The effect of oleuropein on working and passive avoidance memory in the pentylenetetrazole-induced seizure animal model

Parvin Sadate Hosseini[1], Maryam Rafieirad[2*], Somayeh Esmaeili[1]

1. Department of Psychology, Izeh Branch, Islamic Azad University, Izeh, Iran
2. Department of Biology, Izeh Branch, Islamic Azad University, Izeh, Iran

***Corresponding author:**Tel: +98 9163116542; Fax: +98 6143643374
Address: Department of Biology, Izeh Branch, Islamic Azad University, Izeh, Iran
E-mail: Rafieirad.m@gmail.com
Received: 2019/02/28 revised: 2019/03/28 accepted: 2019/05/3

Abstract

Introduction: Epileptic seizures are product of abnormal electrical discharges of the brain. Electrical wave productive of epileptic seizure generates disturbances in brain data processing circuits and these patients suffer from memory impairment. In this study, the effect of different doses of oleuropein on the treatment of memory impairment Caused by frequent seizures was in male rats investigated.

Materials and methods: Forty rats were randomly divided into four groups of 10 (the negative control group received normal saline, the positive control group received diazepam 1 mg/kg and the two treatment groups received doses 10 and 20 mg/kg of oleuropein). Thirty minutes after administration of different doses of oleuropein or saline or diazepam, pentylenetetrazole (PTZ) at a dose of 85 mg/kg was injected intraperitoneal into rats, and after creating seizure and animal survival, tests of memory were performed. One-way ANOVA and Tukey's tests were the procedures used to analyze the results.

Results: In both tests, the memory of the control group (normal saline recipient) decreased significantly ($P<0.001$). The administration of 10mg/kg oleuropein shows a significant increase in periodic behavior measurements by maze y ($P<0.01$). Both doses of 10 and 20 mg/kg increased passive avoidance memory ($P<0.001$).

Conclusion This study shows that the oleuropein has an appropriate anticonvulsant effect and improves the working memory and passive avoidance in epileptic rat and Future studies appear to be necessary to understand further how the mechanism of its effect.

Keywords: Oleuropein, Memory, Pentylenetetrazole, Rat

Introduction

Approximately 4 billion people (50% of the world population) live in Asia that about 23 million people of them are involved with epilepsy. The higher incidence of epilepsy in Asia compared to Western countries may be due to an increased risk of central nervous system infection through diseases such as cerebral malaria, neurocysticercosis, meningitis, encephalitis, tuberculosis, and human immunodeficiency virus (HIV) (1). Cognitive impairments such as losing memory, mental deficits, and lack of attention are commonly associated with epilepsy (2). Causes of cognitive impairment in epilepsy may include underlying causes of epilepsy, such as subclinical seizures, or

Copyright © 2019 Journal of Basic Research in Medical Science. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/) which permits copy and redistribute the material, in any medium or format, provided the original work is properly cited.

[Downloaded from jbrms.medilam.ac.ir on 2026-11-12]

electroencephalography, and side effects of the central nervous system because of the use of AED (antiepileptic drugs) (3); therefore, early diagnosis and management of the cognitive impairments are highly essential especially for premature seizures. Patients with epilepsy are at risk of early death resulting from dementia or stroke, especially during the first two years after the seizure (4). Study of epilepsy in rodents is performed using seizure generating medicines such as Pentylenetetrazole (PTZ) that produce seizure and damage in CA1 and CA3 areas of the hippocampus (5). Pentylenetetrazole prevents the combination of the chloride ion nuclear with the GABA-A receptor, which has seizure effects after repeated or single-dose administration. Also, it affects several neurotransmitter systems such as GABAergic and glutamatergic systems (6). Existence of cognitive disorder and memory impairment and cognitive deficiencies in the experimental models of epilepsy has been confirmed previously in the laboratory animals (7). In various studies, from different amounts of polyphenols of species medicinal has been called as the most critical compounds of secondary with antioxidant properties that by inhibition of free radicals prevents the oxidative stresses and occurrence of cardiovascular disease, tuberculosis, atherosclerosis, neurological diseases and cancer. The main active substance in the olive leaf, which is responsible for many of its therapeutic properties, is oleuropein. Oleuropein acts on the chain of lipid release of peroxyl radicals (8). The antioxidant capacity of oleuropein is four times more than vitamin C and two times more than green tea or extract of grape seed (9). So, more research is needed to examine the cognitive effects of oleuropein in clinical and laboratory studies. Given the different nature of cognitive trauma in the epilepsy model and seizure model and since the impact of oleuropein hasn't been examined yet on the improvement of these impairments, so in the present study, the effect of oleuropein on the improvement of the working memory disorder and passive avoidance in the Pentylenetetrazol-induced seizure animal model, is investigated.

