Effect of oleuropein and swimming practice on motor disorder induced by 6-hydroxydopamine toxin in mature male rats

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Received; 15/01/2020 Revised; 27/02/2020 Accepted; 19/03/2020

Abstract

Introduction: Parkinson's disease is caused by damage to the nervous system. Oleuropein and exercise have protective effects on the nervous system. The purpose of the present study was to examine the effect of oleuropein and swimming practice on motor disorder induced by 6-hydroxydopamine toxin in mature male rats.
Materials and methods: In this experimental study, 40 rats were divided into control, Parkinson's, swimming practice (five sessions per week and 30 minutes per session), oleuropein (20mg/kg), and combined swimming practice and oleuropein groups. Parkinson's disease was induced by injection of 8µg 6-hydroxydopamine neurotoxin (6-OHDA) in the medial forebrain bundle (MFB) of the left brain of the rats. At the end of the administration period, catalepsy, step length, muscle stiffness, and motor coordination were measured using the rotarod test to assess motor disorders.
Results: Four weeks of oleuropein administration, four weeks of swimming practice, and four weeks of combined swimming practice and oleuropein use significantly improved the motor disorders induced by 6-OHDA administration. Moreover, four weeks of swimming practice and oleuropein showed a significant increase in motor balance test and a significant decrease in the rotarod test respectively compared to the administration of oleuropein ($P<0.05$). However, there was no significant difference in the swimming practice group.
Conclusion: The effects of swimming exercise, which is a high activity physical activity, were more tangible than those of oleuropein and did not show a significant difference with co-administration of oleuropein and swimming exercise, so it can be considered as an effective treatment for preventing the multiple complications of Parkinson's disease.

Keywords: Parkinson's disease, Oleuropein, Swimming practice, Motor disorder, 6-Hdroxydopamine

Introduction

Neuronal analysis of Parkinson's disease causes resting vibrations, bradykinetic movement, muscle stiffness, and postural imbalance. This disease occurs due to the destruction of the secreting cells of a substance called dopamine (catecholamine

neurotransmitter) (1-2). The dopaminergic system of Substantia nigra plays an important role in the practical organization of basal ganglia. The functional defect of this system causes major motor problems in Parkinson's disease (3). Most of this system originates from the dopaminergic neurons of the dense segment of Substantia nigra and is widely distributed in the neo-striatum (caudate and putamen nucleus) (4). Factors such as exercise and natural substances such as antioxidants play an important role in the improvement of this disease. In recent years, it has been shown that there is a strong relationship between diseases and lack of physical movement (5). Oleuropein is one of various polyphenols that is abundant in the olive leaves. This compound prevents cardiovascular diseases by inhibiting the oxidation of membrane lipids and is effective in improving arteriosclerosis by dilating arteries. It has anti-erythemic, anti-inflammatory, and cardiovascular properties improves lipid metabolism, and prevents enzymatic degradation (6). Research has shown that the use of olive leaf extract can protect dopamine neurons and reduce learning disruption in an animal model of Parkinson's disease (7). Exercise reduces the physical and cognitive defects in patients suffering from central nervous system disorders, including stroke and spinal cord injuries (8). Exercise can improve the physical activity, quality of life, strength, balance, and stride length of patients with Parkinson's disease (9). On the other hand, there are reports on the therapeutic effects of exercise on many diseases and problems such as depression, high blood pressure, Alzheimer's disease, and addiction. Exercise can alter the release levels of neurotransmitters such as glutamate, dopamine, acetylcholine, and serotonin in the brain (10). Evidence suggests that exercise activates the dopaminergic system of the brain, increasing dopamine in the corpus striatum. These findings increase the likelihood that exercise can reduce the dopaminergic neuronal vulnerability to 6-hydroxydopamine (11). Exercise activities increase the survival of nerve cells and facilitate the brain performance after damage. Some have reported that extreme sports with high fatigue result in the production of free radicals, while short exercise periods below the maximum and 70% maximum oxygen consumption may reduce lipid peroxidation (12). Therefore, the present study was aimed to evaluate the effect of oleuropein and swimming practice on motor disorders in Parkinson's disease in mature male rats.

