The Effect of Aerobic and Resistance Exercise with Olive Extract on VO2 max, PTEN, and AKT in Rats with Parkinson's disease

Seyedeh Soheila Bolhagh[1], Ramin Shabani[2]*, Donya Sajedi[3]

(1) Ph.D. Candidate in Exercise Physiology, Department of Physical Education and Sports Science, Rasht Branch, Islamic Azad University, Rasht, Iran.

(2)* Professor, Department of Physical Education and Sports Science, Rasht Branch, Islamic Azad University, Rasht, Iran. E-mail: dr.ramin.shabani@gmail.com

(3) Ph.D. in Exercise Physiology, Young Researchers and Elite Club, Rasht Branch, Islamic Azad University, Rasht, Iran.

Abstract:

Introduction: Parkinson's disease is a progressive neurological disorder. This study aimed to investigate the effect of exercise training with the consumption of olive extract on maximal oxygen consumption (VO_2 max), phosphatase and tensin homolog on chromosome 10 (PTEN), and protein kinase B (AKT) in rats with Parkinson's.

Material & Methods: The samples of this experimental study included male Wistar rats (n = 30, 8 weeks old, 200 ± 50 g). Animals were randomly divided into six groups: control (C), Parkinson's (P), Parkinson's with olive extract (P+OE), Parkinson's with exercise (P+E), Parkinson's with olive extract and exercise (P+OE+E), and olive extract with exercise (OE+E) group (n = 5 per group). The training groups performed aerobic and resistance exercises for eight weeks (5 sessions per week) and were given olive extract by gavage. VO_2 max was assessed at the end of the fourth and eighth weeks of exercise. One day after the intervention, rats were sacrificed, and tissues were removed and examined for real-time PCR. One-way analysis of variance and Tukey's post hoc test were used to analyze the data.

Results: The results showed that a period of exercise training combined with the consumption of olive extract significantly increased the VO_2 max in rats with Parkinson's disease ($P < 0.05$). The expression of the PTEN gene decreased in OE+E, P+OE+E, and P+E groups compared to P+OE and Parkinson's groups ($P<0.05$). AKT gene expression in all intervention groups significantly increased compared to the Parkinson's group ($P<0.05$).

Conclusions: The results suggest that a period of exercise with the consumption of olive extract probably can increase the VO_2 max and AKT and decrease PTEN in rats with Parkinson's disease.

Keywords: Exercise, Olive extract, PTEN, AKT, Parkinson's disease

1. Introduction

Parkinson's disease (PD) is a common and progressive disease of the destruction of the nervous system in adults. This mainly occurs in people over 50 years old and is one of the common causes of disability in older people. In general, this disease is caused by the destruction of dopamine-producing cells in the substantia nigra of the midbrain. Considering that the loss of neurons in this disease causes movement and cognitive disorders, but the onset of movement disorders is observed when about 80% of the dopamine-producing cells in the midbrain are destroyed, and the nerve transmission in the basal ganglia of the brain is disturbed (1). The most important symptoms of Parkinson's disease include slow movements, muscle stiffness, tremors, balance disorders, and muscle weakness (2).

Phosphatase and tensin homolog on chromosome 10 (PTEN) is a dual-specificity phosphatase that acts as a tumor suppressor, with protein phosphatase and lipid phosphatase activity that disrupts PI3K activity (3). AKT is a protein serine/threonine kinase that regulates numerous cellular processes. The PI3K/AKT pathway transduces a signal that controls various events involved in cell survival and multiple functions. PTEN overexpression may be closely related to activation of the proteolytic cascade for apoptosis, which could be associated with reduced activation of the cell survival kinase AKT (4). Accordingly, neuronal cell death may be partially attributable to changes in PTEN expression (5). Regulation of apoptosis has been considered in the pathogenesis of many neurodegenerative disorders. Therefore, it is essential to identify the neural pathways controlling apoptosis. AKT stimulation reduces levels of oxidative stress and cell death, suggesting that AKT activation by PTEN inactivation is vital for maintaining its neuroprotective effect, which may form part of the brain's defense machinery against oxidative damage (6-8). PTEN deficiency has been shown to increase several mitochondrial activities, including activation of the PI3K/AKT signaling pathway, of which PTEN may be a negative regulator (9). Furthermore, a ubiquitously expressed PTEN-induced molecular kinase 1 (PINK1) has been shown to play a physiological role in mitochondrial protection, suppression of mitochondrial oxidative stress, oxidative DNA damage, and autophagy (10, 11). Knockdown of PINK1 increases neuronal apoptosis, whereas overexpression of PINK1 reverses it (12-14). PINK1 plays a vital role in regulating mitochondrial function and dynamics, and mutations in PINK1 are associated with the genetic form of Parkinson's disease (15). The protective role of PINK1 in neurons against oxidative stress has also been suggested to develop new strategies for treating neurodegenerative diseases. NADPH oxidase-mediated production of reactive oxygen species (ROS) has been shown to induce oxidation and inactivation of PTEN, leading to upregulation of signaling (16). Similarly, PTEN is oxidized and inactivated by acidosis-induced ROS (17). Inhibition of PTEN by ROS is required to recruit downstream signaling molecules such as AKT in insulin-mediated signaling (18). Recent animal studies show that training with a very intense contraction intensity equivalent to one training session of intense swimming for 120 minutes leads to a sudden increase in phosphorylation and key molecular activities of AKT (19).