Materials and methods

Animals: The present study was carried out using adult male Wistar rats purchased from the proliferation and maintenance center of animals' house in Ahvaz Medical Science University with a weight range of 200 to 250 g. Animals were kept in standard conditions of 20 ± 2°C and 12-hour lighting-darkness cycle (start of light from 7 am). There was adequate access to intensive food from the Tehran Pars Animals Company and Chavdaneh of Shahreza in Isfahan and purified piped water in the animal care center of Islamic Azad University of Izeh, inside standard cages in groups of four. To facilitate the process and for more compatibility with the environment and experimenter, animals were trained daily for a few minutes before the experiment and were divided randomly into 4 equal groups as follows (each one 10): Negative control group: they received normal saline as a placebo 30 minutes before seizure induction. Treatment group number 1: they received oleuropein with 10 mg/kg dosage 30 minutes before seizure induction. Treatment group number 2: they received Oleuropein with 20 mg/kg dosage 30 minutes before seizure induction. Positive control group: they received 1 mg/kg diazepam 30 minutes before seizure induction.

Method of creation chemical kindling: For seizure induction from the Pentylenetetrazole drug (Sigma Company) (85 mg/kg) was used in the form of a solution in normal saline and by the amount of one unit or cc of insulin syringe and intraperitoneal (10).

Shuttle box device: To measure the passive avoidance memory from the shuttle box device, is used. The training

[Downloaded from jbrms.medilam.ac.ir on 2026-11-12]

instrument includes two boxes (bright and dark) that have been separated from each other by a guillotine blade. The dark box floor has been covered by shocking steel bars. Examination of passive avoidance behavior is performed during two consecutive days. The first day is the training session. In training every rat is put within the bright box and 60 seconds after adaptation the separator blade is opened and the delay time is recorded until the rat enters to the dark box. Immediately after entering the rat into the dark box the blade comes down and a shock equal to 75 V, 0.2 MA and 50 Hz is applied for 0.3 seconds. After 5 seconds, the rat is removed from the box and returned to its cage. 24 hours after the training, a retrieving test is done until the long-term memory of the animal is examined; that is, the same work is repeated but no shock isn't applied. The delay memory is computed for a maximum of 300 seconds. The time of the test finishing is 5 minutes after putting in the bright chamber (11).

Maze Y test: The Maze Y test was used to examine working memory. The three arms of the Y-shaped maze have exactly same conditions and each arm is marked with A, B and C letters. Rodents generally prefer to examine new arms. Measurement of the memory process by the maze y is conducted in a relatively dark and quiet room to avoid entering slightest stress to the animal during the test. The animal is put after handling calmly and without stress in one of the three arms and is observed its movements for 5 minutes. The number of times any animal enters in each of the arms will be counted. After 5 minutes, the animal is removed calmly from the maze and the all internal surfaces of the maze will be cleaned by alcohol of 70% in order to prevent the impact of the animal's smelling sense to choose an arm. To evaluate the working memory of the animal, the arms that the animal had entered into them are classified in the triple sequence and is eliminated the groups in which the repeated arm is there. The total number of arms that any animal enters will be determined, such that, the rate of the alternating behaviors is calculated from the sum of the successful entries divided on the total entries each arm minus 2 multiplier in 100 (12).

