

Evaluation of the Protective Effect of Olive Leaf Extracts on Anxiety-like Behaviors in an Animal Model of Parkinson's Disease

S.S. Hosseini (MSc)¹, A. Hajizadeh Moghaddam (PhD)^{*1}

1. Department of Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar, I.R.Iran

Received: Nov 30th 2014, Revised: Feb 4th 2015, Accepted: May 6th 2015

ABSTRACT

BACKGROUND AND OBJECTIVE: Oxidative stress is one of the main causes of Parkinson's disease. Mood disorders such as anxiety are commonly reported in these patients. Olive leaf extracts have strong antioxidative and neuroprotective properties due to the presence of various phenolic compounds. The aim of the present study was to investigate the effects of olive leaf extracts on anxiety-like behaviors in an animal model of Parkinson's disease, using elevated plus maze test.

METHODS: In this experimental study, 42 rats were divided into six groups (seven rats per group): control, sham, patient and treatment (receiving extracts at doses of 50, 100 and 150 mg/kg) groups. The control group did not receive any injections, whereas the sham and patient groups received saline solutions and the treatment group received 50, 100 and 150 mg/kg of olive leaf extracts via gavage for seven weeks. All groups, except the control and sham groups, were administered unilateral 6-hydroxydopamine in the striatum, using a stereotactic device, and an animal model of Parkinson's disease was established. Three weeks after toxin injections, anxiety-like behaviors were assessed, using the elevated plus maze test.

FINDINGS: The injection of 6-hydroxydopamine caused a reduction in the time of animals' entry into the open arm and the number of entries. Administration of different concentrations of the extract caused an increase in the mentioned parameters in all treatment groups, compared to the patient group.

CONCLUSION: The results showed that oral administration of olive leaf extracts caused a significant decrease in anxiety-like behaviors, induced by 6-hydroxydopamine in an animal model of Parkinson's disease.

KEY WORDS: Parkinson, Elevated Plus Maze Test, Anxiety-like Behavior, Olive Leaf Extract.

Please cite this article as follows:

Hosseini SS, Hajizadeh Moghaddam A. Evaluation of the Protective Effect of Olive Leaf Extracts on Anxiety-like Behaviors in an Animal Model of Parkinson's Disease. J Babol Univ Med Sci. 2015;17(7):36-43.

^{*}Corresponding Author: A. Hajizadeh Moghaddam (PhD)

Address: Department of Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar, I.R.Iran

Tel: +98 11 35302453

E-mail: a.hajizadeh@umz.ac.ir

Introduction

Anxiety, as one of the most common mental disorders, is associated with different physical manifestations (1). Oxidative stress in the central nervous system is one of the underlying causes of anxiety. Due to lipid peroxidation, oxidative stress leads to a decrease in membrane fluidity, damages the proteins in the membrane of neurons and thereby changes the release of neurotransmitters and the overall performance of the brain (2). Oxidative stress is also one of the main causes of degeneration in dopaminergic neurons in Parkinson's disease. Parkinson's disease is associated with stress-like behaviors (3, 4), which have unpleasant effects on mobility and quality of life in 40% of patients (5). Injection of 6-hydroxydopamine (6-OHDA) (Sigma Aldrich, USA) into the substantia nigra or the striatum induces an animal model of Parkinson's disease by degenerating dopamine neurons (6). This toxin after a selective entry through dopamine transmitters leads to the damage, inflammation and ultimately death of neurons by producing reactive oxygen species in the neurons of nigrostriatal pathway (7, 8).

According to many studies, use of vegetable products or polyphenol-enriched supplements may delay the initiation or progression of conditions such as Alzheimer's disease, Parkinson's disease and the associated neurological disorders (9, 10). Among natural antioxidants, olive tree is commonly known to have strong antioxidant activities in its oil, fruit and leaf extracts (11). As previous studies have indicated, biophenols in olive possess anti-atherogenic, antioxidant and anti-inflammatory properties, which can prevent or hinder neuronal destruction. Recently, olive oil has been suggested as a sedative, as well (12). On the other hand, many studies have reported the effects of polyphenols in herbal extracts (e.g., green tea extracts). Based on previous research, these compounds could prevent the degeneration of dopaminergic neurons in substantia nigra in an animal model of Parkinson's disease (13).