Pharmacologically, oleuropein, a phenolic compound, is the most active part of olive oil. Various studies have shown that oleuropein has antioxidant and anti-inflammatory effects and can prevent lipid oxidation in laboratory conditions (20).

Various studies have shown that sports training causes neurobiological adaptations and has a neuroprotective effect that increases neurogenesis and angiogenesis (21). Exercise increases the healing process of striatum damage and changes dopaminergic neurotransmission in the striatum system (22). Also, sports activity increases the production of ROS. Reactive oxygen species quickly oxidize macromolecules such as lipids, proteins, carbohydrates, and nucleic acids. On the other hand, the body's antioxidant level decreases to deal with this oxidative stress (23).

Based on the presented research, exercise and olive extract can benefit Parkinson's disease, so the present study investigated the effect of aerobic and resistance training with olive extract on VO2 max, PTEN, and AKT in rats with Parkinson's disease.

2. Materials and methods

The samples of this research included 30 male Wistar rats (8 weeks old, 200 ± 50 g), which were obtained from the Pasteur Institute of Iran. This experimental research was conducted with six groups. After transferring the rats to the laboratory environment, animals were housed in communal cages at 22 ± 1$^{\circ C}$ under a 12-h light/dark cycle (lights on at 07:00) with free access to food and water. All the ethical principles of the present research have been approved by the Animal Ethics Committee of Islamic Azad University, Rasht Branch (Ethical code: IR.IAU.RASHT.REC.1400.028).

Stereotaxic surgery

First, the injection of 6-hydroxydopamine (6-OHDA) into the brains of rats was carried out stereotaxically. In this method, mice were first anesthetized by intraperitoneal injection of xylazine and ketamine. Then, according to the coordinates of the dense region of the substantia nigra, 6-hydroxydopamine (6-OHDA) was slowly injected into each side of the substantia nigra using a Hamilton syringe at a concentration of 5 mg/ml by hand pump. Five minutes after the injection, the needle was slowly removed from the brain.

Exercise program and consumption of olive extract

After transferring to the laboratory environment and getting familiar with the new environment and how to work on the treadmill, the rats were randomly assigned to 6 groups of 5: control, Parkinson's, Parkinson's with olive extract (P+OE), Parkinson's with exercise (P+E), Parkinson's with olive extract and exercise (P+OE+E), and olive extract with exercise (OE+E).
In the olive extract groups, the extract was given by gavage. The intake of olive extract 2 to 3 hours before training was 0.4 cc.

Aerobic and resistance exercises were performed for eight weeks and five weekly sessions (aerobic exercise in the morning and resistance exercise in the evening). Familiarity with aerobic exercises was done in the morning on the treadmill for 15 minutes at a speed of 15 meters per minute. Next, in the first, second, third, fourth, and fifth to eighth weeks, respectively, at a speed of 17 meters per minute, 20 minutes at a speed of 20 meters per minute, 25 minutes at a speed of 22 meters per minute, 25 minutes at a speed of 24 meters per minute and 25 minutes at a speed of 24 meters per minute was performed (24).