Statistical analysis

In this study, the obtained results are expressed as average and standard deviation. First, normality of data distribution was tested using Bartlett and Kolmogorov-Smirnov test and then a statistical comparison was performed between the test groups using one-way ANOVA test. After revealing the significant difference between the groups, among each of the test groups with the related complementary test (Tukey) for analyzing the behavioral test data, including delay times in the passive avoidance test and percentage of alternation in the Maze Y test and the results of the seizure behavior is compared. In all assessments, a difference level of $P< 0.05$ is considered a meaningful response and had the following results. Also, data have been presented as Mean ±SD.

Results

The results obtained from this study showed that in the performance of the rats of different groups, initial delays during passive avoidance behavior test were significantly increased in diazepam group compared to control group ($P<0.001$). Intraperitoneal injection of 10 and 20 mg/kg of oleuropein resulted in a significant increase in passive avoidance memory compared to the control group ($P<0.001$). As shown in Figure 1, the time of initial delays during passage in passive avoidance memory test in the diazepam group was significantly higher than those receiving 10 and 20 mg / kg oleuropein ($P<0.01$) ($P< 0.05$) (Figure 1).

[Downloaded from jbrms.medilam.ac.ir on 2026-11-12]

Figure 2 shows the performance of different groups of rats in the Maze Y test. The rate of behavioral frequency increased significantly in the diazepam group compared to the control group (P<0.001). On the other hand, the percentage of alternation behavior in the treated group with 10 mg/kg dose of oleuropein was significantly increased compared to the control group (P<0.01). The results also showed that the frequency of behavior in the diazepam group compared to the groups treated with doses of 10 mg/kg oleuropeine (P<0.05) and 20 mg/kg oleuropein (P<0.01) was always more.

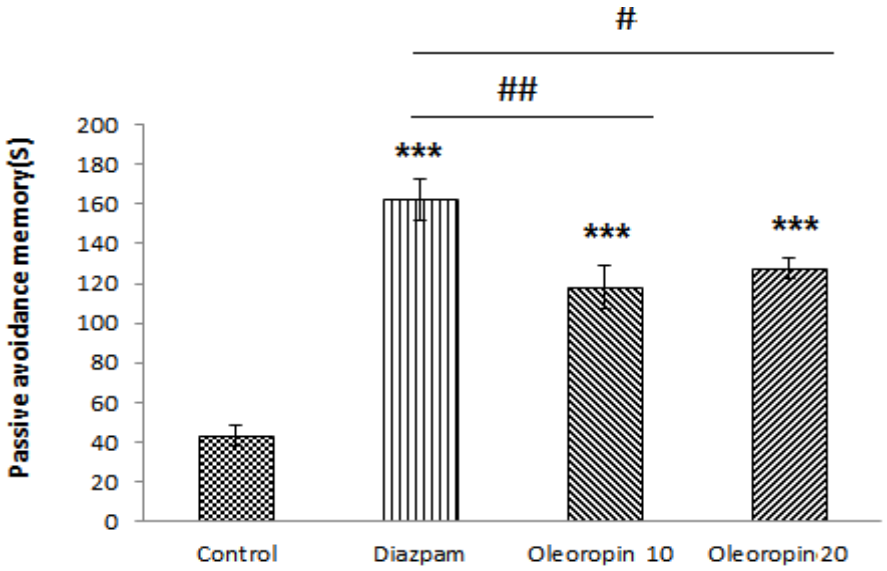


Figure 1. Initial delay during passage in passive avoidance memory test in the control group (normal saline recipients), diazepam and treated with doses of 10 and 20 mg/kg oleuropein.