Materials and methods

In this experimental study, 40 Wistar male rats (200-250g weight) were prepared from Ahvaz Jundishapur University of Medical Sciences. The rats were kept in individual cages under standard conditions of 12 hours of light, 12 hours of darkness, and 21 ± 2 ºC temperature, with free access to enough water and food. They were randomly divided into five groups (each group with 8 rats) as follows: 1- control group (Control); 2- Parkinson's group (PD); 3- Parkinson's group treated with oleuropein consumption (PD+OLE); 4- Parkinson's group treated with swimming practice (PD+EXE); and 5- Parkinson's group treated with swimming practice and oleuropein consumption (PD+OLE+EXE).

The swimming practice and swimming practice plus oleuropein consumption groups swam for 4 weeks (five times per week and 30 minutes per session) in a swimming bathtub (special for obligatory swimming in rats) (13). The oleuropein consumption and swimming practice plus oleuropein consumption groups received oleuropein orally for 4 weeks (20 mg/kg).

Animal model of PD

The animals were anaesthetized by intraperitoneal injection of 90 mg/kg hydrochloride ketamine + 10 mg/kg xylazine. Then, the rats' heads were fixed by stereotaxy for the brain surgery. Afterward, 2 μI of 6-hydroxydopamine (6-OHDA) neurotoxic drug, purchased from the American Sigma Company, was injected into the medial forebrain bundle **(**MFB) on the left side of the brain of the animals (14). Apomorphine (Sigma Co., USA) was dissolved in the normal saline containing 0.01% ascorbic acid. This drug was injected subcutaneously at 0.05 mg/kg body weight (apomorphine was used to confirm the parkinsonization of animals). Next, 5-10 minutes after apomorphine injection, the rotations of the animal on the damaged side were counted and recorded for 15 minutes (15). In the end, two hands of the animal were placed on a bar at a height of 9 cm, while the legs were placed on the bottom of the wooden box. The time it took for the animal to take its hands was noted (16).

Apomorphine-induced circling Behavior

The rotational behavior of the rats was tested by injecting 2.5 mg/kg hydrochloride apomorphine. Full rotations were measured in a cylindrical case for 60 min in 10-min intervals.

Rotarod test (motor balance test)

The rotarod test is done to measure the motor performance and coordination. Animals were placed on the rotarod device bar whose movement speed is variable (rotation rate of the rod was 5 rpm). Then, the rotation rate was increased gradually up to 40 rpm during 5 minutes. Animals were already familiar with this test. The training included 3 sessions in 3 consecutive days (one session per day) and each session included 2 separate tests. The interval between the two tests per day was 45-60 minutes and the time tolerated on the rotary bar of the rotarod device (with increasing speeds) was recorded in seconds and compared between different groups (15, 17).

Stride length test

This device consists of a dark wooden box with a sliding door with 20×17×10 cm dimensions and a narrow tunnel with 45×10×4.5 cm dimensions. The ends of the tunnel were open and the boundary between the square section and the tunnel is also separated by a guillotine blade. A square plastic box with ink floor was placed at the open end of the tunnel and the floor of the tunnel was covered with a white strip paper with ¾ cm width. Next, the fingers of the rat's motor organs were placed in an ink box with its tail and hand upward. The animal was then guided to the tunnel and as soon as it entered the dark box, the guillotine blade was released and the animal was jammed inside the tunnel floor to prevent it from returning and walking on the paper inside the dark box. Then, the paper tape was removed from the tunnel floor to dry the fingerprints of the rats; thus, the length of the steps was recorded on the paper. It should be noted that the animal became familiar with the box before the test (18).

