

Comparison of the Effect of Hydroalcoholic Extract of Olive (*Olea Europaea*) Leaf and Levodopa on Reducing Parkinson's Symptoms in the Animal Model

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ARTICLE INFO	ABSTRACT
<p><i>Article type:</i> Research Paper</p> <hr/> <p><i>Article History:</i> Received: 29 Dec 2019 Accepted: 31 Aug 2020 Published: 12 Sep 2020</p> <hr/> <p><i>Keywords:</i> Parkinson, Olive leaf Levodopa Alternative drug Rearing test Bar test Mice</p>	<p>Introduction: Today, the use of medicinal herbs to prevent and slow down the progression of Parkinson's disease has attracted the attention of researchers. This research seeks to answer the question of whether the extract of leaf of olive can be used as an appropriate alternative to levodopa in reducing the symptoms of Parkinson's.</p> <p>Methods: In this study, 60 small mice were assigned in 6 groups including control, Parkinson, levodopa, and 3 groups receiving the hydroalcoholic extract of olive leaf in three doses of 50, 100 and 200 mg/kg. All groups except the control group received paroxetine for 19 hours at subcutaneous intervals of 48 hours. After induction of Parkinson's by Rotenone, the drug group received levodopa ½ mg/kg of mice weight, and the extract groups received an intraperitoneal injection in three doses of 50, 100 and 200 mg/kg of mice, respectively, and 55 minutes after injection, each mice was individually evaluated behavioral by both Rearing test and bar test, and standard assessment indicators were checked and recorded through the analysis the review and recorded.</p> <p>Results: The results show that extract in a dose of 100 mg/kg in bar test compared with the drug group and other doses of the extract significantly increased the holding time of the bar by the mouse which indicating a reduction in the symptoms of Parkinson's.</p> <p>Conclusion: Based on the results, it seems that the hydroalcoholic extract of the olive leaf with a dose of 100 mg/kg of mice weight can be considered as a suitable alternative to levodopa in reducing Parkinson's symptoms.</p>

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Introduction

Parkinson's disease is a central nervous system disorder in older adults, characterized by "progressive muscle stiffness, shake, and loss of motor skills. After Alzheimer's disease, Parkinson's disease is considered as the most common malignant neurological disease. The progression of this disease is progressive, with an average age of 55 years, and its incidence increases dramatically with age; in this disease, dopamine secreting cells in the black body disappear and in the absence of dopamine, body movements become irregular. Parkinson's symptoms appear in both physical and psychological categories, with apparent symptoms such as motionlessness, slowness of movement, muscle stiffness, resting tremors, and decreased reflexes. Also, various degrees of

visual hallucinations and cognitive symptoms such as depression, sleep disturbance, anxiety, indifference, sensory disturbances, delusions, and aggression are among the psychological symptoms of this disease (1).

The main risk factors of Parkinson's are age and environmental factors. Several genes have been identified for hereditary Parkinson's disease that led to new treatments. Dopamine replacement therapy and commonly used drugs reduce motor impairment significantly and have a positive effect on the quality of life (2).

The primary treatment of Parkinson's disease is based on replacing the dopamine deficiency with this substance itself. Because dopamine cannot pass through the Blood-brain barrier, it can use its metabolic precursor (levodopa). For the substance not to be converted to dopamine

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before entering the central nervous system, using Dopa decarboxylase inhibitors reduces the levodopa metabolism by up to 75%. This inhibitor is combined with levodopa and in the form of a levodopa-carbidopa series (3).

Levodopa improves the slackness of the movements (Bradykinesia) and muscle stiffness (Rigidity) of Parkinson's patients more than Tremor (4). However, with prolonged use of levodopa, symptoms of Parkinson's disease recur again, and disorders due to chronic drug use appear as wearing off levodopa effects and dyskinesia. Therefore, achieving an appropriate therapeutic approach that can be safely and continuously used for Parkinson's patients is important (5).

The formation of free radicals in mitochondria and metabolic reactions are among the factors that lead to cell death and disease. Anti-oxidants such as vitamins C and E and plant extracts can prevent the formation of free radicals. Therefore, the use of plants with antioxidant activity is one of the ways to protect the body against oxidative damage caused by free radicals (6). The use of natural antioxidants to prevent Parkinson's disease is vital so that searching for natural antioxidants is of great importance (7).