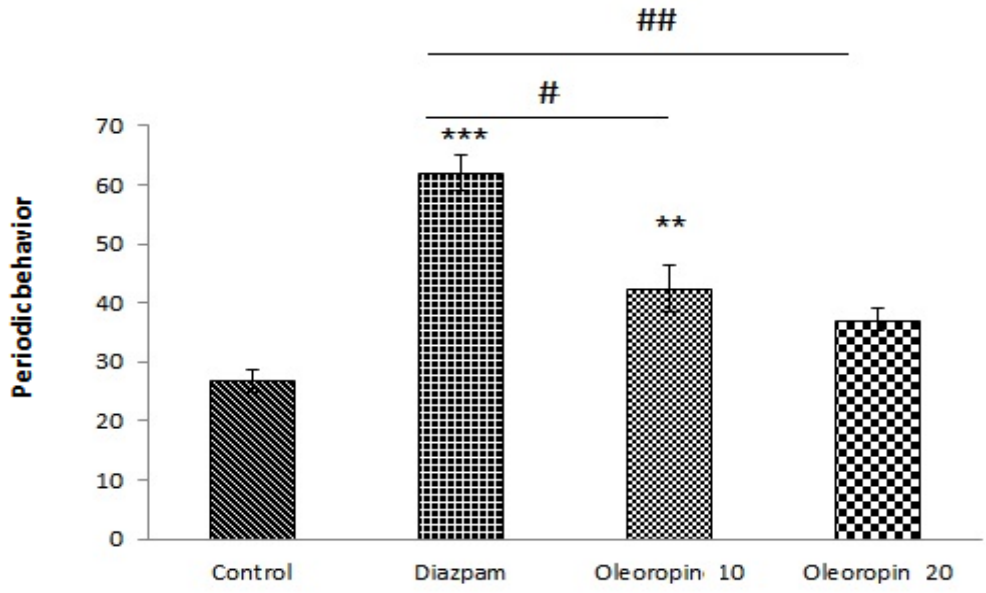


Figure 2. Percentage of periodic behavior in maze Y test in the control group (normal saline recipients), diazepam and treated with doses of 10 and 20 mg/kg oleuropein.

[Downloaded from jbrms.medilam.ac.ir on 2026-11-12]

