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Research Article

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Study of the Effect of Concurrent Therapy of Spironolactone With Levodopa on Depression Disorder in Male Rats

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Abstract

Background: Depression has a high prevalence and high mortality and a tremendous negative impact on people's quality of life. This study was considered to examine the effect of levodopa and spironolactone (SPIR) in 6-hydroxydopamine (6-OHDA)-induced depression-like behavior.

Methods: Parkinson was induced by the unilateral intra-nigral injection of 6-OHDA (8 µg/2 µL/rat) in the central region of the substantia nigra pars compacta (SNc). In the levodopa treatment group, 21 days after the injection of 6-OHDA, the rats were treated with (i.p.) injections of levodopa (15 mg/kg) for 14 consecutive days. In the other group, only SPIR (25 mg/kg) was added to levodopa (15 mg/kg) as a concurrent therapy according to the treatment protocol. Anxiety and depression-like behavior were assessed by behavioral tests such as passive avoidance task (PAT), open field test (OFT), tail suspension test (TST), and forced swimming test (FST).

Results: Intra-nigral injection of 6-OHDA in the SNc increased anxiety and depression-like behavior. Our results showed that the use of levodopa (15 mg/kg) treatment significantly attenuated depression-like behavior induced by 6-OHDA in FST ($P<0.001$) and TST ($P<0.001$) in rats. Moreover, levodopa (15 mg/kg) + SPIR (25 mg/kg) significantly reduced the symptoms of depression-like behavior induced by 6-OHDA in OFT ($P<0.05$), FST ($P<0.001$), and TST ($P<0.001$) tests in rats.

Conclusion: Based on the findings, the intra-nigral injection of 6-OHDA into rats could cause anxiety and depression-like behavior in the fourth week onwards. Treatment with levodopa was able to attenuate stress and depressive symptoms. Additionally, our results revealed that SPIR could improve the effect of levodopa on depression-like behavior. Base on the results of this study, it is suggested that levodopa and SPIR could have some improving effects on 6-OHDA-induced depression-like behavior in parkinsonian rats.

Keywords: Depression, Parkinson's disease, 6-OHDA

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Introduction

Neurological disorders may precede the behavioral symptoms in patients with Parkinson's disease (PD), including depression, anxiety, and cognitive impairment and usually presenting with immobile PD symptoms (1, 2). The epidemiological research suggests that depression can be mainly heritable, and chronic intense stress has been recognized as one of the key factors of major depression (3,4). There are multiple hypotheses about the biology of depression. The biological etiology of depression is closely associated with monoamine neurotransmitter deficiency and the abnormal function of neurotransmitter receptors (5). Recent studies have also exposed that depression is related to hypothalamic-pituitary-adrenal axis disorders (6). The two corticosteroid receptors, which are most commonly expressed in the limbic part of the brain, are the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). MR is predicted to be occupied under hemostatic conditions while GR is significantly occupied under stressful conditions (7). Previous research evaluated the role of GR in responding to stress and reported a link between high corticosterone or decreased anterior brain GR levels and depression (8).

Levodopa is the most incredibly effective mediatior in terating the PD. However, two controversies remain on whether levodopa causes motor problems and whether levodopa is toxic. Levodopa is toxic to cultured dopamine neurons, and this may be a problem in PD. Clinical trials have not clarified this condition, but there are a limited number of firm documents to suggest that levodopa is toxic in vivo or PD. Further, evidence suggests that levodopa is associated with motor complications due to the short half-life of the drug (and its potential to stimulate dopamine receptors) rather than to specific properties of

the molecule (9).

Spironolactone (SPIR) is an MR blocker and has been employed as potassium-sparing diuretics (10). MRs are expressed on the immune cells (e.g., microglia) and their function in the induction of neuro-inflammation is known (11,12). Moreover, researchers have investigated the effects of MR blockers on improving memory performance (13). The current study aimed to examine the effect of levodopa and SPIR concurrent therapy on depression-like behaviors in the rats injected with the intra-neural infusion of a 6-hydroxy dopamine (6-OHDA, 8 µg/2 µL/rat) into the central region of the substantia nigra pars compacta.