Today, the use of medicinal herbs to prevent and slow down the progression of Parkinson's disease has attracted the attention of researchers. The phenolic compounds in plants are secondary metabolites that, by inhibiting oxidation, cause the removal of free radicals, and the use of these antioxidants in the diet plays an important role in the prevention of chronic diseases due to oxidative stress. Olive leaf extract contains various phenolic compounds that induce various pharmacological effects. These compounds have broad antioxidant features and eliminate free radicals (8).

The history of treating diseases with medicinal herbs dates back to the history of human life on the planet. The demonstration of the adverse effects of chemical drugs, environmental pollution, expensive synthetic drugs, and lack of access have led many people around the world to satisfy their therapeutic needs from medicinal herbs. In recent years, these factors have led to extensive research on special strains of these plants that have beneficial effects on many human diseases. Currently, 25% of drugs in the world drug market have herbal sources.

OLE is a significant source of natural antioxidants; it has effective antioxidant activity

against different reactive species and protects human erythrocytes against oxidative damage (9).

Treatment with OLE prevents the increases in the levels of MDA, significantly improves the SOD, CAT, and GPx levels in the midbrain, and prevents the depletion of the TH-positive neurons. (10).

The main active component in olive leaf and its extract is oleuropein, a natural product of the secoiridoid group. Several studies have shown that oleuropein possesses a wide range of pharmacologic and health-promoting properties including antiarrhythmic, spasmolytic, immunostimulant, cardioprotective, hypotensive, anti-inflammatory, antioxidant, and anti-thrombic effects (11).

The olive plant is one of the plants that has been used since ancient times as an herbal medicine for the treatment of many diseases. In previous studies, the antioxidant, antidiuretic, and anti-hepatitis effects of olive leaf extract have been proven. Also, in pharmacological studies of olive leaves on animals, the decreasing effects of hypertension and uric acid, as well as weight gain, have been observed. Some studies have revealed the painkilling effects of olive leaf extract, and have shown a possible mechanism for this effect through the calcium/calmodulin route (12).

Therefore, using herbal medicines for the treatment of many psychiatric disorders can be a good alternative to chemical drugs because of the chemical drugs' side effects and requires further research on the effectiveness of medicinal plants. On the other hand, due to economic benefits and other benefits of experimental studies on animals, including the genetic similarity of mice with the human genome collection, many medical and biological research and experiments are conducted on mice and laboratory samples (13). Hence, researchers have developed Parkinson's disease model on these animals. Methods for creating the Parkinson's model are divided into three main groups: genetic model, viral model, and neurotoxin-induced models. The latter method is cheaper and simpler than the other two methods, which is why it has more application among researchers. The toxins of this group are MPTP, hydroxydopamine, Rotenone, and Paraquat. Based on previous studies, the use of Paraquat and Rotenone models has expanded since they are closer to real conditions. Among the animal models, the systemic model of

Rotenone reliably simulates the behavioral symptoms and physiopathology of Parkinson's disease (14).

Regarding the review of theoretical research and the lack of a scientific study on the effect of comparison of olive leaf extract with levodopa on the reduction of Parkinson's symptoms in the animal model, this research seeks to answer the question of whether the extract of leaf of the olive tree is used as an appropriate alternative to levodopa in reducing the symptoms of Parkinson's in an experimental animal mouse model?

Materials and Methods

This research is an experimental type. The statistical population of the present study was 60 male and female mice (Balb/C) weighing 25-30 g. They were randomly selected and distributed in 6 groups. Mice were kept in standard cages made of polycarbonate with a stainless-steel grille for 2 weeks under the same conditions and free access to water and food, natural light period, temperature $23\pm 2^{\circ}\text{C}$, and humidity of about 60% to be compatible with the environment. Water and food were provided to them appropriately. The floor of the cages was also planted with sawdust and was replaced every two days. These conditions continued during the experiment.

Extraction of Olive Leaf

Dried Olive leaf was powdered, and an ethanol extract was prepared by the modified method of Fuhrman. Briefly, leaf powder (1.2 g) was suspended in 3 mL of ethanol in a glass tube-stoppered and was agitated vigorously for 10 min. The mixture was allowed to stand in room temperature for 24 h and after shaking for another 10 min it was filtered through a fluted filter paper into a 100 mL flask. The volume of the filtrate was then raised to 100 mL by the addition of sterile saline. The filter paper was then dried and the amount of the remaining powder on the filter paper was weighed and subtracted from 1.2 g to determine the concentration of the extract (15).

