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Original Research

Effects of Voluntary and Forced Exercise on Anxiety-Related Behaviours and Motor Activity in Parkinson Mouse Model

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ABSTRACT

Objective: The aim of this study was to investigate the effect of two different types of exercise, voluntary and forced, on motor activity and anxiety in a neurotoxic parkinsonian mouse model.

Methods: Parkinsonian mice exposed to neurotoxin underwent voluntary exercise (VE) and moderate forced exercise (FE). The motor activity levels were then measured using the rotarod and pole test. Anxiety was assessed using an open-field test (OFT) and elevated plus maze (EPM) test.

Results: Bradykinesia, a motor dysfunction, was assessed using the pole test. The T_{turn} and T_{total} durations were significantly reduced in Parkinson-induced FE ($p<0.001$). The motor activity was assessed with the rotarod test, and the best improvement was in the long-term FE group ($p<0.001$). The time spent on the opened arms in the EPM test or the time spent in the peripheral zone in the OFT is significantly shorter in the Parkinson groups that performed FE than the VE groups. This suggests that the FE group is more anxious.

Conclusion: The study showed that long-term FE was the best exercise to improve the motor function. Moderate-intensity FE provided restorative effects on the motor symptoms of the disease. However, while this type of exercise increases anxiety, the VE has a healing effect on anxiety. Data obtained in this study showed that exercise provided an effective improvement in motor skills and anxiety behaviors. Thus, exercise is an effective and non-invasive way to be safely recommended by clinicians to all patients with Parkinson's disease.

Keywords: Anxiety, forced exercise, motor activity, Parkinson's disease, voluntary exercise

INTRODUCTION

Parkinson's disease (PD) was first described by the British physician James Parkinson in 1817 as the shaking palsy (1). It is a neurodegenerative disease affecting 2%-3% of the population >65 years (2). PD is physiopathologically characterized by the presence of typical eosinophilic cytoplasmic inclusion bodies (Lewy body) and degeneration in pigmented neurons in the substantia nigra. PD has motor and non-motor symptoms (3). Motor symptoms show four basic features: tremor, rigidity, akinesia (or bradykinesia), and postural instability (4). In addition to the well-described motor features, non-motor symptoms including psychiatric symptoms such as olfactory dysfunction, cognitive impairment, anxiety, sleep disorders, autonomic dysfunction, pain, and fatigue form a significant symptomatic burden (5).

An effective pharmacologic treatment to cure the disease completely has not yet been found despite the extensive research on PD. Levodopa is the most effective treatment for motor symptoms (2). However, many studies in the literature report that exercise has a therapeutic effect on motor (6) and non-motor symptoms of PD (7).

Physical activity is defined as any bodily movement produced by the skeletal muscle and causing energy expenditure. Physical activity in daily life can also be divided into occupational, sportive, conditioning, and domestic work activity. Therefore, exercise is a subtype of physical activity that is planned, structured, and routinized (8). On the other hand, physical inactivity, a common risk factor for chronic diseases, is the fourth risk factor leading to death in the world, because it is accounted for 6% of deaths globally.

A lifestyle is determined by various factors such as physical exercise, social interaction, and nutrition. These factors have a positive effect on people's mood. Physical exercise is an effective tool to slow down the physical and cognitive decline caused by illnesses. Regular physical exercise reduces anxiety and increases physical and mental development. It is also recommended as a

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complementary treatment for problems such as anxiety, depression, and attention-deficit-accompanying multifactorial diseases like PD or Alzheimer disease. Although the mechanism of the healing effect of regular exercise has not been fully explained due to inadequate studies conducted on humans, data indicate that it is necessary to increase physical activity (9). However, it is still unclear which type of exercise contributes most to these healing effects.

The aim of this study was to investigate the effect of physical exercise types on motor activity and anxiety in an animal model of neurotoxic PD. This model of PD was created by four intraperitoneal injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (4×20 mg/kg, at 12 h intervals). Two types of exercise (voluntary exercise [VE] and forced exercise [FE]) were used in the mice. An open-field test and an elevated plus maze (EPM) test were applied to measure anxiety levels. In addition, the mice were subjected to the rotarod and pole test to determine the development of motor activity.