Olive leaf extracts are enriched with many phenols such as oleuropein, luteolin-7-glucoside, apigenin-7-o-glucoside, verbascoside and hydroxytyrosol, which neutralizes superoxides and hydroxyl radicals under laboratory conditions. Under these circumstances, hydroxytyrosol normally removes peroxyl radicals on the surface of the

membrane, and oleuropein functions on the lipid distribution chain of peroxyl radicals (14).

The effects of polyphenols in cognitive processes and neural destruction are applied through their interactions with neuronal and glial signaling pathways, which lead to the disruption of gene expression and cell death (9). In this study, we aimed to investigate the protective effects of olive leaf extracts on anxiety-like behaviors in an animal model of Parkinson's disease, using elevated plus maze test.

Methods

This experimental study was conducted on 42 Wistar rats, weighing 200-250 g. The animals were purchased from Amol Pasteur Institute (Amol, Iran) and kept in separate cages (12 hr of light and 12 hr of darkness), with free access to water and food. All the experiments were performed in accordance with the ethical codes of Bioethics Committee of Mazandaran University of Medical Sciences, Mazandaran, Iran. The rats were divided into six groups (seven rats per group): control, sham, patient and treatment (receiving olive leaf extracts at doses of 50, 100 and 150 mg/kg) groups (15, 16). The sham and patient groups received saline solutions via gavage, whereas the treatment groups received hydroalcoholic extracts of olive leaves on a daily basis between 10 a.m. and 12 p.m.

Three weeks after the injections, all rats, except the control group, were anesthetized via intraperitoneal injection of ketamine (70 mg/kg) and xylazine (4 mg/kg). Afterwards, the rats underwent surgery, using a stereotaxic device. Cannulation was conducted unilaterally in the striatum (right hemisphere), based on Watson and Paxinos rat brain atlas with the following coordinates: anterior-posterior position (1 mm), lateral position (2.5 mm) and depth of 4.5 mm relative to bregma (17).

After a couple of days, the rats in patient and treatment groups were administered 10 mg of 6-OHDA in 2 μ l of saline (containing 0.1% ascorbic acid) via a Hamilton syringe. The sham group also received saline solution by Hamilton syringe. Treatment with the extracts continued for three weeks after the surgery in treatment groups.

Behavioral tests

Apomorphine-induced rotational behaviors:

Apomorphine (apomorphine hydrochloride

hemihydrate, Sigma-Aldrich, USA) is an agonist for dopamine, which causes a rotation towards the opposite side of the area damaged by 6-OHDA (18). The rotational behavior induced by apomorphine was investigated 21 days after the surgery in all groups. The animals were stored in a cylindrical chamber (28 cm in diameter and height of 38 cm) in order to familiarize them with the environment.

Afterwards, apomorphine with a concentration of 0.5 mg/kg, along with saline (containing 0.1% ascorbic acid), was intraperitoneally injected in rats (19). One minute after the injection, the rats were placed inside the chamber and the number of rotations towards the damaged area and the opposite side was measured within an hour. The number of pure rotations was calculated as the difference between rotations in two directions.

Elevated plus maze test: In this study, elevated plus maze test was used to investigate anxiety-like behaviors. The used device was a wooden, cross-shaped maze. This maze was equipped with two open arms (without walls) and two closed arms (with a 30 cm wall). The length and width of the open arm were 50 and 10 cm, respectively. Also, the open arms were attached to closed arms (10 cm in diameter) in the center area. The apparatus was placed on a foundation at a height of 50 cm above the ground. A low-light lamp was used as the source of light in the room during the experiments (15). Before the onset of experiments, the rats were kept in a room in which the device was located (for five minutes) in order to familiarize them with the environment. The animals were placed in the center of the device on the opposite side of the open arm. They were allowed to move in the open and closed arms for five minutes. The percentage of time spent in open arms (OAT) and the percentage of open-arm entries (OAE) were regarded as standard parameters for the evaluation of anxiety (20). These parameters were calculated as follows:

$$\text{OAE} = (\text{Open arm entries} / \text{open} + \text{closed entries}) \times 100$$

$$\text{OAT} = (\text{Time of entry to the open arm} / \text{time of all entries}) \times 100$$