Materials and Methods

Chemicals

Levodopa and SPIR powder were supplied by Pharmacy Galen. (Tehran, Iran) and Abu-Raihan Pharmaceutical Company (Iran), respectively. Levodopa powder was dissolved in distilled water. SPIR powder was dispersed in 20% polyethylene glycol. In addition, 6-OHDA was provided by Sigma-Aldrich Company (USA) and dissolved in distilled water containing 0.02% ascorbic acid (as activator and antioxidants). Likewise, ketamine and xylazine were obtained from Alfasan Company (The Netherlands). All other chemicals were of the highest analytical grade and supplied by Merck (Germany).

Study Approval and Animals

25 male Wistar rats were utilized in this study. All animal experiments and handling were conducted by the National Institutes of Health guidelines and committee on the care and use of the laboratory animals of the council on animal care at the University of Hamedan, Iran (IR.UMSHA.REC.1399.018). Adult male Wistar rats weighing 200 ± 250 g were housed in the groups of ten with a 12:12 hours light-dark cycle at room temperature (22 ± 2°C) with ad libitum access to standard rodent chow and tap water.

Experimental Design

All animals were allowed to adapt to laboratory conditions for at least one week. The rats were randomly divided into five groups of eight for tests as follows:

- Group 1: Control group (intact animals);
- Group 2: Sham group (surgery without the injection of 6-OHDA and received vehicle);
- Group 3: Parkinsonian group (received an intra-nigral injection of 6-OHDA: 8 µg/2 µL/rat);
- Group 4: 6-OHDA + L-DOPA 15 mg/kg (i.p, for 2 weeks);
- Group 5: 6-OHDA + (L-DOPA 15 mg/kg, i.p + SPIR 25 mg/kg, i.p, for 2 weeks as concurrent therapy).

Surgical Procedures

In general, the stereotaxic surgery model and 6-OHDA injection were performed according to previous research (14). Briefly, for the induction of PD, the unilateral injection of 6-OHDA hydrochloride into the rat brain was performed using stereotaxic surgery.

Behavioral Tests

All the rats experienced depression-like behavioral tests after the ending of the trial. The protocol included passive avoidance task (PAT), open field test (OFT), tail suspension test (TST), and forced swimming test (FST), respectively. The behavioral tests were performed according to studies previously performed (14,15).

It is important to note that in the case of the treatment groups, first, 6-OHDA was injected, and then treatments were started for 2 weeks after 3 weeks when the recovery period was over, and behavioral tests were evaluated at the end of the study.

Passive Avoidance Task

This test was accomplished to evaluate anxiety (15), and the apparatus is a two-way shuttle-box divided by a guillotine door. A 40-W bulb irradiates one of the chambers whereas another chamber is dark. On the training day, the rats were separately sited in the light space, facing the wall opposite the guillotine door. The door was silently pull down when the rat arrived in the darkened space, and the animal received a 0.5 mA foot shock for two seconds through the grid floor. On the test day, the rat was again placed in the light area without applying a foot shock. Sep-through latency was noted each time, and the length of time, which the rats spent in the dark space, was recorded as spent time in the dark compartment.

Open Field Test

The OFT was used to evaluate the anxiety of rats (16,17). In the OFT, behavioral responses such as the total traveled distance, the mean velocity, the spent time in the center zone, and the center entries were examined on rats. The apparatus was made of a white acrylic field (surface area: 50×50 cm and wall height: 38 cm) with low ambient lighting. First, the rats were placed in the open ground and allowed to roam the area for 5 minutes. The total traveled distance, the mean velocity, the spent time in the center zone, and the center entries were recorded by a video-tracking camera (18,19).

Tail Suspension Test

This test assesses depression-like behaviors (20). This experiment was executed according to the prior study (21). During a five-minute session, the rats were hung and held at a point two cm from the end of their tails so that their bodies were hung in the air. Observations were recorded from the second minute onwards. The length of time, which the rats spent in a hanging position with no desire to fight, was recorded as the duration of immobility.