Testing the 3- and 9-cm platforms

In the next step, the right hand of the animal was placed on a platform with a height of 3 cm, and if the rat did not withdraw its hand from the platform for at least 10 seconds, it was scored 0.5. Then the animal's left hand was placed on the platform at a height 3 cm. If the rat did not withdraw its hand from the platform for at least 10 seconds, it got score 0.5 again. The animal's right hand was placed on a platform with a height of 9 cm so that the rest of the body did not contact the platform. If the rat did not withdraw its

hand from the platform for at least 10 seconds, it got score 1. Next, the animal's left hand was placed on the platform with a height 9 cm so that the rest of the body did not contact the platform. If the rat did not withdraw its hand from the platform for at least 10 seconds, it got score 1 again (18). In the next step, the animal was placed on a flat surface on a table or on the mosaic of the laboratory floor. If it started to walk, it got score zero and if it did not move or start to move with a touch of a hand, it got score 0.5. The results of this test along with those of the 3- and 9-cm platforms were considered for evaluating and scoring muscle stiffness (18).

Results

The level of motor coordination in the Parkinson's group showed a significant decrease compared to the control group ($P<0.001$), and treatment with 20 mg/kg body weight oleuropein could significantly increase this motor coordination compared to the Parkinson's group ($P<0.05$). As shown in Figure 1, the level of motor coordination in the Parkinson's group treated with swimming practice showed a significant increase compared to the Parkinson's group ($P<0.001$). Further, the level of motor coordination in the Parkinson's group treated with swimming practice and oleuropein showed a significant increase compared to the Parkinson's group ($P<0.001$) and the Parkinson's group treated with oleuropein ($P<0.05$).

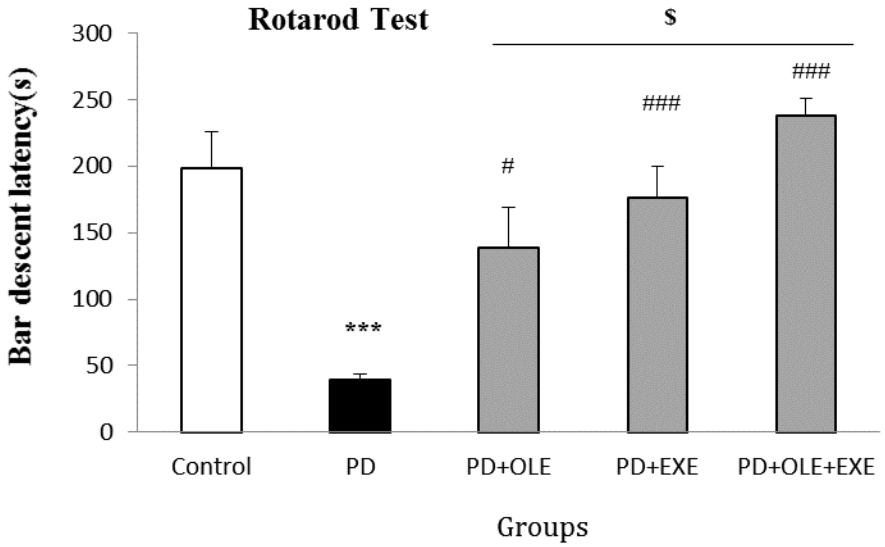

Figure 1. Effect of four weeks of systemic administration of oleuropein and swimming practice on motor coordination (rotarod) in an animal model of Parkinson's disease. Results were presented as mean ± SEM. One-way ANOVA and Tukey's test (in each group, n=8) were used.
*** Significant difference with healthy control group (Control) ($P<0.001$).
#, ### Significant difference with Parkinson's group (PD) ($P<0.05$ and $P<0.001$, respectively).
$ Significant difference between Parkinson's group treated with oleuropein (PD+OLE) and Parkinson's group treated with swimming practice and oleuropein (PD+OLE+EXE) ($P<0.05$).