After the first week of familiarization with the ladder, a resistance training program on the ladder three times, including climbing the ladder four times, with a 30-second rest between each time, was considered. The state of the resistance training load was as follows:

The second, third, fourth, fifth, sixth, seventh and eighth weeks, respectively, were carried out with a load of 30%, 70%, 100%, 110%, 120%, 130% and 140% of the mouse's body weight (25).

Maximal oxygen consumption (VO_2 max) values were evaluated using a treadmill. The zero slope was used to determine the VO_2 max. The speed obtained in the last stage when the rat was unable to run was calculated as the maximum running speed of the rat, which was ten stages; each step was increased by 3%, and the time in each set was 3 minutes. The VO_2 max was determined at the end of the fourth and eighth weeks of training.

Tissue preparation

One day after the end of the intervention, the rats were anesthetized after 12 hours of fasting by intraperitoneal injection of anesthetic xylazine and ketamine after 2 to 8 minutes and then underwent surgery. The tissues were removed and placed in 10% formalin, and the other half was placed in a microtube (nitrogen bank) and stored at -80 degrees for real-time PCR.

PTEN and AKT gene expression by Real-Time PCR method

RNA extraction was performed in all investigated groups according to the manufacturer's protocol (Qiagen, Germany) to conduct molecular investigations at the gene expression level. After extracting RNA with high purity and concentration from all studied samples, cDNA synthesis steps were performed, and then the synthesized cDNA was used to perform the reverse transcription reaction. The quantitative Real Time-PCR method measured the expression levels of PTEN and AKT. The primers were verified by the NCBI BLAST Tool. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as a control gene. The cycle threshold evaluated the expression ratio of the studied genes in this study (CT: Cycle Threshold) comparative method.

Statistical analysis

A one-way analysis of variance (ANOVA) was conducted to compare the mean differences among groups on all variables. Tukey’s multiple comparison test was used to distinguish the difference in the means between the groups. All values are expressed as mean ± standard deviation (SD). A level of $P < 0.05$ was set for the statistical significance of all tests. Statistical analysis was done using Prism version 6 software.

3. Results

Results of VO2 max changes

The statistical analysis results showed that VO_2 max in the OE+E group and P+OE+E group, as well as the P+E group, showed a significant increase compared with the P+OE and the Parkinson's group ($P < 0.05$). The increase in VO_2 max in the OE+E group was also significant compared to the P+E group ($P < 0.05$). Also, all the intervention groups showed a significant difference compared to the control group ($P < 0.05$) (Figure 1).

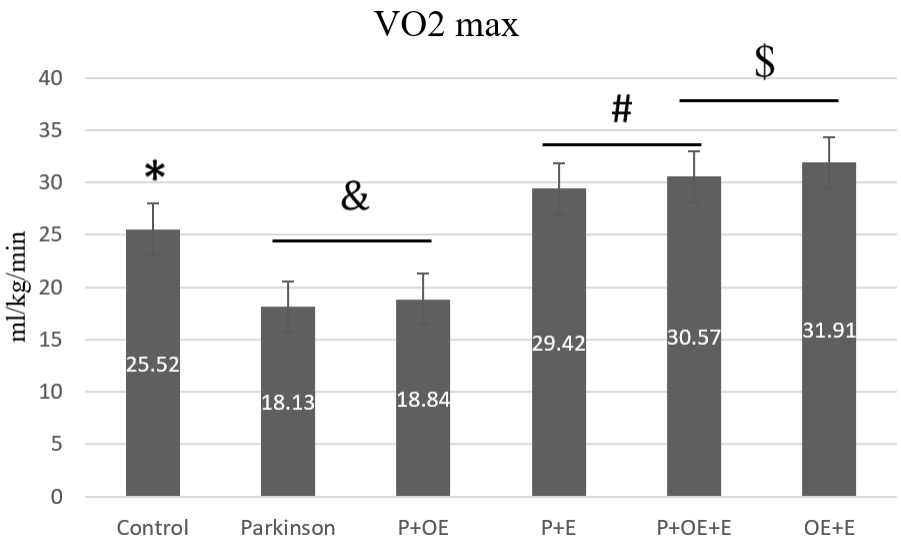

Figure 1. The VO2 max changes of different studied groups. The values are displayed as the means and SD of the means (means ± SD). Other signs represent statistically significant differences between the mean values ($P < 0.05$), but the same signs do not.

P+OE: Parkinson's plus olive extract, P+E: Parkinson's plus exercise, P+OE+E: Parkinson's plus olive extract plus exercise, OE+E: olive extract plus exercise.