Forced Swim Test

The FST evaluates depression-like behaviors (22). Plexiglas cylinders (height: 40 cm and diameter: 18 cm) were applied in this test. The rats were held in these cylinders, which contained 25 cm of water at 25-30°C (23). The animals were egressed from the cylinder after five minutes, and the whole time of immobility was calculated accordingly. Observations were recorded from the second minute onwards. When rattan animals remained floating passively in the water, it was considered immobile.

Statistical Analysis

The statistical analysis of the data set was performed using GraphPad Prism software, version 8.4. Data were expressed as the mean ± SEM and analyzed by one-way ANOVA in each experiment. In the case of significant variations ($P < 0.05$), the values were compared by the Tukey test.

Results

Effect of Levodopa and Levodopa + SPIR as Concurrent Therapy on the Depressive-like Behavior on PAT in Parkinsonian Rats

The current study investigated the effects of the administration of 6-OHDA on PAT performance in depressive-like behavior in rats. Based on the results, 6-OHDA-injected rats displayed an increase in spent time in the dark compartment ($P < 0.001$, Figure 1) when compared to the control group. Treatment with levodopa (15 mg/kg) and levodopa (15 mg/kg) + SPIR (25 mg/kg) represented no significant difference in spent time in the dark compartment compared with the lesion group (Figure 1).

It was found that 6-OHDA-injected rats demonstrated a noticeable decrease in step-through latency (STLr) time in comparison with the control group ($P < 0.001$, Figure 2). However, treatment with levodopa (15 mg/kg) and levodopa (15 mg/kg) + SPIR (25 mg/kg) showed no significant difference in STLr time compared with the lesion group (Figure 2).

Effect of Levodopa and Levodopa + SPIR as Concurrent Therapy on the Anxiety on OFT in Parkinsonian Rats

The induced anxiety by 6-OHDA injection was assessed using an OFT. The total traveled distance, the mean velocity, the spent time in the center zone, and the center entries were examined in the OFT test. In general, the total traveled distance by the parkinsonian rats (the 6-OHDA group, 10.23 cm) was and significantly lower than that of the control group (18.78 cm, $P < 0.05$, Figure 3). The total traveled distance in parkinsonian rats treated with 15 mg/kg levodopa (12.72 cm) was no significantly larger compared to the parkinsonian group (Figure 3). However, treatment with 15 mg/kg levodopa + 25 mg/kg SPIR (15.67 cm) significantly ($P < 0.05$) increased the total traveled distance in the OFT compared to the 6-OHDA groups and SPIR could improve the therapeutic effect of levodopa (Figure 3).

Overall, the mean velocity of the parkinsonian group (3.37 cm/s) was significantly lower than that of the control group (6.17 cm/s, $P < 0.05$, Figure 4). On the other hand, the mean velocity in parkinsonian rats treated with 15 mg/kg levodopa (4.04 cm/s) was not significantly larger compared to the parkinsonian group (Figure 4).

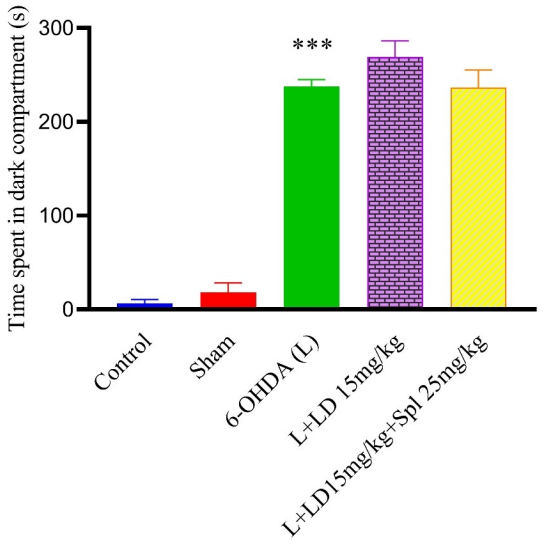

Figure 1. The Behaviors in 6-OHDA-treated Rats and the Effect of Levodopa on Passive Avoidance Apparatus. *Note*. SEM: Standard error of the mean; 6-OHDA: 6-hydroxydopamine. The behaviors in the levodopa-treated rat in the spent time in the dark compartment(s) (n = 5). The results are presented as the mean ± SEM. ***$P < 0.001$ as compared to the control group.