In this study, the total number of entries into the arms was regarded as a factor indicating rats' mobility. The low number of entries to the open arms and the little time spent in these arms were indicative of stress-like behaviors. For statistical analysis and intergroup comparisons, one-way ANOVA and Tukey's test were performed. All calculations were

performed, using SPSS version 16. P-value less than 0.05 was considered statistically significant. Moreover, in order to illustrate the figures, SigmaPlot version 12.5 was used.

Results

The results of apomorphine-induced rotational test: The results of apomorphine-induced rotational test showed that the number of induced rotations in the control and sham groups was lower than 30. No significant difference was observed in the number of complete rotations between these groups. In addition, the number of complete rotations in the patient group significantly increased, compared to the control group ($P < 0.001$). Use of olive leaf extracts also significantly reduced the number of rotations towards the opposite side in treatment groups, receiving extracts at doses of 50, 100 and 150 mg/kg ($p < 0.001$) (table 1).

Table 1. The mean number of total rotations, induced by the intraperitoneal injection of apomorphine three weeks after the surgery

Groups	The mean number of pure rotations (Mean \pm SE)
Control group	12.71 \pm 3.03
Sham group	13.71 \pm 3.03
Patient group (10 μ g per rat)	137.85 \pm 25.75***
Treatment group (50 mg/kg)	50.14 \pm 9.09***+++
Treatment group (100 mg/kg)	17.85 \pm 3.23+++
Treatment group (150 mg/kg)	15.71 \pm 4.49+++

*** Significant difference with the control group ($P < 0.001$)

+++ Significant difference with the patient group ($P < 0.001$)

The results are expressed as Mean \pm SE (n=7)

The effects of 6-OHDA injection on anxiety-like behaviors: The results of 6-OHDA administration on anxiety-like behaviors within three consecutive weeks showed that OAT and OAE parameters significantly reduced in treatment groups, compared to the control group ($P < 0.001$). These results indicated the occurrence of anxiety-like behaviors in an animal model of Parkinson's disease, which were amplified as time passed.

The effects of olive leaf extracts on anxiety-like behaviors by the end of the first week: OAT% and

OAE% in treatment groups, receiving different doses of olive leaf extracts, and patient group significantly reduced in the first week, compared to the control group. However, data related to rats' mobility did not reveal any significant differences between these groups, compared to the control group (fig 1).

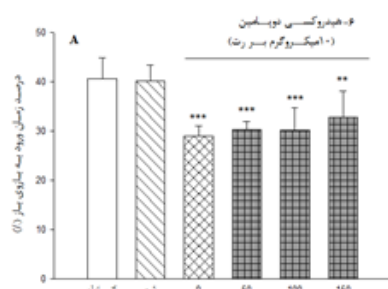


Figure A: The percentage of time spent in the open arm

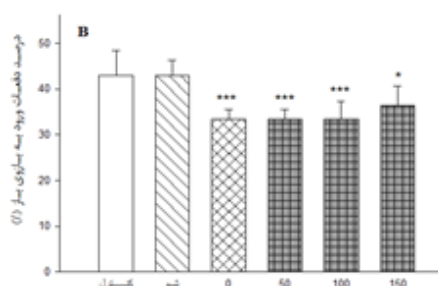


Figure B: The percentage of the number of entries to the open arm

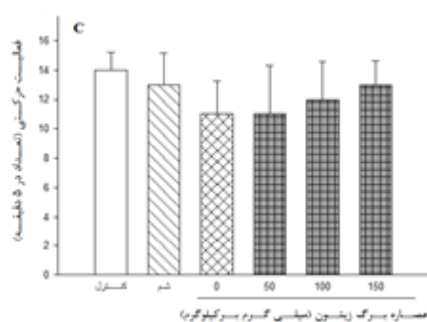


Figure C: Physical mobility

Figure 1. The effects of different doses of olive leaf extracts on anxiety-like behaviors, induced by 6-OHDA in elevated maze plus test in the first week Significant difference with the control group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