Experimental Groups

1. The group received olive leaves extract at a dose of 50 mg/kg body weight (n = 10)
2. The group received olive leaves extract at a dose of 100 mg/kg body weight (n = 10)
3. The group received olive leaves extract at a dose of 200 mg/kg body weight (n = 10)

4. Levodopa group: This group was included in samples that were treated with 1.2 mg/kg body weight levodopa. (n = 10)

5. Parkinson group: This group was included in samples that received Rotenone Parkinson's toxin and did not receive any medicine. (n = 10)

6. The control group (without Parkinson's induction): This is a group of normal mice who did not receive any injectable drugs or drugs. (n = 10)

Considering that no similar research has been done, three doses were selected.

This research has been approved by the Code of Ethics No. IR.IAU.NAJAFABAD.REC.1398.113.

Parkinson's Induction Method

For the induction of Parkinson's model, Rotenone, sunflower oil, dimethyl-sulfoxide (Sigma, USA) were used. First, the rotenone was dissolved in dimethyl sulfoxide and then diluted in sunflower oil to be injectable at doses of 1, 2, and 3 milligrams per kilogram. To induce Parkinson's disease, animals received Rotenone subcutaneously at doses of 1, 2, and 3 mg/kg in 48 hours intervals for 19 days.

Evaluation of Parkinson's Symptoms Reduction method

Rearing Test

This test was performed to measure motor skills in mice. Naturally, when the mouse is placed in a new environment, it stays on two legs based on its search behavior and touches the walls of the environment with its front organs. The performance of Parkinson's mice decreases in this experiment. In this experiment, each mouse was individually placed in a transparent glass cylinder (20 cm in diameter and 40 cm in height) for 5 minutes each time, and for each mouse, the number of times it stands on two legs and placed one or both arms above the shoulder surface and touched the walls were counted. The performance of the mouse was filmed in a quiet environment with a video camera and was evaluated by a person who was non-informative in the experimental group (16).

Bar Test

This experiment was also performed to measure muscle soreness in mice. To do this, mouse's two hands were placed on a bar at a height of 10 cm (i.e., half the height at which the animal is in Rearing's position), and time was recorded as long as the animal takes one or both of its arms

out of the bar. The maximum time considered for this test was 180 seconds (17).

Injection Method

To induce Parkinson's disease, animals received Rotenone subcutaneously at doses of 1, 2, and 3 mg/kg, at intervals of 48 hours for 19 days.

1. Extract groups that received olive leaf extract after induction of Parkinson's in three doses of 50, 100, and 200 mg/kg body weight intraperitoneally.
2. The levodopa drug group received a 1.2 mg/kg bodyweight drug intraperitoneally after induction of Parkinson's disease.
3. Parkinson's group: This group was included in samples that only Parkinson's disease was induced and no drug injection was performed on them.
4. Control group: The function of this group was evaluated without the creation of Parkinson's.

Statistical Analysis

In this research, data analysis was performed through a statistical package of the social

sciences. To analyze the collected data, the data were analyzed in two descriptive and inferential levels. In the descriptive part, the mean and standard deviations of the variables were calculated and in the inferential level, the analysis of variance (ANOVA) and Tukey's post hoc test was used.

Obtained data were analyzed using the SPSS program, at 5% probability level.

Results

Descriptive Statistics

In this research, a cylinder was used in the Rearing and bar tests in the bar test to examine the progress of the mice and the number of standing on the two legs in the cylinder and the holding time of the 10 cm bar in the mice were evaluated. The mean and standard deviation of the cylinder and bar test results of six groups are presented in Table 1.

