<0.05). In the treated groups, the concentration of nitrite decreased significantly compared to the depressed rats. According to the observations made among these groups, the level of NO in the ECT and ECT+ Mel 1000 treatment rats decreased 20 and 5 times compared to the depressed group (P<0.05).

3.2.5. Concentration of catalase in the brain

According to table 2, the concentration of catalase in the depressed group compared to the control group is an 86% decrease (P<0.05). In all treatment animal groups an increase in the level of CAT was observed compared to the depressed animals. Catalase level in the ECT and ECT+Mel 1000 treatment groups were increased by 85% and 74% in comparing to the depressive rats (P<0.05).

Table 2. Biochemical stress oxidative in the brain tissue of depressed and treatment groups.

Test/group	Cont	Dep	ECT	Mel 600	Mel 1000	ECT+Mel 600	ECT+Mel 1000
MDA	1.66.1±0.17	21.3±1.4*	1.63±0.21$$	8.06±0.57$$	5.74.3±0.44$$	3.61±0.42$$	2.09±0.18$$
SOD	64.04±1.7	9.30±1.61*	64.18±4.29$$	36.86±4.8$$	50.47±4.62 $$	47.66±274$$	59.67±1.3$$
GSH	28.6±3.4	32.2±0.4 *	26.3±1.6	55.5±8.9$$	10.38±1.8	10.2±9.7 $$	17.6±1.4 $$
NO	0.84±0.36	19.9±1.7*	0.99±0.44	9.87±0.92	6.89±0.47	6.38±0.71$$	3.84±0.31$$
CAT	23.6±5.2	31.65±1.1	21.43±1.1$$	66.6±2.51	89.9±4.2	11.4±9.6 $$	12.6±8.3 $$

The table shows the amount of MDA, SOD, GSH, NO and CAT in the homogenous brain tissue of the control, depressed and treatment groups. The numbers of each house in the table represent the average value of the data ± the standard error of the mean. * indicates the significant difference of that group compared to the control group with P<0.05 and $$ indicates the significant difference of that group compared to the depressed group with P<0.01, n=8.

4. Discussion

In the present research, similar to the study conducted by Yang et al. in 2017, rats subjected to depression with the CUMS model showed some degrees of depression and anxiety in the aforementioned behavioral tests (11). With the difference in this study, the OAE index did not have a significant result in the elevated plus maze test. Also, in the conducted studies, the electroshock treatment method was effective on the prefrontal region in depressed rats and improved their behavioral performance. In this research, similar to what was mentioned in the study of Luo et al. in 2012, an increase in the amount of sucrose consumption in the sucrose preference test, an increase in the number of crossing the open arm in the elevated plus maze test was observed in the group treated with electroshock compared to the depressed group (12). But the results of Luo's study in the forced swimming test in the mentioned groups have shown a decrease in immobility time and the number of passing through the center in the open box chamber, which opposes with the results of this research. The possible reason for this discrepancy may be related to human error during the test. The constructive effect of ECT is due to the fact that the electrical stimulation of the brain from the skull through electric current leads to a decrease in the resting potential of the membrane of the cell body of neurons and an increase in depolarization, and ultimately increases the excitability of the cortex (3). Therefore, it was proven that the animals which were received ECT can antagonized the depression like behaviors. Also, accordingly to the results of the Haj Rasouli reports, our study showed an anti-depressant behavioral activity by using *M. officinalis* (7). In today's society,