The results are expressed as Mean ± SE (n=7)

The effect of olive leaf extract on anxiety-like behaviors by the end of the second week: Similar to the first week, a significant decline was reported in OAT% and OAE% parameters in treatment groups,

receiving different doses of olive leaf extracts, compared to the control group by the end of the second week. The evaluation of anxiety-like behaviors by the end of the second week indicated that the oral administration of different doses of olive leaf extracts caused a significant decline in anxiety-like behaviors, induced by the toxin in the animal model of Parkinson's disease (Figure 2).

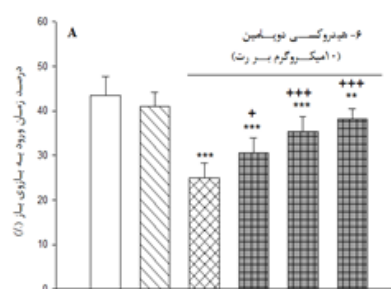


Figure A: The percentage of time spent in the open arm

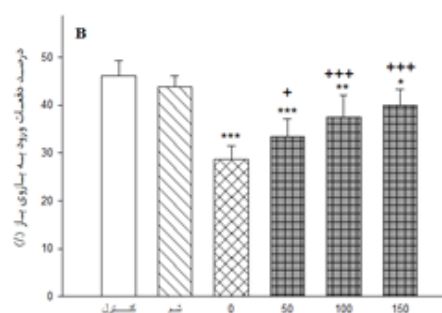


Figure B: The percentage of the number of entries to the open arm

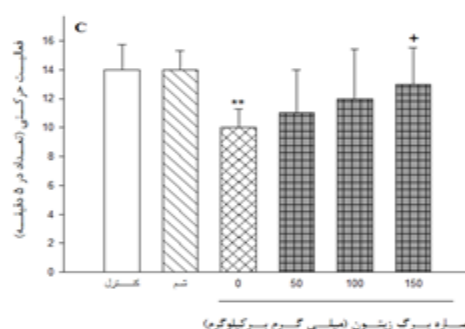


Figure C: Physical mobility

Figure 2. The effects of different doses of olive leaf extract on anxiety-like behaviors, induced by 6-OHDA in elevated plus maze test during the second week Significant difference with the control group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Significant difference with the patient group (+ $p < 0.05$, ++ $p < 0.01$, +++ $p < 0.001$)

The results are expressed as Mean ± SE (n=7)

The effects of olive leaf extracts on anxiety-like behaviors by the end of the third week: Similar to the second week, a significant increase was reported in OAT% and OAE% parameters in treatment groups, compared to the patient group. This indicated the effect of the extracts on decreasing anxiety-like behaviors, induced by 6-OHDA. The evaluation of anxiety-like behaviors by the end of the third week showed that the oral administration of different doses of leaf extracts not only significantly reduced anxiety-like behaviors, but also normalized these behaviors (Figure 3).