METHODS

Animals
This study was carried out at Gaziantep University Experimental Animal Center laboratories, and C57BL/6 mice (male, weighing 25-30 gr, 6-10 months old) were used. All mice were housed at 23±2°C in a 12-hour ligh-dark cycle with free access to standard rodent food and water.

Groups
The mice were randomly divided into eight experimental groups, as follows: (1) Control (n=10); (2) MPTP (n=10); (3) 4VE+Saline+4VE (n=10); (4) 4FE+Saline+4FE (n=8); (5) MPTP+4VE (n=8); (6) MPTP+4FE (n=8); (7) 4VE+MPTP+4VE (n=8); and (8) 4FE+MPTP+4FE (n=8). Descriptions of the groups are given below (*).

*Control group: Animals were housed in the cage as a sedentary, and no intervention was performed.

MPTP group: MPTP was intraperitoneally injected to mice at the beginning of the experiment, and the mice were housed in the cage as sedantary.

MPTP+4VE group: MPTP was intraperitoneally injected to mice at the beginning of the experiment, and VE was performed for 4 weeks.

MPTP+4FE group: MPTP was intraperitoneally injected to mice at the beginning of the experiment, and FE was performed for 4 weeks.

4VE+Saline+4VE: After VE was performed for 4 weeks, saline was intraperitoneally injected, and VE was performed for 4 weeks again.

4FE+Saline+4FE: After FE was performed for 4 weeks, saline was intraperitoneally injected, and FE was performed for 4 weeks again.

4VE+MPTP+4VE: After VE was performed for 4 weeks, MPTP was intraperitoneally injected, and VE was performed for 4 weeks again.

4FE+MPTP+4FE: After FE was performed for 4 weeks, MPTP was intraperitoneally injected, and FE was performed for 4 weeks again).

Experimental protocol
Body weights were measured once a week by a digital scale.

Experimental Parkinson model
1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) neurotoxin induces dopaminergic neurodegeneration. Thus, it was used to obtain the Parkinson model. MPTP hydrochloride (M-0896, Sigma-Aldrich, St. Louis, Mo, USA) was applied with intraperitoneal injections to all MPTP groups to generate the Parkinson model in animals. MPTP was dissolved in saline (pH 7.4) as 3 mg/mL. To reduce the risk of death, the applied dose was 4×20 mg/kg every 12 hours (10).

Exercise Protocols

Forced exercise
A custom design treadmill system for the FE was produced according to dimensions and specifications, which have been commonly used in the literature. The maximum speed was up to 16 m/min for this treadmill, which had 8 lanes, 38×5×5 cm each (Figure 1) (11).

The animals were run at 10 m/min for 5 days before starting the experiment. If they did not comply with the experimental protocol, they were excluded from the experimental group (12). The exercise protocol was performed at a speed of 15 m/min for 40