Discussion

Results obtained from this study showed that prescribing oleuropein has a performance similar to the positive control group and can be effective on the epileptic caused by PTZ through intensifying the inhibitory processes and reduction of stimulation transfer and result in the improvement of working memory and also the ability of preserving information and keeping them in the passive avoidance test. Cholinergic nicotine receptors in the brain have a high capability to calcium ions passage, and some of them are in pre-synaptic position for GABAergic neurons. Reduction of GABAergic activity results in an increase of neurons' irritability (13). Increase of irritability in the local epilepsy may be relevant to an immunity reaction (for example, the creation of glutamate receptor antibodies) or be excess germination of damaged axons (Mossy axons in hippocampus) (14). Today, in the treatment of epilepsy, they use compounds that have three mechanisms of action: GABAergic enhancement, reduced glutamate stimulation and ionic flow modulation, especially sodium, calcium and chlorine ions (15). One of the most common causes of epilepsy and seizure in humans and animals is weakening of the GABAergic system (16). The activation of benzodiazepine receptors is done by stopping up the epileptic seizures through strengthening the brain's GABAergic system. Pentylenetetrazole (PTZ) seizure is induced by suppressing the flow of iodine-mediated chloride by GABA on the GABA A receptor and subsequently reduce the chloride ion (17). Therefore, drugs that the function of the GABAergic system enhance through the GABA A receptor can be effective in preventing epilepsy induced by Pentylenetetrazole (18). Benzodiazepines, such as diazepam, act with such mechanism (18, 19). Similar compounds of benzodiazepines have been reported in the many plant compounds, and most of the compounds identified are flavonoids (20). Recently, the effect of flavonoids through binding to benzodiazepine receptors that can have the same effects as benzodiazepines is posed (21). In particular, a large number of polyphenols and sequeyridoeyds found in olive oil, It contains oleacanthal, Hydroxy Tyrasole and oleuropeine Which is strongly responsible for the health effects of the Mediterranean regime (22). Many studies in rodents have shown that rich foods of polyphenols, particularly natural olive oil and red grape juice, improves learning disorder and age- dependent memory and also reduces oxidative damage in the brain (23). Oleuropeine is the main material of Structure the olive leaf and its fruit that it has beneficial effects, including antioxidant properties, anti-inflammatory, anticancer and anti-apoptosis effects (24). Reports indicate that the oleuropeine improved cognitive function in the Alzheimer-pseudo model in C-elegans and $TgCRND_8$ mice and is reduced amyloid β aggregation (25). Also, many studies have shown that Preventive treatment with oleuropeine has antioxidant effects against damage caused by Ischemic reperfusion (26), oxidative stress induced by colchicine in the area brain hippocampus (27) and aging (23). The treatment effect of oleuropeine on free radicals may occur through the formation of hydroxyl groups and direct neutralization of free radicals. Many of the pharmacologic effects of oleuropein have been attributed to its antioxidant activity (24). The role of oleuropein as a Neuroprotective compound has also been studied in Alzheimer's in vitro models. Neuropathological Indicators of Alzheimer's disease include the accumulation of extracellular neurotic plaques (which consists of amyloid β peptides) and intracellular neurofibrillary coils (its the main combination is protein tau hyperphosphorylation) (28). Oleuropein has this ability which prevents tau protein fibrillation at in vitro (29). Oleuropein also prevents the formation of

[Downloaded from jbrms.medilam.ac.ir on 2026-11-12]

amyloid β accumulation and cytotoxicity by inhibiting the formation of toxic oligomers (30). In another study, it was observed that the injection of oleuropein inside the brain, is able to reduce amyloid β toxicity and inflammation (31). Also, the injection of oleuropein is reduced oxidative stress caused by reperfusion-ischemia of the hippocampal region (26). In another study, oleuropein reduced oxidative damage in the Substantia Nigra in old rats by increasing of the activity of antioxidant enzymes (32). The role of this substance in the prevention of oxidative stress can be one of the effective mechanisms in the suggested effects. It is possible that a part of the desired effect of this drug on the memory and learning in this study is through prevention of the weakening of cholinergic system and reinforcing it in the epileptic rats that, in turn, requires more examinations. In this regard, it has been found that the generation of the experimental model of epileptic in laboratory animals over time results in weakening the performance of Cholinergic system associated with learning and memory and prescribing antioxidants probably can shift the level of activity of this system toward normal that this can be occur about oleuropein.

Conclusion

The results of this study indicate that administration of oleuropine in epileptic animals improves the ability to keep information and remind them in the passive avoidance memory, and improve the working memory in epileptic animals.

Acknowledgement

This study is based on a master's thesis, with code 15320706962065 and was conducted at the Biology Research Laboratory of Islamic Azad University, Izeh branch.

Conflict of interest

No conflict of interest exists.