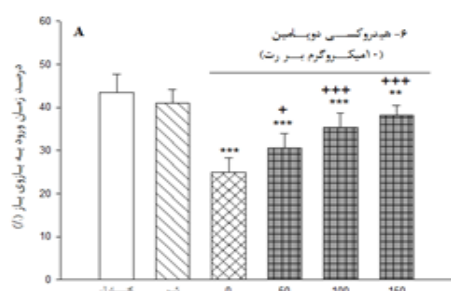


Figure A: The percentage of timespent in the open arm

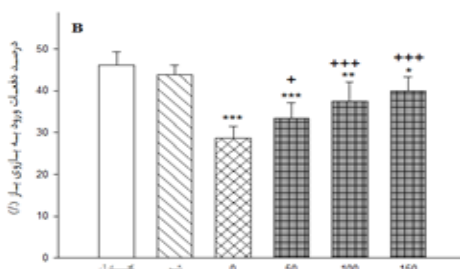


Figure B: The percentage of the number of entries to the open arm

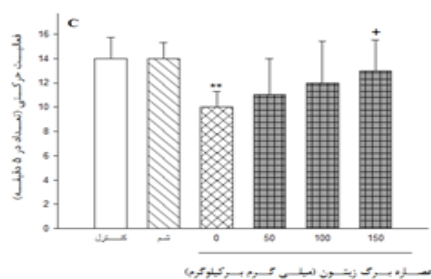


Figure C. Physical activity

Figure 3: The effects of different doses of olive leaf extract on anxiety-like behaviors, induced by 6-OHDA in elevated plus maze test in the third week of the experiment Significant difference with the control group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) Significant difference with the patient group (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) The results are expressed as Mean \pm SE (n=7)

Discussion

The results of the present study showed that one month of treatment by different doses of olive leaf extracts did not have any significant effects on anxiety-like behaviors in an animal model of Parkinson's disease during the first week of evaluation. Overall, injection of 6-OHDA into the brain of rats is being widely applied for establishing an animal model of Parkinson's disease. The main mechanism of 6-OHDA toxicity is related to the production of oxygen radical species through autoxidation. Also, 6-OHDA injection into the striatum can gradually degenerate dopaminergic neurons (22).

As many studies have indicated, oxidative stress is an effective factor for decreased cognitive function imaging (23). Deumens et al. also showed that the injection of 6-OHDA could be used as a suitable model for investigating the symptoms of Parkinson's disease. Use of this injection could also add to the current information about the pathological mechanisms of such neurological disorders (24). In this regard, Tadaiesky and colleagues reported that the damage caused by 6-OHDA in the striatum of rats could increase anxious responses in elevated plus maze test (25).

Polyphenols in herbal extracts have various antioxidant and anti-anxiety properties (26, 27). In this regard, Shrivastava et al. reported that piperine, due to its antioxidant properties, has protective effects against Parkinson's disease (induced by 6-OHDA) through an anti-apoptotic mechanism (17). In addition, some studies have revealed the positive effects of herbal extracts on anxiety disorders. Shah Gagan et al. investigated the anti-stress properties of the methanolic extract of *Cymbopogon citratus* leaf and suggested that the existence of flavonoid compounds leads to an increase in OAE and OAT parameters in elevated plus maze test (28). In another study, the relaxing effects of *Passiflora actinial* leaf extracts were reported (29). According to previous studies, olive leaf extracts are also enriched with polyphenol compounds (26, 30) and have strong protective neuronal activities, which improve oxidative stress and nerve damage in animals (31).

In the present study, the evaluation of behavioral parameters by the end of the second and third weeks showed that over time, use of different doses of olive leaf extracts significantly reduced stress-like behaviors in treatment groups. By the end of the

sixth week of extract use at higher doses, anxiety-like behaviors returned to the normal level, which is probably related to the protective effects of this compound on the induced damage in Parkinson's model.

Many studies have shown that pre-treatment with polyphenols in olive leaf significantly prevents the production of super oxides and nitric oxides after ischemia and prohibits lipid peroxidation (32). In fact, these flavonoids can probably protect nerve cells via different mechanisms such as stimulating the regeneration of damaged neurons and increasing the performance of remaining neurons. On the other hand, flavonoids may affect protein modulation and lipid kinase signaling pathways by inhibiting MAP kinase signaling cascades such as p38 or ERK1/2 (9). The results of the present study showed that the use of olive leaf extract in the long run can significantly protect the neurons against damages

caused by 6-OHDA, due to its antioxidant properties. Thereby, these extracts reduce the induced behaviors by 6-OHDA. Researchers have suggested the protective effect of this extract against neurological disorders such as Parkinson's disease.