Figure 1. Treadmill system for forced exercise

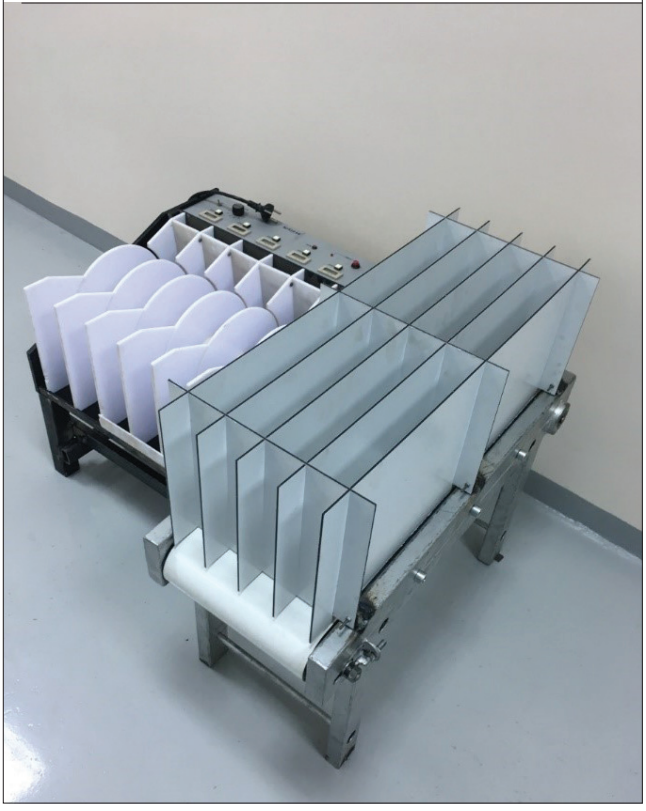
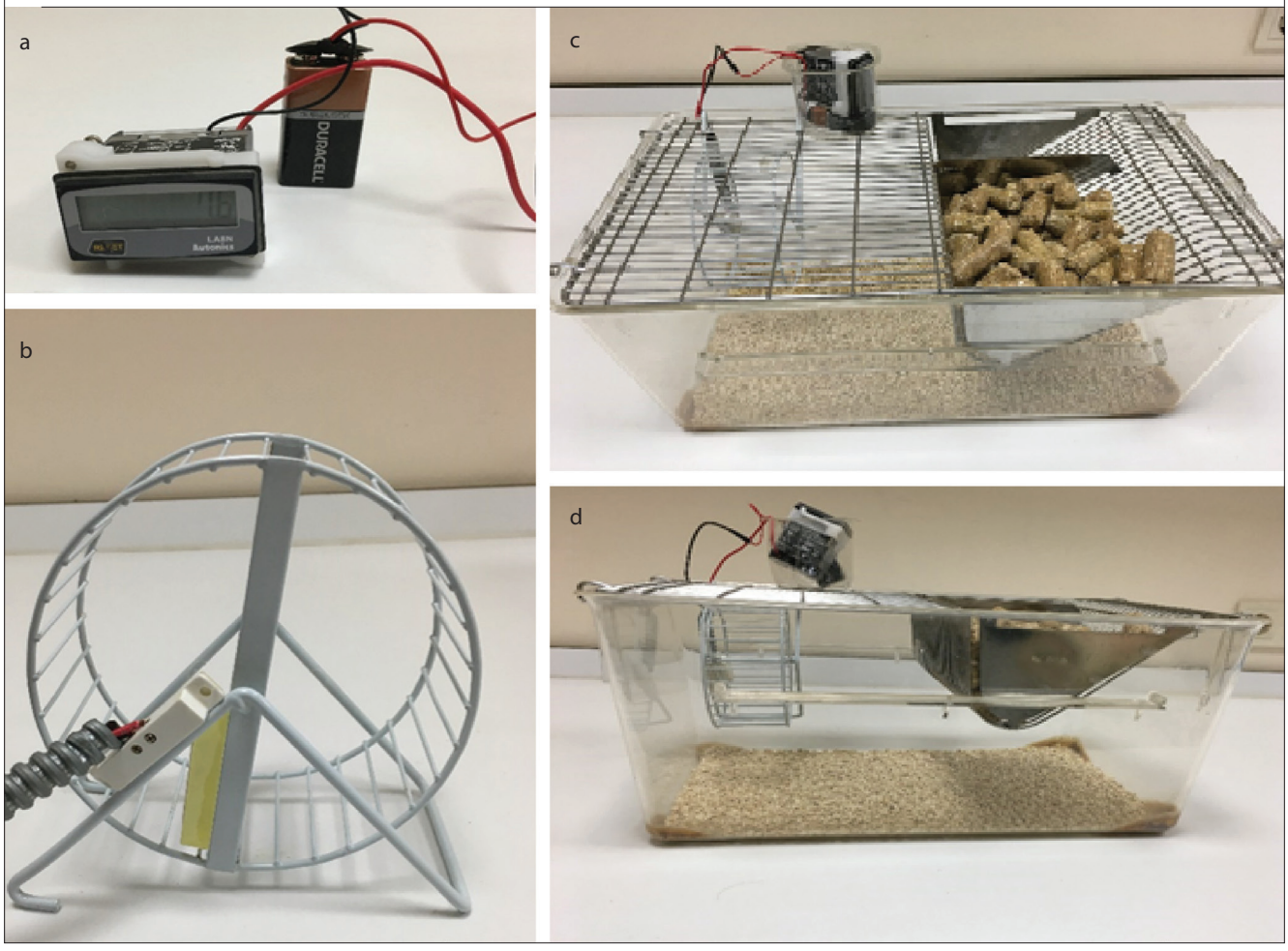