References

1. Mac TL, Tran DS, Quet F, Odermatt P, Preux PM, Tan CT. Epidemiology, aetiology, and clinical management of epilepsy in Asia: a systematic review. Lancet Neurol. 2007; 6(6): 533-43**.** doi.org/10.1016/51474-4422(07)70127-8.
2. Kim EH, Ko TS. Cognitive impairment in childhood onset epilepsy: up-to-date information about its causes. Korean journal of pediatrics. 2016; 59 (4):155-64. doi: 10.3345/kjp.2016.59.4.155.
3. Shehata GA, Bateh Ael A, Hamed SA, Rageh TA, Elsorogy YB. Neuropsychological effects of antiepileptic drugs (carbamazepine versus valproate) in adult males with epilepsy. Neuropsychiatr Dis Treat. 2009; 5: 527-33**.** doi.org/10.2147/NDT.S5903.
4. Trinka E, Kwan P, Lee B, Dash A. Epilepsy in Asia: Disease burden, management barriers, and challenges. Epilepsia. 2019; 60 Suppl 1:7-21. doi: 10.1111/epi.14458.
5. Amigo I, Kowaltowski AJ. Dietary restriction in cerebral bioenergetics and redox state. Redox Biol. 2014; 2: 296-304**.** doi.org/10.1016/j.redox.2013.12.021.
6. Naseer MI, Shupeng L, Kim MO. Maternal epileptic seizure induced by pentylenetetrazol: apoptotic neurodegeneration and decreased GABAB1 receptor expression in prenatal rat brain. Mol Brain. 2009; 2: 20**.** doi.org/10.1186/1756-6606-2-20.
7. Nassiri-Asl M, Mortazavi SR, Samiee-Rad F, Zangivand AA, Safdari F, Saroukhani S, et al. The effects of rutin on the development of pentylenetetrazole kindling and memory retrieval in rats. Epilepsy Behav. 2010;

[Downloaded from jbrms.medilam.ac.ir on 2026-11-12]

18 (1-2): 50-3**.** doi.org/10.1016/j.yebeh.2010.03.005.
8. Stacey Lockyer PY, Spencer JPE and Rowland I. Olive leaf phenolics and cardiovascular riskreduction: Physiological effects andmechanisms of action. Nutrition and Aging. 2012; 1 (2): 125–40**.** doi: 10.3233/NUA-2012-0011.
9. Ozkaya F, Ozkaya M. Oleuropein using as an additive for feed and products used for humans. 2011**.** doi.org/10.4172/2157-7110.1000113.
10. Etemadi Kermani S, Rafieirad M. The The effect of intraperitoneal injection of ferulae angulata hydroalcoholic extract on pentylenetetrazole-induced epilepsy in male mice. 2018; 17 (1): 107-14**.** doi: 10.22118/JSMJ.2018.59644.
11. Rafieirad M, Valipour CS. [Gallic acid improves the memory and pain in diabetic rats]. Yafte 2013, 15(2): 33-41. (Article in Persian)
12. Borzou Z, Edalatmanesh MA. The evaluation of brain derived neurotrophic factor and working memory in valproic acid animal model of autism. Shefaye Khatam. 2015; 3 (4): 10-16. doi: 10.18869/acadpub.shefa.3.4.10.
13. Annegers JF, Grabow JD, Groover RV, Laws ER Jr, Elveback LR, Kurland LT. Seizures after head trauma: a population study. Neurology. 1980; 30 (7): 683-9. doi:10.1212/wnl.30.7.683.
14. Rissman R, Mobley W. Implication for treatment: $GABA_A$ receptors in aging, Down syndrome and Alzheimer's disease. J Neurochem. 2011 May; 117(4): 613–622. doi: 10.1111/j.1471-4159.2011.07237.x.
15. Diniz T, Cabral Silva J, Raquel S, Ribeiro F, Pacheco A, Mendes de Freitas R, et al. The Role of Flavonoids on Oxidative Stress in Epilepsy. Oxid Med Cell Longev. 2015;2015:171756. doi: 10.1155/2015/171756.
16. Stafstrom CE, Carmant L. Seizures and epilepsy: an overview for neuroscientists. Cold Spring Harbor perspectives in medicine. Cold Spring Harb Perspect Med. 2015; 5(6). doi: 10.1101/cshperspect.a022426.
17. Greenfield LJ, Jr. Cold Spring Harb Perspect Med. 2015; 5(6). pii: a022426. doi: 10.1101/cshperspect.a022426. 2013; 22(8):589-600**.**
18. Kiasalari Z, Khalili M, Roghani M, Sadeghian A. antiepileptic and antioxidant effect of brassica nigra on pentylenetetrazol-induced kindling in mice. Iranian journal of pharmaceutical research: Iran J Pharm Res. 2012; 11(4): 1209-17. doi: 10.22037/IJPR.2012.1212**.**
19. Sieghart W. [GABA(A) benzodiazepine receptors and epilepsy]. Wien Klin Wochenschr. 1990; 102(7):197-201. (Article in German)
20. Wasowski C, Marder M. Flavonoids as GABAA receptor ligands: the whole story? J Exp Pharmacol. 2012; 4: 9–24. doi: 10.2147/JEP.S23105.
21. Hanrahan JR, Chebib M, Johnston GAR. Flavonoid modulation of GABA(A) receptors. Br J Pharmacol. 2011; 163(2):234-45. doi: 10.1111/j.1476-5381.2011.01228.x.
22. Pitt J, Roth W, Lacor P, B Smith A, Blankenship M, Velasco P, et al. Alzheimer's-associated Abeta oligomers show altered structure, immunoreactivity and synaptotoxicity with low doses of oleocanthal. Toxicol Appl Pharmacol. 2009; 240(2):189-97. doi: 10.1016/j.taap.2009.07.018.
23. Pitozzi V, Jacomelli M, Zaid M, Luceri C, Bigagli E, Lodovici M, et al. Effects of dietary extra-virgin olive oil on behaviour and brain biochemical parameters in ageing rats. Br J Nutr. 2010; 103(11):1674-83. doi: 10.1017/S0007114509993655.
24. Omar SH. Oleuropein in Olive and its Pharmacological Effects. Sci Pharm. 2010; 78(2):133-54. doi: 10.3797/scipharm.0912-18.
25. Diomede L, Rigacci S, Romeo M, Stefani M, Salmona M. Oleuropein Aglycone Protects Transgenic C. elegans Strains Expressing Aβ42 by Reducing Plaque Load and Motor