depression is considered a very serious disease and can be diagnosed with certain psychological and behavioral symptoms. In this connection, the reduction of brain norepinephrine and serotonin are the most involved in the pathophysiology of mood disorders in depressive mans (13). In this study, the anti-depressant effects caused by the administration of hydroalcoholic extract of M. officinalis plant, probably can be attributed to the increase in the amount of the neurotransmitter serotonin, which probably leaves its antidepressant effect with the mechanism of inhibition of reabsorption of serotonin (7). However, it was shown that GABA is one of the neurotransmitters that has important inhibitory effects on the central nervous system (CNS) and is necessary for the balance between the states of nervous stimulation and inhibition and normal brain function. There was shown that the presence of monoterpenes in M. officinalis extract can inhibits the enzyme acetylcholinesterase, and this effect terminate to higher cholinergic system activity through nicotinic and muscarinic receptors that could finally reduce the depressive behaviors (14). Also, it must be minded that the convulsive symptoms caused by the injection of pentylene tetrazole in rat could markedly prevented by application of M. officinalis (15). Regarding the combined effect of ECT with the hydroalcoholic extract of M. officinalis plant with doses of 600 and 1000 mg, we observed that there was a significant increase in the amount of sucrose consumption in the sucrose preference test and the number of crossing the center in the open box test in group tested by ECT+Mel 600 mg/kg. In this regard, a brief decrease in the immobility time in the forced swimming test and a significant increase in the number of passes and the time spent in the open arm in the elevated plus maze test were observed in both groups, and this effect was observed in the group treated with ECT combined with a dose of 1000 mg/kg of M. officinalis. It appeared more meaningful, because in the open box test, which requires activity and movement, neurons must be stimulated. Since anti-depressant drugs have an excitatory effect and the mechanism of action of M. officinalis is the same as anti-depressants such as fluoxetine, so this plant probably stimulates postsynaptic neurons at lower doses by inhibiting the reabsorption of serotonin and increasing its amount in the synaptic space (16). On the other hand, there are reported that shown the extract could increase the GABAergic brain activity, which could explain resulted from open arm and elevated plus-maze tests (7). Also, since there was indicated that the forced swimming test causes a significant increase in the amount of serotonin in the plasma, so we could relate any behavioral immobility time reaction in forced swimming test attribute to the changes in the amount of serotonin in the CNS (17).

Another step in this study was to measure the amount of oxidative stress in the brain tissue of animals suffering from depression under treatment and comparing it with the baseline level of these values in control group. Damage to macromolecules during oxidative stress occurs mainly through peroxidation of lipids, oxidation of proteins and DNA, as well as dysfunction and destruction of enzymes and cell membranes. Therefore, in many researches, including the current one, the amount of stress oxidant agents production were augmented which is along with increase the depressive and anxiety behaviors occurrence. However, mention finding could be minded as a para-clinical treatment for patients who suffer from depression disease.

References

1. Menken M, Munsat TL, Toole JF. The global burden of disease study: implications for neurology. Archives of Neurology. 2000; 57(3): 418-20.
2. Kamalipour M, Akhoundzadeh S, Rezazadeh SA. Herbal medicines in the treatment of depression and anxiety. Journal of Medicinal Plant. 2008; 7(4): 1-7.
3. O'Donovan S, Kennedy M, Guinan B, O'Mara S, McLoughlin DM. A comparison of brief pulse and ultrabrief pulse electroconvulsive stimulation on rodent brain and behaviour. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2012; 37(1): 147-52.
4. Salehi I, Hosseini SM, Haghighi M, Jahangard L, Bajoghli H, Gerber M, et al. Electroconvulsive therapy (ECT) and aerobic exercise training (AET) increased plasma BDNF and ameliorated depressive symptoms in patients suffering from major depressive disorder. Journal of Psychiatric Research. 2016; 76: 1-8.
5. Pierce J. A disease once sacred. A history of the medical understanding of epilepsy. Oxford University Press; 2002; 47-52.
6. Luo J, Min S, Wei K, Cao J, Wang B, Li P, et al. Propofol prevents electroconvulsive-shock-induced memory impairment through regulation of hippocampal synaptic plasticity in a rat model of depression. Neuropsychiatric Disease and Treatment 2014: 1847-59.
7. Hajrasouliha S, Khakpour S. Comparison of antidepressant effect of Melissa officinalis L. hydroalcoholic extract with fluoxetine in male mice. Medical Science Journal of Islamic Azad Univesity-Tehran Medical Branch 2020; 30(4): 418-24.
8. Petit-Demouliere B, Chenu F, Bourin M. Forced swimming test in mice: a review of antidepressant