Figure 2. a-d. Wheel-running system for voluntary exercise



minutes per day (6 m/min for 5 min, 9 m/min for 5 min, 12 m/min for 20 min, 15 m/min for 5 min, 12 m/min for 5 min) and 5 days/week with 0° of inclination for the FE groups. Thus, each animal in the FE group run 450 m/day. This exercise is defined as a moderate-intensity exercise in the literature. FE was not applied to the sedentary mice groups; however, they were transported to the training room daily so that they were exposed to the same environmental stress with animals from the exercise group (13).

Voluntary exercise

A wheel-running system, which includes a 5" wheel and a magnetic counter and two electrodes, is produced for each cage as a custom design (Figure 2). Each animal from the VE groups was singly housed. The mice were allowed for 5 days to acclimate before the experiment. The number of wheel rotations was recorded every day at 10 am for each cage (14, 15).

Motor Performance Tests

Pole test

The pole test, performed according to Ogawa et al. (16), is a useful test evaluating the degree of bradykinesia, a typical and basic sign of Parkinsonism. The diameter of the metal bar is 0.8 cm, and the length is 50 cm. Before the experiment, this bar was wrapped with gauze to prevent slipping and make it more suitable for animal. A mouse was placed head upward at the top of the bar. The mouse traveled along the pole freely and came down to the floor (pre-trial). After animals were allowed to get used to the test system with two or three pre-trials, two measurements were taken. T_{turn} is the duration between the time point when the mouse is placed on top of the bar and when it turns the head down. T_{total} is the duration between the time point when the mouse is placed on top of the bar and when it comes down on the ground of the cage. The pole test was applied on the 7th days after the MPTP or saline injection for determining the bradykinesia (10).

Rotarod test

The rotarod test has been accepted widely, and it provides a simple drug-free evaluation of overall motor deficits in rodent models of a disease, such as PD, and it may offer a useful quantitative test to assess the efficacy of therapeutic strategies (17). This test was performed to evaluate motor functions 7th days after the MPTP or saline injection. After mice were placed on the rod (Ugo Basile Mouse Rotarod Cat. No. 47650, Varese, Italy) by hand, the speed was increased gradually from 5 rpm to 40 rpm during 300 seconds. The falling time of the mice from rod was recorded.

The experiment would be terminated for the animal which fell 3 times in 300 seconds. If a mouse never fell down from rod, the time was recorded as 300 seconds, and the experiment was not repeated again for this mouse (6).

Locomotor activity tests

Elevated plus maze test

The EPM test is a widely used test to measure anxiety in mice. The EPM apparatus was composed of four plastic arms (two open and two closed arms), arranged as a cross. The length of each arm is 45 cm and the width is 10 cm (9). Each mouse was placed into the center of the maze, and its behavior (spending time in the closed and open circuit, total distance moved, and velocities of motion and immobility) was recorded by a camera (The Axis M1145-L Network Camera system) for 5 minutes once every 2 weeks. The Etho Vision XT 11.5 was used to analyze the EPM data. The propensity to avoid the open arms is considered as an index of anxiety (18).

Open-field test

The OFT, developed by Hall (19) in 1934, is one of the most commonly used tests to detect changes in the emotional state of an animal before and after any procedure. It consists of a square platform measuring 90×90 cm and a camera system. The Axis M1145-L Network Camera system was used to record behavioral data, and the Etho Vision XT 11.5 was used to analyze the data. All animals were left in the center one by one, and behavioral data (the total distance moved, velocity, spent time at the center and periphery) were recorded for 5 minutes once every 2 weeks. In this test, the preferential exploration of the peripheral area of the open-field was considered an index of anxiety (18).

In both systems, the area was cleaned with 30% ethanol after each animal was tested, and it was waited to dry before the new animal underwent the experiment (20).

