European Journal of Therapeutics https://doi.org/10.58600/eurjther.20232902-1594.y

Original Research

The Effect of Forced Exercise on Striatal and Serum Serotonin Levels in a Parkinson's Mouse Model

Nadide Özkul Doğru[1], Hatica Kübra Yiğit[1], Ayşegül Burçin Yıldırım[2], Hasan Ulusal[3], Mustafa Örkmez[3], Elif Yiğit[1], Hakim Çelik[4]

[1] Department of Physiology, Gaziantep University, Faculty of Medicine, Gaziantep, Turkey

[2] Department of Histology and Embryology, Gaziantep Islam Science and Technology University, Faculty of Medicine, Gaziantep, Turkey

[3] Department of Medical Biochemistry, Gaziantep University, Faculty of Medicine, Gaziantep, Turkey

[4] Department of Physiology, Harran University, Faculty of Medicine, Şanlıurfa, Turkey

ABSTRACT

Objective: The general treatment approach for Parkinson's disease (PD) is L-dopa administration. While L-dopa only relieves dopaminergic deficiency, it has no effect on the serotonergic system, which is thought to be impaired in the disease. The limitations of current treatment methods have made it necessary to discover new approaches to the treatment of the disease. Studies conducted in recent years report that different types of exercises lead to improvements in the symptoms of PD. Exercise decreases serotonin levels and increases dopamine levels. However, the effect of exercise on serotonin and dopamine levels in PD and its effect on non-motor symptoms such as anxiety and depression are unknown.

Methods: PD is created using MPTP. The exercise groups were given challenging treadmill exercises for six weeks. Serotonin and dopamine levels were measured in the striatum and serum. Parkinson's symptoms were examined with pole test and behavioral tests.

Results: Exercise significantly reduced bradykinesia, increased motor activity, and decreased anxiety behaviors in the exercise groups. While exercise increased striatal dopamine levels in all exercise groups, there was no difference in striatal serotonin levels. However, the serotonin serum level decreased in the PD model group. While treadmill exercise increased striatal dopamine levels in the Parkinson's mouse model, it did not cause any change in striatal serotonin levels. However, the decrease in serum serotonin level was determined only in the MPTP group.

Conclusion: The fact that the decrease in serotonin level was only in the disease group and the lower level of anxiety observed in behavioral experiments suggested that regular treadmill exercise was the reason. However, this improvement was not observed in cases where the anxiety level was very high.

Keywords: forced exercise, serotonin, treadmill exercise, Parkinson's disease, dopamine

INTRODUCTION

Dopaminergic neuronal loss is known as the main cause of Parkinson's disease (PD), and motor and non-motor symptoms are seen in this disease. Motor symptoms include bradykinesia, rigidity, and tremor, while non-motor symptoms include a variety of symptoms such as cognitive impairment, autonomic dysfunction, sleep disturbances, depression, and anxiety [1-3]. It is known that 60% of people with PD have non-motor symptoms [4]. While depression affects approximately 25-40% of people with PD, anxiety is seen in 40% [5]. Some studies also state that mood disorders appear years before the motor symptoms of the disease appear [6]. However, non-motor symptoms are generally not treated [7]. The pathophysiology underlying mood disturbances such as anxiety and depression in PD is still not fully understood. However, there is evidence showing that the cholinergic, dopaminergic, and noradrenergic systems in the brain have an effect on mood disorders [8]. It is known that dysfunction in the serotonergic [5-hydroxytryptamine) system contributes to mood disorders and cognition disorders [9]. An old systemic review stated that the use of antidepressants treated depression in PD, but the results were not consistent with each other. In addition, it has been reported that the drugs used show side effects at a rate of about 26% [8].

Physical activity's affirmative effects on health have been known for years. Regular physical exercise increases synaptic plasticity,

How to cite: Doğru NÖ, Yiğit HK, Yıldırım AB, Ulusal H, Örkmez M, Yiğit E, Çelik H (2023) The Effect of Forced Exercise on Striatal and Serum Serotonin Levels in a Parkinson's Mouse Model. Eur J Ther. 29(2):179-187. https://doi.org/10.58600/eurjther.20232902-1594.y

Corresponding Author: Nadide Özkul Doğru **E-mail:** nadideozkul@hotmail.com

Received: 17.05.2023 • **Accepted:** 26.05.2023 • **Published Online:** 27.05.2023

pISSN: 2564-7784
eISSN: 2564-7040

modulates neurogenesis, and increases brain perfusion. It has even been reported that exercise has antidepressant and anxiety-reducing effects [10, 11].
In this research, we researched the effect of forced treadmill exercise on serotonin and its relationship with non-motor functions like anxiety and depression in a mouse model of Parkinson.

METHODS

Animal: 24 adult C57Bl/6 male mice were tested in the experiment. Standard rodent chow and water were placed in the cages of the animals. Animals were fed ad-libitum. The procedure of the experiment was confirmed by "the animal care and usage committee of Gaziantep University" and was in accordance with the "Declaration of Helsinki and International Association for the Study of Pain Guidelines". 24 mice were casually separated into four groups. Groups are 1) Control (CON), 2) MPTP (MPTP), (3) Control+Exercise (CON+EXE), and (4) MPTP+Exercise (MPTP+EXE).

Parkinson Model: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is known as a neurotoxin. It was used to induce dopaminergic neurodegeneration in this experiment. The MPTP-Parkinson model very closely reflects the clinical symptoms of PD. MPTP (M-0896, Sigma-Aldrich, St. Louis, Mo. USA) solution was prepared with normal saline at 3mg/ml and injections were administered 4 times at a dose of 20 mg/kg intraperitoneally to reduce the risk of animal loss due to the toxin [12,20].