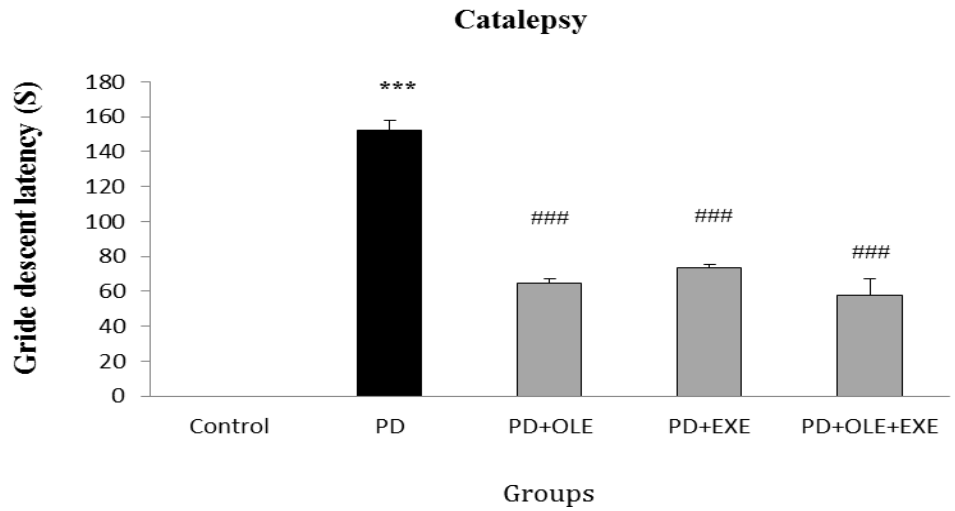

Figure 2. Effect of four weeks of oleuropein administration and exercise on catalepsy in an animal model of Parkinson's disease.
*** Significant difference with healthy control group (Control) (P<0.001).
Significant difference with Parkinson's group (PD) (P<0.001).

In this test, non-movement disorder was significantly increased in the Parkinson's group than in the control group (P<0.001), and treatment with 20 mg/kg body weight oleuropein could significantly increase this motor disorder compared to the Parkinson's group (P<0.05). As shown in Figure 1, the level of motor disorder in the Parkinson's group treated with swimming practice showed a significant decrease compared to the Parkinson's group (P<0.001). Moreover, the swimming practice with oleuropein consumption showed a significant decrease in the immobility created in Parkinson's group compared to the Parkinson's group (P<0.001).

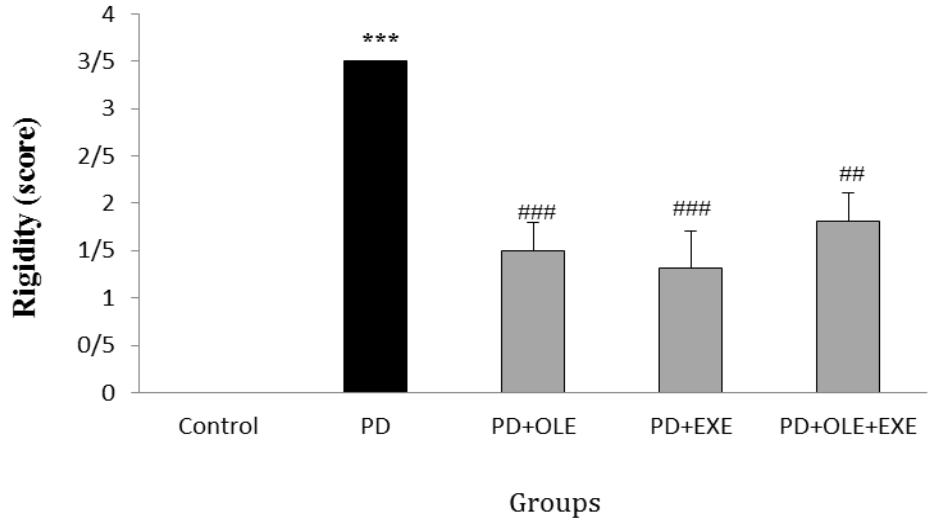

Figure 3. Effect of four weeks of oleuropein administration and swimming practice on muscle stiffness (rigidity) in Parkinson's disease.
*** Significant difference with healthy control group (Control) (P<0.001).
##, ### Significant difference with Parkinson's group (PD) (P<0.01 and P<0.001, respectively).