Statistical Analysis

Data were presented as the mean±SEM. The Statistical Package for the Social Sciences (SPSS) Statistics 20 (IBM Corp.; Armonk, NY, USA) was used to compare the means of data acquired. Motor performance was measured by using rotarod and pole test. Differences in motor performance among the groups were analyzed using the one-way analysis of variance and followed by the Bonferroni or Tamhane T2. Motor behaviours and anxiety was evaluated by using open field and elevated plus maze tests. Differences in motor behaviour among the groups were analyzed using Kruskal-Wallis test and followed by the "pairwise multiple comparisons" test. A statistically significant difference was considered for $p<0.05$.

RESULTS

Body Weight Changes

There was no significant difference in the body weights of the groups (Data not shown).

Motor Performance

Bradykinesia

The changes in the bradykinesia are presented in Figures 3 and 4. The T_{turn} is reduced in the long-term exercise (4FE+MPTP+4FE) group compared to other MPTP groups (Figure 3). Also, the T_{total} is reduced in all FE groups compared to VE groups and the MPTP group. However, T_{total} was significantly longer in the MPTP, 4VE+Saline+4VE, MPTP+4VE, and 4VE+MPTP+4VE groups than in the control, as shown in Figure 4 ($p<0.001$).

Rotarod

The injection of MPTP impaired the ability of mice to stay on the rotating rod for 5 min. There was a significant difference between

Figure 3. T_{turn} values for determining of bradykinesia by the pole test. Data are the means±SEM. Statistical analysis was conducted using the Kruskal–Wallis, followed by pairwise multiple comparisons against the indicated group

*p<0.001 vs. control002E

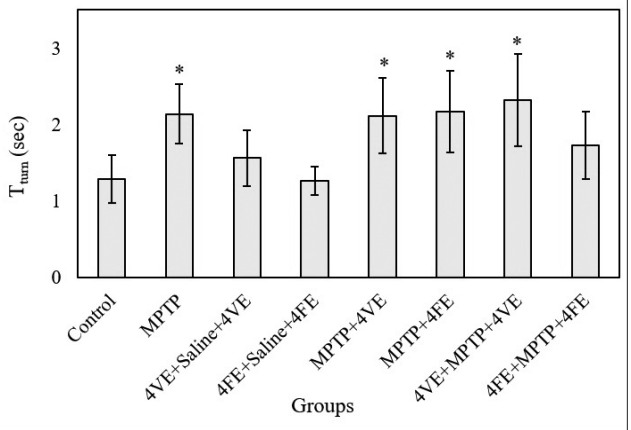


Figure 4. T_{total} values for determining of bradykinesia by the pole test. Data are the means±SEM. Statistical analysis was conducted using the one-way analysis of variance, followed by the Bonferroni test against the indicated group

*p<0.001 vs. control

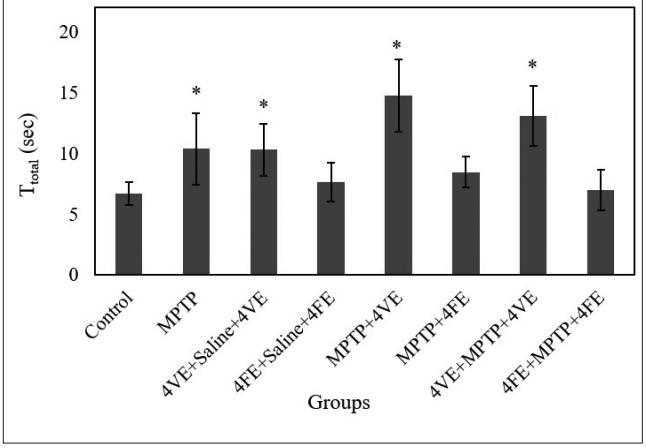


Figure 5. Rotarod performance at 7 days after the MPTP injection. Data are the means±SEM. Statistical analysis was conducted using the one-way analysis of variance, followed by Tamhane's T2 test against the indicated group
*p<0.001 vs. MPTP