Table 1: Mean and standard deviation of results of the cylinder and bar tests in control groups, Parkinson's, Levodopa drug, Olive leaf extract with doses of 50, 100 and 200

Group	Cylinder		Bar		
	Indexes	Mean	Standard deviation	Mean	Standard deviation
Control		71.1	6.2	27.5	8.93
Olive leaves extract at a dose of 50 mg		36.6	5.44	9	5.65
Olive leaves extract at a dose of 100 mg		49.1	6.67	23.8	6.51
Olive leaves extract at a dose of 200 mg		19.4	5.01	13.4	5.5
Levodopa		57.9	7.17	5.4	1.64
Parkinson		41.2	7.62	2.5	1.17

ANOVA Test

The results of Table 2 shows that the mean number of standing on two legs in a cylinder as one of the symptoms of Parkinson's in 50 mg of olive leaf extract was 36.6, in a dose of 100 mg was 49.1 and in a dose of 200 mg was 19.4. The mean of this mark in the control group was 71.1, in the levodopa group was 57.9 and, in the Parkinson, group was 41.2. Therefore, it can be concluded that the mean number of standing on two legs in the control group is higher than the other groups and in the extract group of the olive leaf with a dose of 200 mg is lower than the other groups. The length of holding the 10 cm bar in the 50 mg group of olive leaf extract is 9, in a dose of 100, 23.8, and the 200 mg dose is 13.4. While the mean of this period in the control group was 27.5

and in the groups of the Levodopa and Parkinson drugs, it was 4.5 and 2.5 respectively. Thus, this time or test results of the bar in the control group are higher than the other groups and in the Parkinson's group lower than the other groups. Based on the findings, the mean scores of the variables of Parkinson's symptoms, such as the frequency of standing on the two legs in the cylinder, and the holding time of the bar in the mice test in six groups (levodopa, Parkinson's, control and olive leaf extract with three doses of 50, 100 and 200 mg) has a significant difference (P = 0.001). The results show that nearly 82.3% of the individual differences in the research variables used to examine the symptoms of Parkinson's are related to the difference between the six groups. Therefore, it can be concluded

that the research hypothesis has been confirmed, which means that the extract of the olive leaf can have favorable effects on the reduction of Parkinson's symptoms in the animal model compared to levodopa.

The results generally show that group membership or dosage of olive leaf extract and levodopa drugs improve Parkinson's symptoms

and the groups are different, but for comparison of groups with different doses of olive extract, the Tukey's follow-up test is used and the results are presented below. Paired comparison results using the Tukey test for comparison of the 50 mg/kg body weight of olive leaf extract with control groups, levodopa, and Parkinson's group are presented in Table 2.

Table 2: a paired comparison of the 50 mg dose of olive leaf extract to other groups

variable	groups	Mean differences	standard deviation error	Sig	Confidence interval	
					min	max
Cylinder	Control	** -34.5	2.87	0.001	-42.98	-26.01
	Levodopa	** -21.3	2.87	0.001	-29.78	-12.81
	Parkinson	-4.6	2.87	0.601	-13.08	3.88
Bar	Control	** -18.5	2.5	0.001	-25.9	-11.09
	Levodopa	3.6	2.5	0.705	-3.8	-11.007
	Parkinson	5.6	2.5	0.117	-0.907	13.9

0.01 (p<) ** 0.05 (p<*)

ANOVA test

The results of the paired comparison of the mean differences in Table 2 shows that in the cylinder test, the difference between the group of olive leaf extract (50 mg) and the control group (P <0.01) and levodopa (P <0.01) is significant, but the difference with the Parkinson's group is

insignificant. The difference between a dose of 50 mg of olive leaf extract and control group in the bar test was significant (P <0.01), but it was not significant for levodopa and Parkinson groups. Paired comparison results using the Tukey test for comparison of the 100 mg/kg body weight of olive leaf extract with control groups, levodopa, and Parkinson's group are presented in Table 3.

Table 3: a paired comparison of the 100 mg dose of olive leaf extract to other groups

Variable	Groups	Mean differences	Standard deviation error	Sig	Confidence interval	
					min	max
Cylinder	Control	**22-	2.87	0.001	-30.48	-13.51
	Levodopa	*8.8-	2.87	0.038	-17.28	-0.21
	Parkinson	7.9	2.87	0.082	-0.58	16.38
Bar	Control	-3.7	2.5	0.681	-11.1	3.7
	Levodopa	**18.4	2.5	0.001	1.99	25.8
	Parkinson	**21.3	2.5	0.001	13.89	28.7

0.01 (p<) ** 0.05 (p<*)

ANOVA test

The results of the paired comparison of the mean differences in Table 3 shows that in the Cylinder test, the difference between the group of olive leaf extract (100 mg) with control groups (P <0.01) and levodopa (P <0.05) is significant but its difference with the Parkinson's group is insignificant. Also, the difference between the

100 mg dose of the olive leaf extract group in the bar test was significant with levodopa (P <0.01) and Parkinson's (P <0.01), but insignificant with the control group. Paired comparison results using the Tukey test for comparison of the 200 mg/kg body weight of olive leaf extract with control groups, levodopa, and Parkinson's group are presented in Table 4.