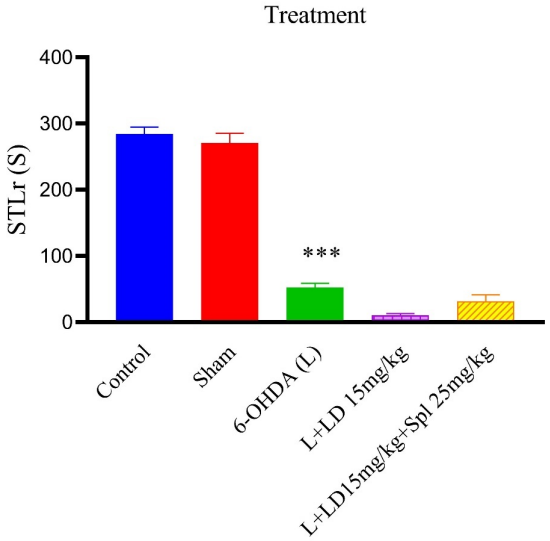

Figure 2. The Behaviors in 6-OHDA-treated Rats and the Effect of Levodopa on Passive Avoidance Apparatus. *Note*. SEM: Standard error of the mean; STL: Step-through latency; 6-OHDA: 6-hydroxydopamine. The behaviors in levodopa-treated rats in STLr (s) (n = 5). The results are displayed as the mean ± SEM. ***$P < 0.001$ as compared to the control group.

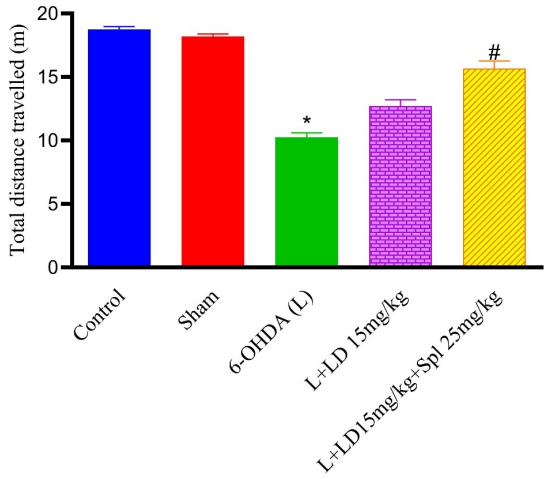

Figure 3. The Behaviors in 6-OHDA-treated Rats and the Effect of Levodopa on Anxiety Symptoms in the Open Field Test. *Note*. SEM: Standard error of the mean; 6-OHDA: 6-hydroxydopamine. The behaviors in levodopa-treated rats in the total traveled distance (n = 5). The results are represented as the mean ± SEM. $^{\#}P < 0.05$ as compared to parkinsonian rats; $^{*}P < 0.05$ as compared to the control group.

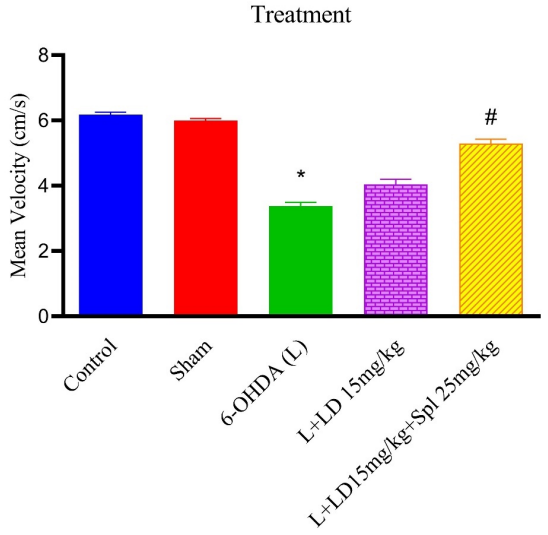

Figure 4. The Behaviors in 6-OHDA-treated rats and the Effect of Levodopa on Anxiety Symptoms in the Open Field Test. *Note*. SEM: Standard error of the mean; 6-OHDA: 6-hydroxydopamine. The behaviors in levodopa-treated rats in the mean velocity (n = 5). The results are provided as the mean ± SEM. $^{\#}P < 0.05$ as compared to parkinsonian rats; $^{*}P < 0.05$ as compared to the control group.