Gene expression of PTEN and AKT

The results of statistical analysis showed that the expression of the PTEN gene in the OE+E group and P+OE+E group, as well as the P+E group, showed a significant decrease compared with the P+OE and Parkinson groups ($P < 0.05$) (Figure 2).

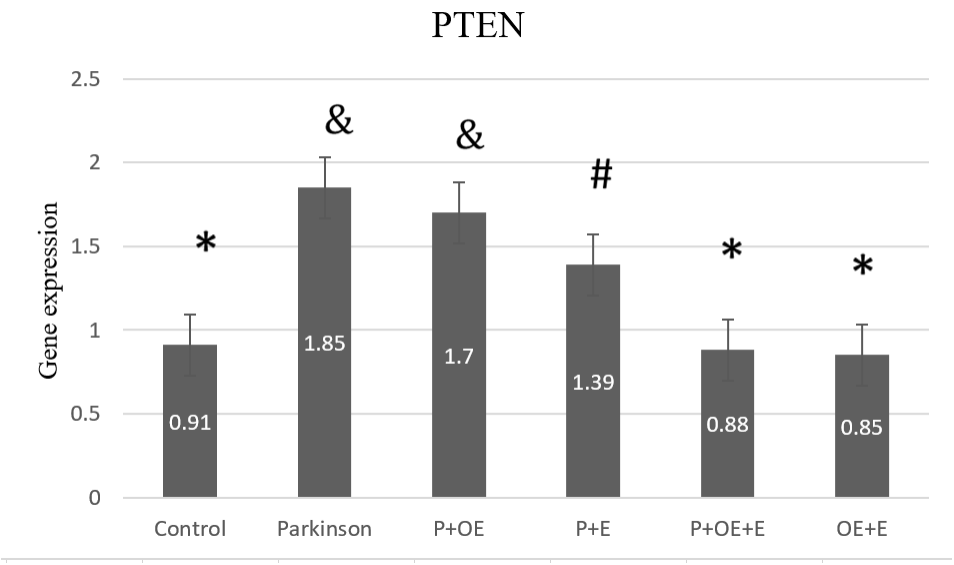

Figure 2. The mRNA expression of PTEN in different studied groups. The values are displayed as the means and SD of the means (means ± SD). Other signs represent statistically significant differences between the mean values (P < 0.05), but the same signs do not.
P+OE: Parkinson's plus olive extract, P+E: Parkinson's plus exercise, P+OE+E: Parkinson's plus olive extract plus exercise, OE+E: olive extract plus exercise.

Also, the gene expression changes in AKT from different research groups are shown in Figure 3. The results showed a significant increase in AKT in the OE+E, P+OE+E, P+E, and P+OE groups, compared with the Parkinson's group (P < 0.5).

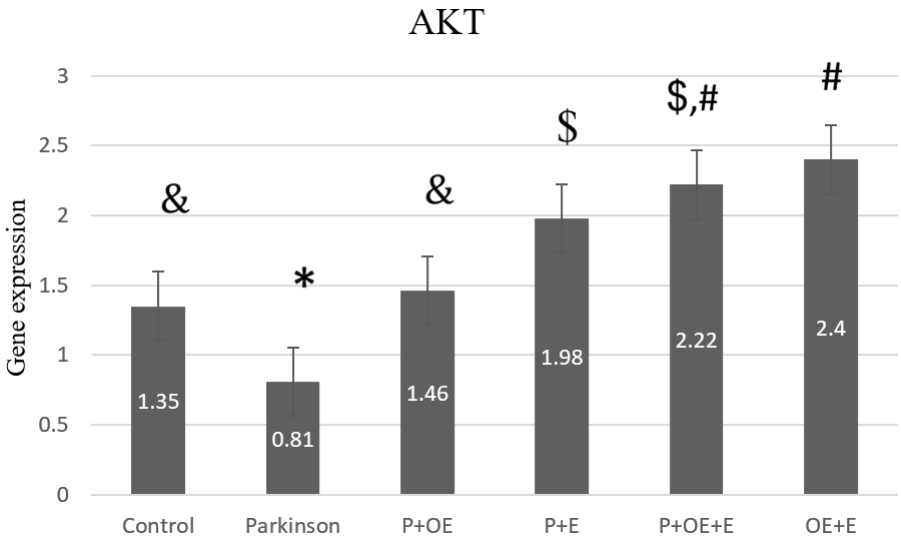

Figure 3. The mRNA expression of AKT in different studied groups. The values are displayed as the means and SD of the means (means ± SD). Other signs represent statistically significant differences between the mean values (P < 0.05), but the same signs do not.