Table 4: a paired comparison of the 200 mg dose of olive leaf extract to other groups

Variable	Groups	Mean differences	Standard deviation error	Sig	Confidence interval	
					min	max
Cylinder	Control	** -51.7	2.87	0.001	-60.18	-43.2
	Levodopa	** -38.5	2.87	0.001	-46.98	-30.01
	Parkinson	** -21.8	2.87	0.001	-30.28	-13.31
Bar	Control	** 14.1	2.5	0.001	-21.5	-6.69
	Levodopa	** 8	2.5	0.027	0.59	15.4
	Parkinson	** 10.9	2.5	0.001	3.49	18.3

0.01 (p<) ** 0.05 (p<*)

ANOVA test

The results of a paired comparison of the mean differences in Table 4 shows that in the cylinder test, the difference between the group of olive leaf extract (200 mg) and all three control groups, levodopa and Parkinson are significant. The difference between the dosage of 200 mg of olive leaf extract group in the bar test was also statistically significant with all three control groups, levodopa, and Parkinson's.

Briefly, the results show that the difference between the olive extract group of 50 mg and 100 mg with the control group and levodopa group is significant, and is insignificant with Parkinson's group. But there is a significant difference between the olive extract of 200 mg in the rearing test with all of the three groups of control, levodopa, and Parkinson. In the bar test, the group of 50 mg dose of olive leaves extract has a significant difference with the control group but its difference is insignificant with levodopa and Parkinson's drug groups. Also, the difference in dosage of 200 mg of extract of olive leaf extract with all three control groups, levodopa, and Parkinson's drug is significant in the bar test.

Discussion

According to the results obtained in the cylindrical test, the mean number of standing on the two legs in the control group is higher than in all groups and in two groups of the 200 mg dose group of olive leaf extract was lower than the other groups. In the bar test, the holding time of the 10 cm bar in the control group was higher compare to all the groups and in the Parkinson's group, it was lower than the other groups. Since the increase in the number of standing on the two legs in the cylinder in the rearing test or the increase in the holding time of the bar in the bar test is considered to be an indicator of the reduction of Parkinson's symptoms, the

benchmark for reducing Parkinson's symptoms is that if simultaneously both indicators, the dumiceion of holding the bar and the number of standing on the two legs in the cylinder, increases or one of them have an insignificant difference with the control group or the levodopa recipient group, it is considered as a significant change in the reduction of Parkinson's symptoms.

Regarding the effect of hydroalcoholic extract of olive leaf with a dose of 50 mg/kg body weight compared to levodopa on reducing the symptoms of Parkinson's disease, the results show that the group extracts olive leaves at a dose of 50 mg/kg body weight has a significant difference with control and the levodopa drug group and its difference with the Parkinson's group is not significant. In the bar test, the difference between the group of olive leaf extract with a dose of 50 mg/kg body weight with the control group is significant, but with the levodopa and Parkinson's drug group it is insignificant. Therefore, it can be concluded that the hydroalcoholic extract of the olive leaf with a dose of 50 mg/kg body weight cannot be similar to levodopa in reducing Parkinson's symptoms. In a comparison between the results of this study and the results of studies related to this variable, the results of this research are inconsistent with the results of previous studies. Doses that were used in previous studies are different from the number of doses that are used in the present study, and this suggests that the effectiveness of the amount of effective ingredient in the compounds in the plant depends on a certain dose of it. Examples include recent studies such as the effect of resin extract on Catatonia (the most common symptoms of Parkinson's disease) caused by Perphenazine in labomiceory mice (18), the effect of BlackBerry extract on Parkinson's disease symptoms in male mice the effects of Achilles yarn on the Parkinson's model created by 6-hydroxydopamine in male mice