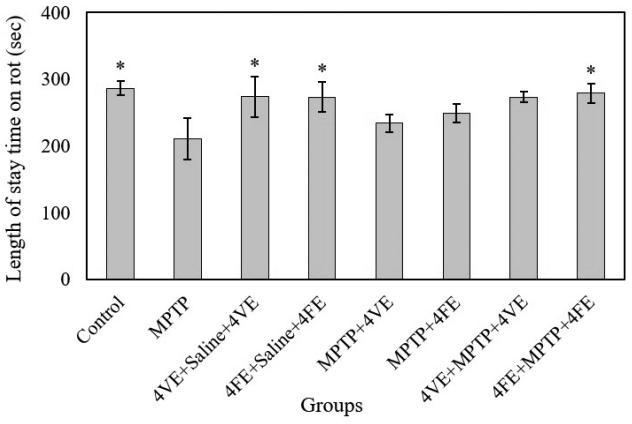
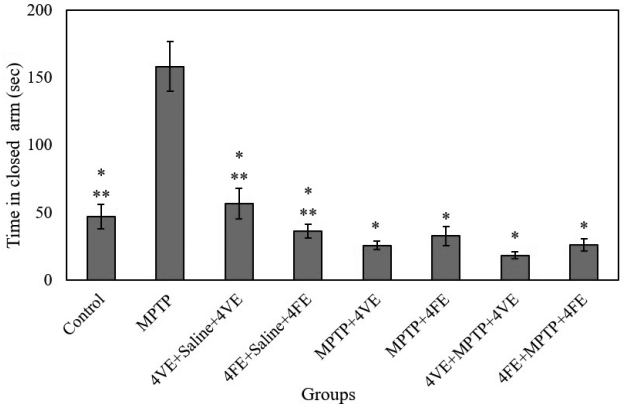


Figure 6. The total time of groups spent in the closed arm of the elevated plus maze test. Data are the means±SEM. Statistical analysis was conducted using the Kruskal–Wallis test, followed by pairwise multiple comparisons against the indicated group
*p<0.001 vs. MPTP; **p<0.001 vs. 4VE+MPTP+4VE
MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 4VE: Group performed voluntary exercise during 4 weeks; SEM: Standard error of mean



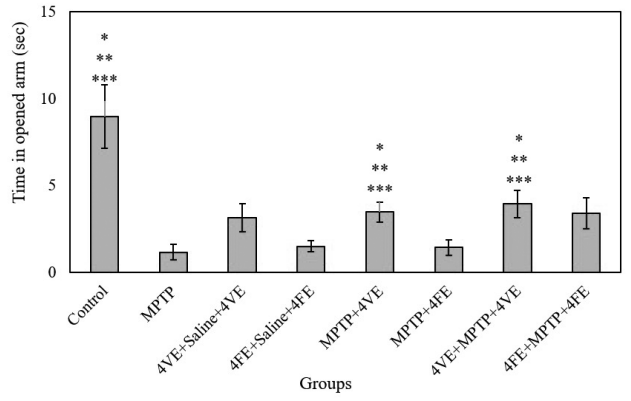
the 4FE+MPTP+4FE and all of the other MPTP groups, as shown in Figure 5 (p<0.001). Long-term FE especially prolonged the duration of the staying on the rod.

Locomotor activity

Elevated plus maze test

Elevated plus maze is one of the most popular tests for evaluating anxiety. The total time spent by animals in the closed arm of the EPM test was shown in Figure 6. Accordingly, there was a significant decrease in the time spent in the closed arm in all groups compared to the MPTP group (p<0.001). Namely, anxiety was reduced in all exercise groups.

Figure 7. The total time of groups spent in the open arm of EPM. the elevated plus maze test. Data are the means±SEM. Statistical analysis was conducted using the Kruskal–Wallis test, followed by pairwise multiple comparisons against the indicated group
*p<0.001 vs. control; **p<0.001 vs. MPTP, ***p<0.001 vs. MPTP+4FE



The time spent in closed arm in the EPM test gives information about animal's anxiety. If the animal is anxious, it stays longer than normal in the closed arm. The spent time in the closed arm in the MPTP group was significantly prolonged compared to the all other groups, as presented in Figure 6. Namely, animals from the MPTP group were more anxious than the others. However, the time spent in the closed arm in the 4VE+MPTP+4VE group was significantly reduced compared to the control, 4VE+Saline+4VE, and 4FE+Saline+4FE groups.