P+OE: Parkinson's plus olive extract, P+E: Parkinson's plus exercise, P+OE+E: Parkinson's plus olive extract plus exercise, OE+E: olive extract plus exercise.

4. Discussion

The results of the present study showed that a period of exercise with the consumption of olive extract increases VO2 max and AKT and decreases PTEN in rats with Parkinson's disease.

In line with the present research conducted by De Souza et al., he investigated the effect of three types of endurance, strength, and endurance-strength training protocols on the expression of the AKT gene in the skeletal muscles of rats. The research findings showed that in the combined training group, an increase of 87% in AKT protein was observed (26).

Regular exercise is a well-known method of preventing age-related neurodegenerative diseases. There is evidence that regular exercise training has beneficial effects, including neuroprotection and improvement of spatial memory, on the brain in laboratory animals (27).

Regular exercise reduces the level of oxidative stress, and this can also reduce the expression level of PTEN (28). Therefore, exercise can induce a chronic response and reduce oxidative stress (29). A physiological explanation for this phenomenon is related to the production of neuroprotective factors that optimize antioxidant mechanisms, thus slowing the progression of Parkinson's (30). Previous studies have reported the reduction of parameters related to oxidative stress in human (31) and animal (32) models under aerobic and strength training, respectively. In a study conducted with Wistar rats suffering from induced Parkinson's, aerobic exercise (8 weeks) increased the level of antioxidant enzymes (superoxide dismutase and catalase) and reduced oxidative damage to lipids and proteins (32). Based on these results, it seems that chronic exercise can create beneficial adaptations regarding the production of free radicals, increase antioxidant protection in patients with Parkinson's disease, and protect them against the harmful effects of ROS. Research suggests exercise can reduce the risk of neuronal damage in Parkinson's (33).

Research has reported that olive leaf extract is rich in polyphenol compounds (34, 35) and has a vigorous neuron protection activity that improves animal oxidative stress and nerve damage (36). The flavonoids of this plant can protect nerve cells through various mechanisms, such as stimulating the regeneration of damaged neurons and increasing the function of the remaining neurons. On the other hand, flavonoids may affect protein modulation and lipid kinase signaling pathways by inhibiting the MAP kinase signaling cascade (37). In this regard, Hosseini et al. showed that swimming exercise with the consumption of olive extract has interactive effects in increasing the activity of glutathione peroxidase and reducing malondialdehyde in rats (38).

In a different study from the present study, Thomas et al. reported that no significant difference was observed after six weeks of intense interval training in the total antioxidant capacity and malondialdehyde of liver and heart tissue (39). Acikgoz et al. reported that acute activity does not significantly affect brain malondialdehyde (40). Research studies show that exercise changes the antioxidant activity of tissues according to the type of exercise protocol, exercise volume, and the rest periods between exercise programs (41, 42).

5. Conclusion

In general, the results of the present study showed that exercise with the consumption of olive extract can change the factors involved in Parkinson's disease. Therefore, it is possible that the interactive effects of training and the consumption of olive extract can effectively improve VO2 max, PTEN, and AKT levels in Parkinson's disease.

6. Acknowledgments

The authors thank the valuable professors and laboratory members who helped us implement the project.