Conversely, treatment with 15 mg/kg levodopa + 25 mg/kg SPIR (5.3 cm/s) significantly ($P < 0.05$) increased the mean velocity in the OFT compared to the lesion group, and SPIR could improve the therapeutic effect of levodopa (Figure 4).

The time spent in the center zone in the parkinsonian group (26.8 seconds) was considerably lower in comparison with the control rats (45.29 s, $P < 0.05$, Figure 5). The time spent in the center zone in parkinsonian rats treated with 15 mg/kg levodopa (7.40 seconds) and the 15 mg/kg levodopa +25 mg/kg SPIR (9.75 seconds) treated group demonstrated no significant difference in comparison with the lesion group (Figure 5).

The center entries in the parkinsonian group (9) significantly ($P < 0.01$) reduced compared with the control rats (26.14, Figure 6). The center entries in parkinsonian rats treated with the 15 mg/kg levodopa (11.80) and the 15 mg/kg levodopa +25 mg/kg SPIR (15.88) treated group showed no significant difference compared with the lesion group (Figure 6).

Effect of Levodopa and Levodopa + SPIR as Concurrent Therapy on 6-OHDA-induced Depression-like Behavior in TST

The measurement of immobility time in the TST (Figure 7) revealed that 6-OHDA-induced depression-like symptoms in the rat as 6-OHDA administration significantly ($P < 0.001$) increased the immobility time in the parkinsonian group (198.1 seconds) compared with the control groups (119.43 seconds). On the other hand, treatment with 15 mg/kg levodopa in the patient-rat exhibited a significant ($P < 0.001$) reduction in the immobility time in the TST (109.2 seconds) in comparison with the lesion group (Figure 7). Furthermore, treatment with 15 mg/kg levodopa +25 mg/kg SPIR demonstrated a significant ($P < 0.001$) decline in the immobility time compared with the parkinsonian group (77.87 seconds), the related data of which are shown in Figure 7.

Effect of Levodopa and Levodopa + SPIR as Concurrent Therapy on Parkinson-induced Depression-like Behavior in FST

Based on the measurement of the immobility time in the

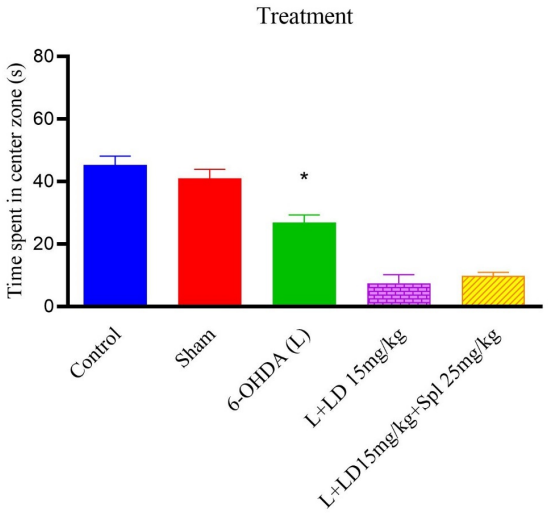

Figure 5. The Behaviors in 6-OHDA-treated Rats and the Effect of Levodopa on Anxiety Symptoms in the Open Field Test. *Note*. SEM: Standard error of the mean; 6-OHDA: 6-hydroxydopamine. The behaviors in levodopa-treated rats in the spent time in the center zone (n = 5). The results are indicated as the mean ± SEM. $^{*}P < 0.05$ as compared to the control group.