activity. Psychopharmacology. 2005; 177: 245-55.
9. Bagewadi HG, Shivaramegowda RM. An experimental study to evaluate the effect of memantine in animal models of anxiety in Swiss albino mice. Journal of Clinical and Diagnostic Research: 2015; 9(8): 11-17.
10. Hulshof HJ, Novati A, Sgoifo A, Luiten PG, den Boer JA, Meerlo P. Maternal separation decreases adult hippocampal cell proliferation and impairs cognitive performance but has little effect on stress sensitivity and anxiety in adult Wistar rats. Behavioural Brain Research. 2011; 216(2): 552-60.
11. Yang Y, Hu Z, Du X, Davies H, Huo X, Fang M. miR-16 and fluoxetine both reverse autophagic and apoptotic change in chronic unpredictable mild stress model rats. Frontiers in Neuroscience. 2017; 11:42-8.
12. Luo J, Min S, Wei K, Zhang J, Liu Y. Propofol interacts with stimulus intensities of electroconvulsive shock to regulate behavior and hippocampal BDNF in a rat model of depression. Psychiatry Research 2012; 198(2): 300-6.
13. Khakpour S, Akhlaghdoust M, Naimi S, Mirlohi S, Seyed-Forootan NS, Foroughi F. Effect of Biebersteinia Multifida root Extract on cholesterol in mice. Zahedan Journal of Research in Medical Sciences. 2013; (15): 17-24.
14. Kennedy DO, Little W, Scholey AB. Attenuation of laboratory-induced stress in humans after acute administration of Melissa officinalis (Lemon Balm). Psychosomatic Medicine. 2004; 66(4): 607-13.
15. Mahmoudi M, Heidari M, Zohour A. Experimental study to evaluate the pretreatment of Melissa Officinalis extract against lethal seizures induced by pentylentetrazole in Wistar rats. Kerman University of Medical Sciences. 2001; 8(3): 88-94.
16. Xu Y, Ku B-S, Yao H-Y, Lin Y-H, Ma X, Zhang Y-H, et al. The effects of curcumin on depressive-like behaviors in mice. European Journal of Pharmacology. 2005; 518(1): 40-6.
17. Kirby LG, Lucki I. Interaction between the forced swimming test and fluoxetine treatment on extracellular 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in the rat. Journal of Pharmacology and Experimental Therapeutics. 1997; 282(2): 967-76.
18. Wu C-R, Tsai C-W, Chang S-W, Lin C-Y, Huang L-C. Carnosic acid protects against 6-hydroxydopamine-induced neurotoxicity in in vivo and in vitro model of Parkinson's disease: involvement of antioxidative enzymes induction. Chemico-Biological Interactions. 2015; 225: 40-6.
19. Singh S, Dikshit M. Apoptotic neuronal death in Parkinson's disease: involvement of nitric oxide. Brain Research Reviews. 2007; 54(2): 233-50.
20. Bajpai A, Verma AK, Srivastava M, Srivastava R. Oxidative stress and major depression. Journal of Clinical and Diagnostic Research 2014; 8(12): 4-9.
21. Lopresti AL, Maker GL, Hood SD, Drummond PD. A review of peripheral biomarkers in major depression: the potential of inflammatory and oxidative stress biomarkers. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2014; 48: 102-11.
22. Kabiri S, Sayyed-Alangi SZ. Comparison of Antioxidant effect of different extracts from Melissa officinalis leaves with immersion and microwave-assisted extractions and its oxidative stability on soybean oil. Innovative Food Technologies. 2015; 2(4): 23-38.