[Downloaded from jbrms.medilam.ac.ir on 2026-11-12]

Deficit. PLoS One. 2013; 8(3): e58893. doi: 10.1371/journal.pone.0058893.
26. Dekanski D, Selakovic V, Piperski V, Radulovic Z, Korenic A, Radenovic L. Protective effect of olive leaf extract on hippocampal injury induced by transient global cerebral ischemia and reperfusion in Mongolian gerbils. Phytomedicine. 2011; 18(13):1137-43. doi: 10.1016/j.phymed.2011.05.010.
27. 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**.** J Physiol Sci. 2016; 66(5):397-405. doi: 10.1007/s12576-016-0437-4.
28. T Lin M, Beal M. Mitochondria dysfunction and oxidative stress in neurodegenerative diseases. Nature. 2006; 443(7113):787-95. doi:10.1038/nature05292.
29. Palazzi L, Bruzzone E, Bisello G, Leri M, Stefani M, Bucciantini M, et al. Oleuropein aglycone stabilizes the monomeric α-synuclein and favours the growth of non-toxic aggregates**.** Scientific Reports. 2018 ; 8(1). doi: 10.1038/s41598-018-26645-5.
30. Rigacci S, Guidotti V, Bucciantini M, Nichino D, Relini A, Berti A, et al. Aβ(1-42) Aggregates into Non-Toxic Amyloid Assemblies in the Presence of the Natural Polyphenol Oleuropein Aglycon. Curr Alzheimer Res. 2011; 8(8):841-52 doi: 10.2174/156720511798192682.
31. Luccarini I, Ed Dami T, Grossi C, Rigacci S, Stefani M, Casamenti F. Oleuropein aglycone counteracts Aβ42 toxicity in the rat brain. Neurosci Lett. 2014; 558:67-72. doi: 10.1016/j.neulet.2013.10.062.
32. 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. doi: 10.6091/ibj.1274.2013.

[Downloaded from jbrms.medilam.ac.ir on 2026-11-12]