References

1. Azizi S, Kargarfard M, Azizi R. Effects of 8 weeks of water-based exercise on the lower limb muscles strength in Parkinson's. Koomesh journal. 2014;16(1):60-6. eng.
2. Durmus B, Baysal O, Altinayar S, Altay Z, Ersoy Y, Ozcan C. Lower extremity isokinetic muscle strength in patients with Parkinson's disease. Journal of Clinical Neuroscience. 2010;17(7):893-6.
3. Haas-Kogan D, Stokoe D. PTEN in brain tumors. Expert review of neurotherapeutics. 2008;8(4):599-610.
4. Wen S, Stolarov J, Myers MP, Su JD, Wigler MH, Tonks NK, et al. PTEN controls tumor-induced angiogenesis. Proceedings of the National Academy of Sciences. 2001;98(8):4622-7.
5. Gary DS, Mattson MP. PTEN regulates Akt kinase activity in hippocampal neurons and increases their sensitivity to glutamate and apoptosis. Neuromolecular medicine. 2002;2(3):261-9.
6. Salinas M, Martín D, Alvarez A, Cuadrado A. Akt1/PKBα protects PC12 cells against the parkinsonism-inducing neurotoxin 1-methyl-4-phenylpyridinium and reduces the levels of oxygen-free radicals. Molecular and Cellular Neuroscience. 2001;17(1):67-77.
7. Fan G-C, Zhou X, Wang X, Song G, Qian J, Nicolaou P, et al. Heat shock protein 20 interacting with phosphorylated Akt reduces doxorubicin-triggered oxidative stress and cardiotoxicity. Circulation research. 2008;103(11):1270-9.
8. Dávila D, Fernández S, Torres-Alemán I. Astrocyte resilience to oxidative stress induced by insulin-like growth factor I (IGF-I) involves preserved AKT (protein kinase B) activity. Journal of Biological Chemistry. 2016;291(5):2510-23.
9. Napoli E, Ross-Inta C, Wong S, Hung C, Fujisawa Y, Sakaguchi D, et al. Mitochondrial dysfunction in Pten haplo-insufficient mice with social deficits and repetitive behavior: interplay between Pten and p53. 2012.
10. Devireddy S, Liu A, Lampe T, Hollenbeck PJ. The organization of mitochondrial quality control and life cycle in the nervous system vivo in the absence of PINK1. Journal of Neuroscience. 2015;35(25):9391-401.

11. Li L, Hu G-k. Pink1 protects cortical neurons from thapsigargin-induced oxidative stress and neuronal apoptosis. Bioscience Reports. 2015;35(1).
12. Oliveras-Salvá M, Macchi F, Coessens V, Deleersnijder A, Gérard M, Van der Perren A, et al. Alpha-synuclein-induced neurodegeneration is exacerbated in PINK1 knockout mice. Neurobiology of aging. 2014;35(11):2625-36.
13. Burbulla LF, Fitzgerald J, Stegen K, Westermeier J, Thost A, Kato H, et al. Mitochondrial proteolytic stress induced by loss of mortal in function is rescued by Parkin and PINK1. Cell death & disease. 2014;5(4):e1180-e.
14. Dai H, Deng Y, Zhang J, Han H, Zhao M, Li Y, et al. PINK1/Parkin-mediated mitophagy alleviates chlorpyrifos-induced apoptosis in SH-SY5Y cells. Toxicology. 2015;334:72-80.
15. Büeler H. Impaired mitochondrial dynamics and function in the pathogenesis of Parkinson's disease. Experimental neurology. 2009;218(2):235-46.
16. Kwak H-J, Liu P, Bajrami B, Xu Y, Park S-Y, Nombela-Arrieta C, et al. Myeloid cell-derived reactive oxygen species externally regulate the proliferation of myeloid progenitors in emergency granulopoiesis. Immunity. 2015;42(1):159-71.
17. Gupta SC, Singh R, Pochampally R, Watabe K, Mo Y-Y. Acidosis promotes the invasiveness of breast cancer cells through ROS-AKT-NF-κB pathway. Oncotarget. 2014;5(23):12070.
18. Seo JH, Ahn Y, Lee S-R, Yeo CY, Hur KC. The major target of the endogenously generated reactive oxygen species in response to insulin stimulation is phosphatase and tensin homolog and not phosphoinositide-3 kinase (PI-3 kinase) in the PI-3 kinase/Akt pathway. Molecular biology of the cell. 2005;16(1):348-57.
19. Castorena CM, Arias EB, Sharma N, Cartee GD. Postexercise improvement in insulin-stimulated glucose uptake occurs concomitant with greater AS160 phosphorylation in muscle from normal and insulin-resistant rats. Diabetes. 2014;63(7):2297-308.
20. Elmarakby AA, Sullivan JC. Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. Cardiovascular therapeutics. 2012;30(1):49-59.
21. Yasuhara T, Hara K, Maki M, Matsukawa N, Fujino H, Date I, et al. Lack of exercise, via hindlimb suspension, impedes endogenous neurogenesis. Neuroscience. 2007;149(1):182-91.
22. O'dell S, Gross N, Fricks A, Casiano B, Nguyen T, Marshall J. Running wheel exercise enhances recovery from nigrostriatal dopamine injury without inducing neuroprotection. Neuroscience. 2007;144(3):1141-51.
23. Battistelli M, Malatesta M, Meschini S. Oxidative stress to promote cell death or survival. Hindawi; 2016.
24. Alaei H, Moloudi R, Sarkaki AR. Effects of treadmill running on mid-term memory and swim speed in the rat with Morris water maze test. Journal of Bodywork and Movement Therapies. 2008;12(1):72-5.
25. Souza MVC, Leite RD, de Souza Lino AD, de Cássia Marqueti R, Bernardes CF, de Araújo HSS, et al. Resistance training improves body composition and increases matrix metalloproteinase