The level of muscle stiffness was significantly increased in the Parkinson's group than in the healthy control group (P<0.001). Treatment with 20 mg/kg body weight oleuropein could significantly decrease muscle stiffness compared to the Parkinson's group (P<0.05). As shown in Figure 3, the level of muscle stiffness in the Parkinson's group treated with swimming practice showed a significant decrease compared to the Parkinson's group (P<0.001). Furthermore, the level of muscle stiffness in the Parkinson's group treated with swimming practice and oleuropein showed a significant decrease compared to the Parkinson's group (P<0.01).

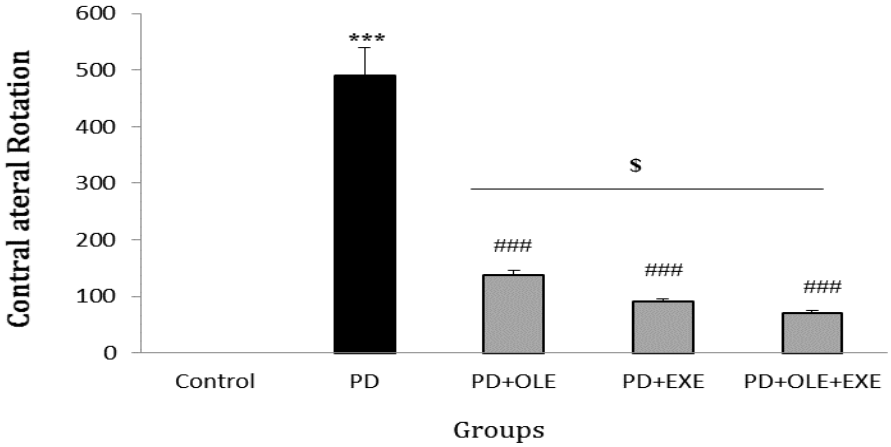

Figure 4. Effect of four weeks of oleuropein administration and swimming practice on rotation in an animal model of Parkinson's disease.
*** Significant difference with healthy control group (Control) (P<0.001).
Significant difference with Parkinson's group, (PD) (P<0.001).
$ Significant difference between Parkinson's group treated with oleuropein (PD+OLE) and Parkinson's group treated with swimming practice and oleuropein (PD+OLE+EXE) (P<0.05).

Rotation rates were significantly increased than in the Parkinson's group in the control group (P<0.001) and treatment with 20 mg /kg body weight oleuropein could significantly decrease this rotation compared to the Parkinson's group (P<0.05). As shown in Figure 4, rotation rate in the Parkinson's group treated with swimming practice showed a significant decrease compared to the Parkinson's group (P<0.001). Further, rotation rate in the Parkinson's group treated with swimming practice and oleuropein showed a significant decrease compared to the Parkinson's group (P<0.01) and the Parkinson's group treated with oleuropein (P<0.05).

The stride length was significantly decreased in the Parkinson's group than in the healthy control group (P<0.001) and treatment with 20 mg/kg body weight oleuropein in the Parkinson's group could significantly decrease the stride length compared to the Parkinson's group (P<0.05). As shown in Figure 5, the stride length in the Parkinson's group treated with swimming practice showed a significant increase compared to the Parkinson's group (P<0.001). Moreover, stride length showed a significant increase in the Parkinson's group treated with swimming practice and oleuropein compared to the Parkinson's group (P<0.01), the Parkinson's group treated with oleuropein alone (P<0.001), and the Parkinson's group treated with swimming practice alone (P<0.001).