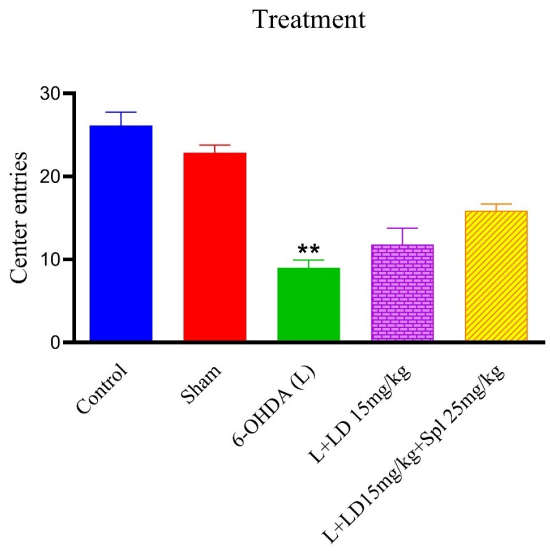

Figure 6. The Behaviors in 6-OHDA-treated Rats and the Effect of Levodopa on Anxiety Symptoms in the Open Field Test. *Note*. SEM: Standard error of the mean; 6-OHDA: 6-hydroxydopamine. The behaviors in levodopa-treated rats in the center entries (n = 5). The results are presented as the mean ± SEM; $^{**}P < 0.01$ as compared to the control group.

FST, 6-OHDA induced depression-like symptoms in the rat, as 6-OHDA administration considerably ($P < 0.001$) increased the immobility time in the parkinsonian group (198.1 seconds) compared to the control group (119.43 seconds, Figure 8). Additionally, in the 15 mg/kg levodopa and 15 mg/kg levodopa +25 mg/kg SPIR treated groups, there was a significant ($P < 0.001$) difference in the immobility time in the FST compared with the lesion group (Figure 8).

Discussion

The vast majority of available treatments for PD have currently improved symptoms while treatments aimed at stopping the disease or correcting the progression of the illness are still in focus. Previous studies have shown that depression and anxiety are essential and joint symptoms in PD and are observed in a high percentage of PD patients (24,25). Given the importance and extent of depression caused by PD, the researchers of this study focused on treating or reducing depression in this disease. Accordingly, new data were presented on the protective effects of SPIR and levodopa against 6-OHDA in depression-like behaviors in PD rats.

Thus, behavioral studies of PAT, OFT, TST, and FST have been generally done in our research. Passive avoidance learning in parkinsonian animals was evaluated in the present study, as avoidance is a core symptom of all anxiety disorders. The result of the experiments showed that 6-OHDA (8 µg/2 µL/rat) could significantly induce the symptoms of anxiety disorders. Previous studies reported that treatment with 6-OHDA caused selective striatal injury, which can lead to learning disabilities and anxiety (26,27), which is totally in line with the finding of the current study. The results further demonstrated that treatment with levodopa (15 mg/kg) decreased the spent time in the dark compartment although it was not significant. Based on the results, although treatment with SPIR (25 mg/kg) + levodopa (15 mg/kg) decreased the spent time in the dark compartment, it was not significant.

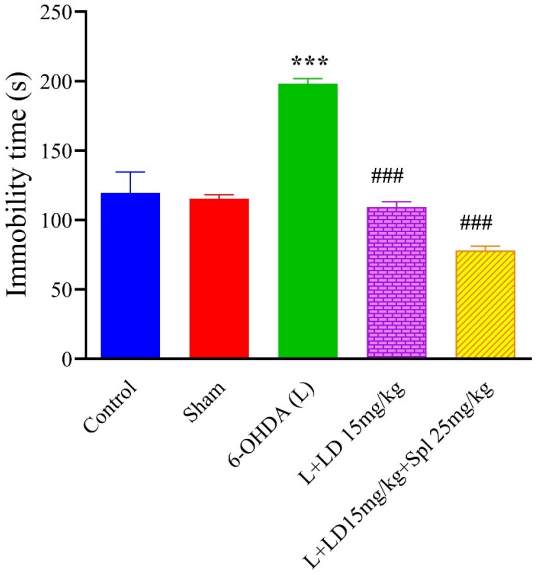

Figure 7. Effect of *Levodopa* on Depression-like Behavior. *Note*. SEM: Standard error of the mean; The behaviors in levodopa-treated rats in the tail suspension test (n = 5). The results are presented as the mean ± SEM; $^{###}P < 0.001$ as compared to parkinsonian rats, $^{***}P < 0.001$ as compared to the control group.