According to the findings of this study, oral administration of olive leaf extracts at higher doses may increase the time and number of entries to the open arm in elevated plus maze test. This indicates rats' recovery from the damage, induced by 6-OHDA, in an animal model of Parkinson's disease.

Acknowledgments

Hereby, we would like to thank the research deputy of Mazandaran University of Medical Sciences for the financial support. We also extend our gratitude to Ms. Sedigheh Khanjani Jelodar for her cooperation in performing this study.

References

1. Bueno CH, Zangrossi Jr H, Viana MB. The inactivation of the basolateral nucleus of the rat amygdala has an anxiolytic effect in the elevated T-maze and light/dark transition tests. *Braz J Med Biol Res.* 2005;38(11):1697-1701 .
2. Bouayed J, Rammal H, Soulimani R. Oxidative stress and anxiety: relationship and cellular pathways. *Oxid Med Cell Longev.* 2009; 2(2):63-7.
3. Dissanayaka NN, Sellbach A, Matheson S, O'Sullivan JD, Silburn PA, Byrne GJ, et al. Anxiety disorders in Parkinson's disease: prevalence and risk factors. *Mov Disord.* 2010;25(7):838-45.
4. Dexter David T & Jenner Peter. Parkinson disease: from pathology to molecular disease mechanisms. *Free Radical Biology and Medicine.* 2013;62, 132-144 .
5. Pontone GM, Williams JR, Anderson KE, Chase G, Goldstein SR, Grill S, et al. Pharmacologic treatment of anxiety disorders in Parkinson disease. *The American Journal of Geriatric Psychiatry.* 2013; 21(6), 520-528 .
6. Jin F, Wu Q, Lu YF, Gong QH, Shi JS. Neuroprotective effect of resveratrol on 6-OHDA-induced Parkinson's disease in rats. *European journal of pharmacology.* 2008; 600(1), 78-82 .
7. Antunes MS, Goes AT, Boeira SP, Prigol M, Jesse CR. Protective effect of hesperidin in a model of Parkinson's disease induced by 6-hydroxydopamine in aged mice. *Nutrition .* 2014; 30, 1415-1422.
8. Bove J, Perier C. Neurotoxin-based models of Parkinson's disease. *Neuroscience.* 2012;211:51-76 .
9. Vauzour D, Rodriguez-Mateos A, Corona G, Oruna-Concha MJ, Spencer JP. Polyphenols and human health: prevention of disease and mechanisms of action. *Nutrients.* 2010;2(11):1106-31.
10. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev.* 2009;2(5):270-8.
11. Abo Ghanema II, Sadek KM. Olive leaves extract restored the antioxidant perturbations in red blood cells hemolysate in streptozotocin induced diabetic rats. *World Acad Sci, Engin Technol.* 2012;6(4):04-29.
12. Obied HK, Prenzler PD, Omar SH, Ismael R, Servili M, Esposto S. Pharmacology of olive biophenols, Chapter 6. In book: *Advances in Molecular Toxicology.* Elsevier; 2012.p.195.
13. Weinreb O, Mandel S, Amit T, Youdim MB.. Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. *J Nutr Biochem.* 2004;15(9):506-16.
14. Lockyer S, Yaqoob P, Spencer JPE, Rowland I. Olive leaf phenolics and cardiovascular risk reduction: Physiological effects and mechanisms of action. *Nutr Aging.* 2012;1(2):125-40.
15. Nekooeian AA, Dehghani GA, Mostafavi H, Khalili A. The effect of hydroalcoholic extract of olive leaves on blood pressure in rat model of two-kidney, one-clip goldblatt hypertension. *Int Cardiovasc Res J.* 2011;5(1):1-6.
16. Al-Attar AM, Shawush NA. Physiological investigations on the effect of olive and rosemary leaves extracts in male rats exposed to thioacetamide. *Saudi J Biol Sci.* 2014;21(5):473-80.
17. Shrivastava P¹, Vaibhav K, Tabassum R, Khan A, Ishrat T, Khan MM, et al. Anti-apoptotic and anti-inflammatory effect of piperine on 6-OHDA induced Parkinson's rat model. *J Nutr Biochem.* 2013;24(4):680-7.
18. Khan MM, Ahmad A, Ishrat T, Khan MB, Hoda MN, Khuwaja G, et al. Resveratrol attenuates 6-hydroxydopamine-induced oxidative damage and dopamine depletion in rat model of Parkinson's disease. *Brain Res.* 2010;1328,139-51.
19. Khuwaja G, Khan MM, Ishrat T, Ahmad A, Raza SS, Ashafaq M, et al. Neuroprotective effects of curcumin on 6-hydroxydopamine-induced Parkinsonism in rats: behavioral, neurochemical and immunohistochemical studies. *Brain Res.* 2011;1368:254-63.
20. Rodgers RJ, Johnson NJ. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. *Pharmacol Biochem Behav.* 1995;52(2):297-303.