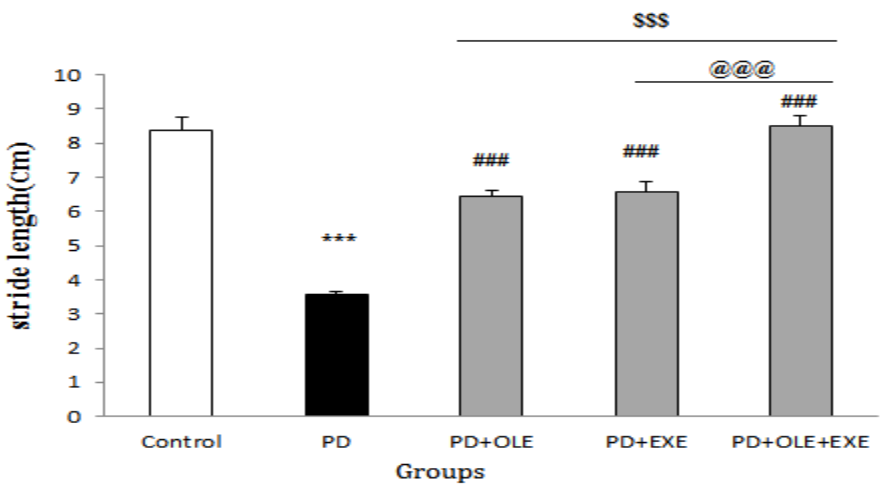

Figure 5. Effect of four weeks of oleuropein administration and swimming practice on stride length in an animal model of Parkinson's disease.
*** Significant difference with healthy control group (P<0.001).
Significant difference with Parkinson's group (PD) (P<0.001).
$$$ Significant difference between Parkinson's group treated with oleuropein (PD+OLE) and Parkinson's group treated with swimming practice and oleuropein (PD+OLE+EXE) (P<0.001).
@@@ Significant difference between Parkinson's group treated with swimming practice(PD+EXE) and Parkinson's group treated with swimming practice and oleuropein(PD+OLE+EXE) (P<0.001).

Discussion

The results of the present study showed that swimming practice significantly increased the rats' motor coordination and stride length, but reduced their motor disorder, muscle stiffness, and rotation rate. It has been reported that exercise and physical activity, by exerting positive effects on dopamine levels, improve the performance of muscular nervous system and anatomical adaptations, break down the negative cycle of disease, aging, and immobility, and improve the performance of patients with Parkinson's disease (19). On the other hand, physical exercise increases the activity of endogenous antioxidant system in the brain and regulates the reduction of glutamate receptors that contribute to stimulatory toxicity. Performing exercise in human subjects leads to the survival of dopaminergic neurons in the black body, thereby increasing the synthesis of dopamine. Also, exercising on a circulating bar reduces the symptoms and signs of Parkinson's disease (20). Dopamine increases the activity of the direct path and motor activity and decreases the activity of the indirect path in the corpus striatum (21). Animal studies have also shown that daily exercise leads to the release of various neurotransmitters in the brain like dopamine (22). In this regard, researchers have argued that exercise increasingly elevates the survival rate, resistance to brain damage, and hippocampal nerve growth (23). Similar with these results, Viosoicovik et al. (2010) investigated the effect of a high-intensity exercise on treadmill on rats with the Parkinson's disease induced by methyl-4-phenyl-1236-tetrahydropyridine (MPTP). Changes in the production of dopamine receptor in the basal ganglia were examined. The results showed that high-intensity treadmill exercise increased the production of dopamine receptor in the corpus striatum. Dopamine receptor specifically causes the transport of dopaminergic neurotransmitters and supports motor performance in the corpus striatum (24). Exercise in water can also be used as a useful and effective