The time spent in the opened arm of the EPM test was reduced in the MPTP, 4FE+Saline+4FE, and MPTP+4FE groups compared to the control (Figure 7). Also, the time spent in the opened arm was reduced in the MPTP and MPTP+4FE groups compared to the MPTP+4VE and 4VE+MPTP+4VE groups.

Open-field test

Mobility decreases when animals are anxious. The total mobility in the MPTP and 4VE+Saline+4VE groups decreased compared to other groups, as shown in Figure 8.

It has been accepted that animals, which spend more time in the central zone, are less anxious, while animals, which spend more time in the peripheral zone, are more anxious. The spent time in the central zone was significantly increased in the control, 4VE+Saline+4VE, 4FE+Saline+4FE, MPTP+4VE and 4VE+MPTP+4VE groups compared to the MPTP groups, as shown in Figure 9.

The time spent in the peripheral zone was increased significantly in the MPTP, 4VE+Saline+4VE, MPTP+4VE, and 4FE+MPTP+4FE groups, compared to control, while it was decreased significantly in the 4VE+Saline+4VE, 4FE+Saline+4FE, MPTP+4FE, and 4VE+MPTP+4VE groups compared to the MPTP group, as presented in Figure 10.

Figure 8. The total mobility of groups in the open-field test. Data are the means±SEM. Statistical analysis was conducted using the Kruskal–Wallis test, followed by pairwise multiple comparisons against the indicated group
*p<0.001 vs. MPTP

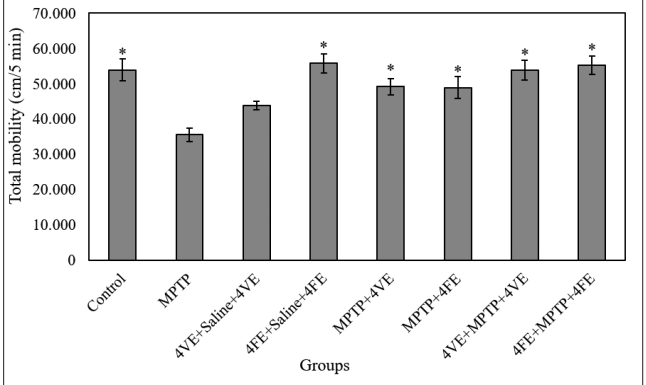


Figure 9. The time spent in the central zone in the open-field test. Data are the means±SEM. Statistical analysis was conducted using the Kruskal–Wallis test, followed by pairwise multiple comparisons against the indicated group
*p<0.001 vs. MPTP

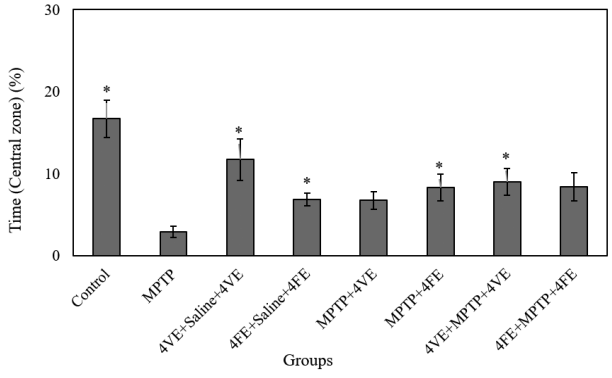
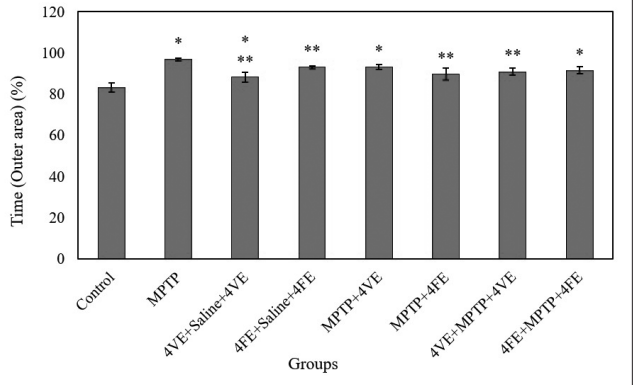