- 21.Rezayat M, Roohbakhsh A, Zarrindast MR, Massoudi R, Djahanguiri B. Cholecystokinin and GABA interaction in the dorsal hippocampus of rats in the elevated plus-maze test of anxiety. *Physiol Behav.* 2005;84(5):775-82.
- 22.Martí MJ, Saura J, Burke RE, Jackson-Lewis V, Jiménez A, Bonastre M, et al. Striatal 6-hydroxydopamine induces apoptosis of nigral neurons in the adult rat. *Brain Res.* 2002;958(1):185-91.
- 23.Rabiei Z, Alibabaei Z, Rafieian-Kopaei M. Effects of matricaria chamomilla extract on motor coordination impairment induced in rat and determination antioxidant properties of chamomile. *J Babol Univ Med Sci.* 2015;17(4):44-50. [In persian].
- 24.Deumens R, Blokland A, Prickaerts J. Modeling Parkinson's disease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway. *Exp Neurol.* 2002;175(2):303-17.
- 25.Tadaiesky MT, Dombrowski PA, Figueiredo CP, Cargnin-Ferreira E, Da Cunha C, Takahashi RN. Emotional, cognitive and neurochemical alterations in a premotor stage model of Parkinson's disease. *Neuroscience.* 2008;156(4):830-40 .
- 26.Sarbishegi M, Mehraein F, Soleimani M. Antioxidant role of oleuropein on midbrain and dopaminergic neurons of substantia nigra in aged rats. *Iran Biomed J.* 2014;18(1):16-22.
- 27.Vauzour D. Dietary polyphenols as modulators of brain functions :biological actions and molecular mechanisms underpinning their beneficial effects. *Oxid Med Cell Longev.* 2012;2012:914273.
- 28.Shah G, Shiri R, Dhabiliya F, Nagpal N, Mann AS. Anti-anxiety activity of cymbopogon citratus (dc.) stapf leaves extracts on the elevated plus-maze model of anxiety in mice. *Pharmac J.* 2010;2(15):45-50.
- 29.Santos Kely Cristina dos, Kurtz Stella Maris Tessaro Figura, Müller Simony, Biavatti DM, de Oliveira RM, de Moraes Santos CA. Sedative and anxiolytic effects of methanolic extract from the leaves of *Passiflora actinia*. *Braz Arch Biol Technol.* 2006;49(4):565-73.
- 30.Aytul KK. Antimicrobial and antioxidant activities of olive leaf extract and its food applications. izmir institute of technology. 2010.available at:<http://library.iyte.edu.tr/tezler/master/biyoteknoloji/T000831.pdf>.
- 31.Dekanski Dragana, Selaković Vesna, Piperski Vesna, Radulovic Zeljka, Korenic Andrej & Radenovic, Lidija. Protective effect of olive leaf extract on hippocampal injury induced by transient global cerebral ischemia and reperfusion in Mongolian gerbils. *Phytomedicine.* 2011; 18(13), 1137-1143 .
- 32.Johri A, Beal MF. Antioxidants in Huntington's disease. *Biochim Biophys Acta.* 2012;1822(5):664-74.