therapeutic approach to improve the muscle strength of the lower part of the body in patients with Parkinson's disease (25). Accordingly, exercise programs may be able to delay or reverse functional disorders in patients with Parkinson's disease. Exercise can improve the physical performance, quality of life, muscle strength, and balance, modify the walking speed , and reduce depression in patients with Parkinson's disease (26). For this reason, exercise in the early stages of the disease can slow down the progression of the disease and delay the onset of symptoms (27). Researchers have argued that physical activity has beneficial effects on the brain health, including energy metabolism, synaptic variability, and increase of protein related to the cognitive function and mitochondrial performance. Exercise can also have a protective effect against several neurological diseases such as Parkinson's and Alzheimer's diseases (28). In any case, it can be concluded that exercise programs improve the balance by affecting other physical factors as well as changing the mechanisms involved in the balance. Performing exercise therapy movement along with common medical treatments can have a positive effect on the motor performance and quality of life in patients with Parkinson's disease, which is useful for these patients (29). There are several reports that oxidative stress plays a role in the pathogenesis of Parkinson's disease by producing free radicals and weakening the antioxidant system of the brain (30). Oxidative stress causes apoptosis and elimination of dopamine cells (30). The findings indicate that the consumption of plant extracts that contain antioxidant compounds can improve the motor and cognitive symptoms induced by the Parkinson's disease (31). Research findings show that oleuropein can alter the balance between the depletion capacity of antioxidant defense system and lipid peroxidation. These results indicate that, before treatment with oleuropein, the hippocampal oxidative biomarkers are reduced the in an animal model of Parkinson's disease (32).

Based on the antioxidant properties of oleuropein, the results of this study showed that the gavage of this material improved motor activities and muscle strength in animals with Parkinson's disease. Studies have shown that olive leaf extract plays an important role in decreasing the death rate of dopaminergic neurons through treatment with 6-OHDA. The olive leaf extract and oleuropein, by reducing the production of reactive oxygen species, reduce caspase 3 activities, the initial Bax polyclonal and initial Bcl-2 monoclonal antibodies, and DNA fragmentation. Moreover, It possibly reduces the damage caused by 6-hydroxydopamine via calcium channel blocking and anti-inflammatory effects. As a rule, it can be used as a useful substance to minimize the neuronal damage caused by toxins that lead to Parkinson's disease (35). Therefore, the results of this study show that the long-term consumption of oleuropein significantly protects the neurons against 6-OHDA-induced damage, likely due to its antioxidant properties, and, as a result, decreases the induction behavior of this toxin. The results of this study also indicated that swimming exercise along with oleuropein consumption for four weeks increased motor coordination and stride length and reduced motor disorders, muscle stiffness, and rotation rate compared to the Parkinson's group. On the other hand, based on the results of one-way analysis of variance, the motor coordination level and stride length increased significantly in the Parkinson's group treated with swimming practice and oleuropein compared to the Parkinson's group treated with oleuropein and Parkinson's group treated with

swimming practice, but the rotation rate decreased significantly. In line with this study, pre-treatment by optional exercise and extract consumption increased the protection of dopaminergic neurons against the damage caused by 6-hydroxydopamine and had a protective role against Parkinson's disease (36). Black body is the main location of dopamine secretion, which plays an effective role in controlling voluntary movements and coordination in skeletal muscle position. Since in this study 6-OHDA destroyed this area of the brain, it reduced the concentration of dopamine and created Parkinson's disease. Oleuropein may improve the disease by compensating for the concentration of dopamine in other areas of the central nervous system. Exercise also plays an important role in improving Parkinson's disease by increasing dopamine levels in the brain.

Conclusion

In general, the findings of the present study showed that swimming practice and oleuropein used concurrently and individually could significantly improve the motor disorder in rats with Parkinson's disease. The results also showed the beneficial effects of swimming on the improvement of motor activity in rats.

Acknowledgments

This article is part of a master's thesis and has been recorded in accordance with the Principles of Research Ethics Working with Animal license No. 10621423962023, Ahvaz branch, Islamic Azad University. In doing so, the authors sincerely appreciate and thank all those who have collaborated in this research.

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