2 activity in biceps and gastrocnemius muscles of diet-induced obese rats. Clinics. 2014;69:265-70.
26. De Souza E, Tricoli V, Bueno Junior C, Pereira M, Brum P, Oliveira E, et al. The acute effects of strength, endurance and concurrent exercises on the Akt/mTOR/p70 S6K1 and AMPK signaling pathway responses in rat skeletal muscle. Brazilian Journal of Medical and Biological Research. 2013;46:343-7.
27. Mattson MP. Neuroprotective signaling and the aging brain: take away my food and let me run. Brain research. 2000;886(1-2):47-53.
28. Dashtiyan AA, Afzalpour ME, Tanideh N, Sepehrimanesh M. The comparison of the effect of vitamin E on the expression of p53/PTEN of prostate gland of male rats in two groups of intensive continuous and intermittent exercise training. Journal of Advanced Biomedical Sciences. 2017;7(3):406-15. Fa.
29. Radak Z, Chung HY, Goto S. Exercise and hormesis: oxidative stress-related adaptation for successful aging. Biogerontology. 2005;6(1):71-5.
30. Friedrich M. Parkinson disease studies yield insights. Jama. 2005;293(4):409-10.
31. Bloomer RJ, Schilling BK, Karlage RE, Ledoux MS, Pfeiffer RF, Callegari J. Effect of resistance training on blood oxidative stress in Parkinson disease. Medicine and science in sports and exercise. 2008;40(8):1385-9.
32. Tuon T, Valvassori S, Lopes-Borges J, Luciano T, Trom C, Silva L, et al. Physical training exerts neuroprotective effects in the regulation of neurochemical factors in an animal model of Parkinson's disease. Neuroscience. 2012;227:305-12.
33. Fallah-Mohammadi Z, Aghasi M, Ahmadi-Kordasiai M. Neuroprotective effects of exercise with hydroalcoholic extraction of Eriobotrya japonica on MANF in the Brainstem of parkinson's rats. Journal of Shahrekord University of Medical Sciences. 2014;16(3).
34. Aytul KK. Antimicrobial and antioxidant activities of olive leafextract and its food applications: Izmir Institute of Technology (Turkey); 2010.
35. 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.
36. Dekanski D, Selaković V, Piperski V, Radulović Ž, Korenić A, Radenović 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.
37. 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.
38. Hosseini F, Hosseini SA, Ahmadi M. The effect of a period of swimming training and chamomile extract on antioxidant status in adult male rats. Qom University of Medical Sciences Journal. 2018;12(6).
39. Songstad NT, Kaspersen K-HF, Hafstad AD, Basnet P, Ytrehus K, Acharya G. Effects of high intensity interval training on pregnant rats, and the placenta, heart and liver of their fetuses. PloS one. 2015;10(11):e0143095.

40. Acikgoz O, Aksu I, Topcu A, Kayatekin BM. Acute exhaustive exercise does not alter lipid peroxidation levels and antioxidant enzyme activities in rat hippocampus, prefrontal cortex and striatum. Neuroscience letters. 2006;406(1-2):148-51.
41. Escribano B, Tunez I, Requena F, Rubio M, De Miguel R, Montilla P, et al. Effects of an aerobic training program on oxidative stress biomarkers in bulls. Veterinarni Medicina. 2010;55(9):422-8.
42. Aksoy Y, Yapanoğlu T, Aksoy H, Demircan B, Öztaşan N, Canakci E, et al. Effects of endurance training on antioxidant defense mechanisms and lipid peroxidation in testis of rats. Archives of andrology. 2006;52(4):319-23.