(19), and the effect of one of the ginseng plants on nervous system disease caused by Parkinson's disease (20). It is also possible to refer to the comparing protective effect of Iranian Blue Propolis extract in Parkinson's disease model in male mice (21), in which experimental groups, were injected extract intraperitoneally in two doses of 50 and 100 mg/kg body weight. The results show that the Iranian Blue Propolis extract in two doses of 50 and 100 mg/kg body weight has a significant protective effect against Parkinson's disease. Regarding the effect of hydroalcoholic extracts of olive leaf with 100 mg/kg body weight compared to levodopa, on reducing the symptoms of Parkinson's disease, the results show that the hydroalcoholic extract of olive leaf with a dose of 100 mg/kg body weight, shows a significant difference in reducing Parkinson's symptoms in mice in the cylindrical test with two control group and the levodopa group based on the frequency of standing on the two legs, and the difference with the Parkinson's group is not significant, and in the bar test, based on the time spent holding the bar, the difference between this experimental group and the levodopa and Parkinsonian group is significant, but it is insignificant with the control group. Therefore, it can be concluded that the hydroalcoholic extract of olive leaf with a dose of 100 mg/kg body weight can be effective in reducing Parkinson's symptoms and, in comparison with the effect of levodopa, has a more favorable effect on reducing the symptoms of Parkinson's disease and treatment with olive leaf extracts depends on the use of a suitable dose. The results of this study are compared with the results of studies related to this compamiceive variable, which is consistent with the results of previous research, which confirms the effectiveness of the amount of active ingredient in existing compounds. For example, it can refer to researches in recent years, such as the anti-Parkinson activity of extract of figs of temples (22), comparing the protective effect of the Iranian Blue Propolis extract and L-Dopa in the Parkinson's disease-induced model of mice (21) and the effects of methanolic extract of fennel seed in the Parkinson's model created by 6-hydroxydopamine in female mice, and the effect of Aloe Vera in a Parkinson's animal model (23). For instance, Kim et al. (2004) investigated the neuroprotective effects of ginkgo Biloba extract

in Parkinson's disease in mice models. In their study, the extracts were injected intraperitoneally with doses of 50 and 100 milligrams. The results of their study showed that ginkgo Biloba extract of 100 mg/kg of body weight could be used as a drug for the treatment of Parkinson's disease (24).

Regarding the effect of hydroalcoholic extract of olive leaf with a dose of 200 mg/kg body weight compared to levodopa, on reducing the symptoms of Parkinson's disease, the results show that hydroalcoholic extracts of olive leaf with a dose of 200 mg/kg body weight have a significant difference with both in-cylinder test and bar test with three doses of control, levodopa and Parkinson's groups in decreasing Parkinson's symptoms in mice. Therefore, it can be concluded that because in none of the two tests there is not an insignificant difference with the control group and the levodopa drug group, the hydroalcoholic extract of the olive tree leaves at 200 mg/kg bodyweight can't be considered as an effective ingredient in reducing Parkinson's symptoms. Accordingly, the results of this study are inconsistent with the results of previous studies. Other related studies have employed different doses than the ones used in this research and have different effects on Parkinson's disease. Some of these studies in recent years are the protective effect of *Silybum marianum* extract in the Parkinson's disease model induced in male mice (25), the neuroprotective effect of Barberry extract on Parkinson's disease model in male mice, and the effect of ginkgo Biloba extract on Alzheimer's and Parkinson's disease on the animal model (26). Aslani and colleagues investigated the effect of Hydroalcoholic extract of Japanese medlar on the levels of CDNF, SOD, and MDA of the cortex in an experimental model of Parkinson's disease in male mice. The Hydroalcoholic extract of Japanese medlar was injected intraperitoneally at a dose of 200 mg/kg, indicating its protective role against Parkinson's disease.

Conclusion

By analyzing the data and statistical analysis, the hydroalcoholic extract of olive leaf in a dose of 100 mg/kg of mice weight in the test bar index compared to the control group significantly increases the holding time of the 10 cm bar which is an index of reducing Parkinson's disease symptoms in an animal model of a small mouse. According to the results of this finding, it can be

concluded that the hydroalcoholic extract of an olive leaf with an appropriate dose compared with levodopa can be effective in reducing the symptoms of Parkinson's disease in the animal model.

The research limitation

This study was performed on Balb/C mice only and the results could not be generalized to other breeds.

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Conflict of Interest

The authors contributing to the present study and this very manuscript have no conflict of interests to declare.

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