Figure 10. The time spent in the peripheral zone in the open-field test. Data are the means±SEM. Statistical analysis was conducted using the Kruskal–Wallis test, followed by pairwise multiple comparisons against the indicated group
*p<0.001 vs. control; **p<0.001 vs. MPTP



DISCUSSION

The presented study revealed that exercise had a healing effect on motor activity and anxiety. A meta-analysis study showed that exercise can increase the physical functions, quality of life, power, balance, and walking speed of patients with PD (6).

The duration of staying on the rod in the MPTP group was shorter than in other groups in the rotarod test used to evaluate motor performance. However, the duration of staying on the rod in the FE group, performing FE during 8 weeks (4FE+MPTP+4FE), was longer (p<0.001). Namely, it was found that performing long-term FE improved motor performance. A study in the United States (2016) reported that FE and anti-parkinson drugs provide similar levels of improvement in the symptoms of the disease. Clinical evaluation of the improvement of upper extremity motor function of patients showed a correlation between the activation fields and the activation scores of FE and anti-Parkinson drugs. These results suggested that FE may be a useful and non-invasive method for the improvement of the PD symptoms (21). Therefore, it was reported that FE is an effective method that partially relieved motor impairment and improved the motor function by reducing loss of dopaminergic neurons and α-synuclein expression in the MPTP animal model in another study (22).

A motor dysfunction, bradykinesia, was assessed by the pole test. Especially FE groups showed significant improvement (p<0.001), whereas the results of VE groups were similar to the MPTP group. Particularly long-term FE seems to have contributed significantly to improved motor functions. In a study on PD, rats performed walking and balance exercises after the PD model, and improvements in theirmotor function were examined with neuro-behavioral evaluations. According to this study; both types of exercise were found to improve motor function when assessed on Days 7, 14, 21, and 28 in the pole test (23).

The longer time spent in the closed arm (24) and decreasing in total mobilization (25) indicate that the animal is more anxious during the EPM test. In the presented study, it was determined that there was an improvement in anxiety and depressive behaviors in both types of exercise. Another study reported that exercise improves the cognitive function and reduces symptoms of depression in elderly people (14). The results of this study are consistent with the results obtained in the presented study.

According to the EPM test results, the time spent in the open arms was reduced in the FE groups compared to the VE groups. This result indicates that animals in the FE groups are more anxious. In a study conducted in 2016, it was shown that the corticosterone level, known as stress hormone, was lower in the VE group performed during 10 days than the forced treadmill- or forced wheel-running exercise groups. Researchers noted that VE worked like a reward system and increased motivation, so it did not increase corticosterone levels (26).

More time spent in the peripheral zone than in the middle area indicates anxiety in the OFT (27). In the presented study, the time spent in the central zone was significantly reduced in the long-term VE group, but there was no significant decrease in the long-term FE group. At the end of the OFT test, it was indicated that VE seems to be more beneficial in improving anxiety than FE. This may be due to an anxiety enhancing effect of FE (23).

All the study data showed that the presence of an improvement in motor skills and anxietic behaviors in all exercise types. In addition, long-term FE is more beneficial for ameliorating motor skills. However, long-term VE has a less significant anxietic effect.

CONCLUSION

In conclusion, FE has a positive effect on motor activity when done for a long time. VE has an ameliorative effect on anxiety. Thus, exercise is a non-invasive method that clinicians can safely recommend to all patients with PD.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gazintep University (no: 2017/14, date: 07.06.2017).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - N.Ö.D., R.B.; Design - N.Ö.D.; Supervision - N.Ö.D., R.B.; Resources - N.Ö.D.; Materials - N.Ö.D.; Data Collection and/or Processing - N.Ö.D.; Analysis and/or Interpretation - N.Ö.D., R.B.; Literature Search - N.Ö.D.; Writing Manuscript - N.Ö.D.; Critical Review - N.Ö.D., R.B.

Conflict of Interest: The authors have no conflicts of interest to declare.

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