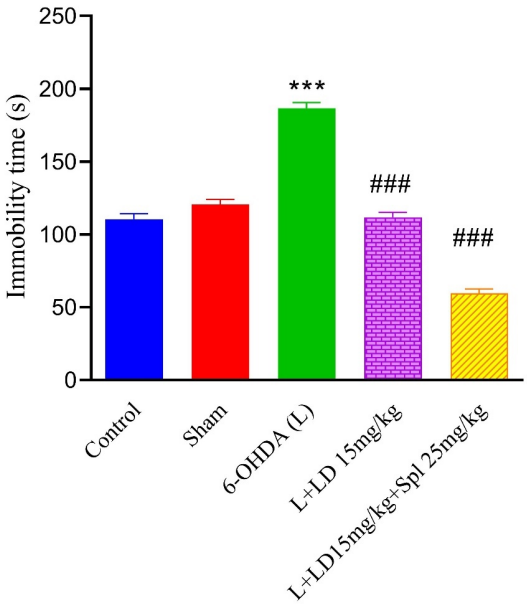

Figure 8. Effect of Levodopa on Depression-like Behavior. *Note*. SEM: Standard error of the mean; FST: Forced swimming test. The behaviors in levodopa-treated rats in the FST (n = 5). The results are demonstrated as mean ± SEM; $^{###}P < 0.001$ as compared to parkinsonian rats, $^{***}P < 0.001$ as compared to the control group.

The results of a previous study displayed that SPIR as therapy is effective in improving symptoms in response to stress in bipolar disorders in all patients (28), which is consistent with our findings.

In our research, the 6-OHDA treatment produced significant stress and depression behavior symptoms in PD. The results of our study confirmed the hypothesis that the use of 6-OHDA induces depression-like behaviors. Another study also approved that treatment with 6-OHDA causes motor dysfunction and depressive symptoms due to PD (29). Treatment with SPIR and *levodopa* increased the total traveled distance and the average velocity in OFT, suggesting that treatment could improve the balance and stress and depression behavior symptoms in the 6-OHDA-injected rats. The mentioned motor irregularities are supposed to be related to the excessive level of striatal dopamine which may be due to the injury of gamma-aminobutyric acid-ergic neurons in the striatum that are effective in controlling the whole striatal output (29,30).

Further, to establish the emotional behavioral faults in PD induced-depression, the rats were tested using FST and TST (31). In terms of behavioral studies, inactivity indicates behavioral despair and is one of the most important factors in describing depression (32). 6-OHDA-treated rats represented augmented immobility time in both FST and TST, which can indicate a depression-like state. Similarly, previous studies approved that treatment with 6-OHDA causes emotional behavioral deficits in PD (33, 34). Moreover, treatment with levodopa and levodopa + SPIR reduced immobility time in tests, implying the possible role of drugs in alleviating depression and improving the mood of the rat.

Among other related parameters that can be mentioned for behavioral despair is the active factor (swimming or trying) that indicates the escape search behavior. It has been described that the augmented time of swimming is associated with the serotonergic system activation whereas longer struggling is linked to augmented catecholaminergic neurotransmission (35). These results are supported by other studies, showing that SPIR improves mitochondrial dysfunction in diabetic rats (36).

Conclusion

The results of this study showed that the intra-nigral injection of 6-OHDA in rats could cause anxiety and depression-like behaviors from the fourth week onwards. Our study explained that levodopa could improve the symptoms of Parkinson-induced depression-like behaviors. It seems that treatment with levodopa could attenuate anxiety and depressive symptoms in PAT, OFT, TST, and FST. On the other hand, the results of this study also demonstrated that the use of SPIR in the treatment of stress and depression behavior symptoms caused by 6-OHDA with levodopa can be more effective compared to the use of either alone, and the use of this drug with levodopa has a more essential effect on reducing stress and depression behavior symptoms. Base on the results of this study, it is suggested that levodopa could have some improving effects on 6-OHDA-induced depression-like behaviors in parkinsonian rats. Overall, our study findings indicated that SPIR may be a prospective drug in terms of PD-induced depression therapy.

Conflict of Interests

The authors report no conflict of interests.

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