Exercise Protocol: The treadmill (The Ugo Basil, 47300) was used to apply a forced exercise protocol to the animals and had dimensions of 38x5x5 cm. Adaptation exercise was trained at 10 m/min for 5 days to measure the exercise compliance of the animals before applying the exercise protocol. Animals that did not comply with exercise were not included in the experimental group [13]. The mice in the exercise groups exercised five days a week. The exercise continued regularly for six weeks. Mice were subjected to moderate-intensity treadmill exercise for 40 minutes per day [14]. The exercise protocol is as follows: Speed for exercise is 15 m/min (5 min 6 m/min, 5 min 9 m/min, 20 min 12 m/min, 5 min 15 m/min, and 5 min 12 m/min) with 0° inclinations.

Pole test: The pole test is used for the assessment of bradykinesia symptom [15-19]. Animals injected with MPTP were applied to the pole test on the 7th day of the experiment. The application of the pole test is as follows: A rod's length, used in the pole test, is 50 cm, and its diameter is 0.8 cm. The mouse was put on the end of the rod with its head facing up. The time until he turned his head was considered Tturn, and the time until he turned his head down was considered Ttotal. The average of the three measurements in the experiment was recorded.

Open Field Test (OFT) and Elevated Plus Maze (EPM) Test: Both tests are used to qualify locomotor activities, and mood disorders such as anxiety and depression. EPM is a plus-shaped experimental setup with 2 open and 2 closed arms at a specific height from the ground, and OFT consists of a 50 x 50 cm square platform. A camera system is placed in full view of both test platforms to record behavioral data [26, 27]. Recording behavioral data were taken by the Axis M1145-L network camera system, and analysis was made using Etho Vision XT 11.5 in this study. Each mouse was put in the midpoint of the open field cage or the middle zone of the elevated place maze for testing all parameters, and the camera continued to record for 5 minutes. The area in both systems was completely cleaned with a 10% ethanol solution to wipe away the animal odors at the end of each experiment, and a new animal was included in the experiment after it was allowed to dry thoroughly [27].

Tissue preparation: All mice were anesthetized after a 6-week treadmill exercise. Xylazine was injected at a dose of 10 mg/kg, and ketamine was injected at a dose of 100 mg/kg. Injections are applied intraperitoneally for anesthesia. Animals were guillotined and euthanized 48 hours after the last exercise to exclude the acute effects of exercise.

Brain dissection and tissue homogenization: Brains were removed, and striatum tissues were immediately isolated [28]. Brain tissues and serum were preserved at -80°C until homogenization. The striatum was homogenized for 30 seconds, using a sonicator (Branson Sonifier® UNITS Model S-150D) on ice. Then, centrifugation (Thermo Scientific MicroCL Centrifuge) was performed at 5,000 g for 5 minutes at 8°C for the tissues, and supernatants were collected. Protein determination (BioTek, Synergy H1 Microplate Reader) was performed using 2 microliter samples from each different homogenate.

Determination of dopamine and serotonin levels: Dopamine and serotonin levels were determined by a sandwich enzyme-linked immunosorbent assay (ELISA) (Mouse Serotonin ELISA Kit, FineTest, China Cat: No: EM1465, Mouse Dopamine ELISA Kit, FineTest, China Cat: No: EM1712), following the indication provided by the manufacturer. Serotonin and dopamine levels were examined in both the striatum and serum.

Statistical assessment: Statistical Package for the Social Sciences (SPSS) software for Windows (version 20.0) was used for data analysis. Whether the data had a normal distribution or not was determined by the Kolmogrov-Smirnow test. A one-way analysis of variance (ANOVA) test was applied to normally distributed data. Kruskal Wallis was used for the analysis of non-normally distributed data. The Tukey Post Hoc Test was applied to parametric data to determine the differences between groups. Dunn's test was used to determine the differences between groups in the data that was not normally distributed. Data are presented as mean±SD. Data were considered significant if the p-value was less than 0.05 ($p<0.05$).

RESULTS

Bradykinesia assessment: The pole test, which measures impairments in starting and maintaining movement, shows two parameters. In our results, a significant prolongation was observed statistically in the time to start movement in the MPTP and MPTP+EXE groups compared to the CON and CON+EXE (Figure1). Total shows the time it takes to start and maintain

movement. This value was significantly longer only in the MPTP group compared to the CON and CON+EXE groups (Figure 2).

Motor performance assessment: Motor activities were evaluated with EPM and OFT tests. Mean distance moved in EPM (Figure 3) and velocity in OFT (Figure 4) decreased only in MPTP group compared to other groups.

Anxiety and depression assessment: Anxious and depressed animals spend longer in closed arms and shorter in open arms in EPM. Anxious and depressed animals spend longer in the EPM in closed arms and shorter in open arms. Figure 5 shows the average time spent by the groups in the open arms. According to Figure 5, the time spent in the open arms of the MPTP group in EPM decreased compared to the CON and CON+EXE groups. However, no significant difference was found between the disease model group and the exercise disability model group. In Figure 6, it is seen that the time spent in closed arms decreased only in the CON group compared to the MPTP and MPTP+EXE groups.

Anxious and depressed animals spend longer time in OFT in the peripheral area and shorter in the middle area. Compared to the other groups, the MPTP group passed less time in the middle area (Figure 7) and more time in the peripheral area (Figure 8).

Assessment of Dopamine and Serotonin Levels: Both neurotransmitter levels were measured in the striatum and serum. It was found to be statistically significantly reduced in striatal dopamine levels in the PD model group compared to the other groups (Figure 9). However, there was no difference in serum dopamine levels among groups (the figure is not shown). It was found to be statistically significantly reduced in serum serotonin levels in only the MPTP group compared to the control group (Figure 10). However, there was no difference in striatal serotonin levels among groups (the figure is not shown).

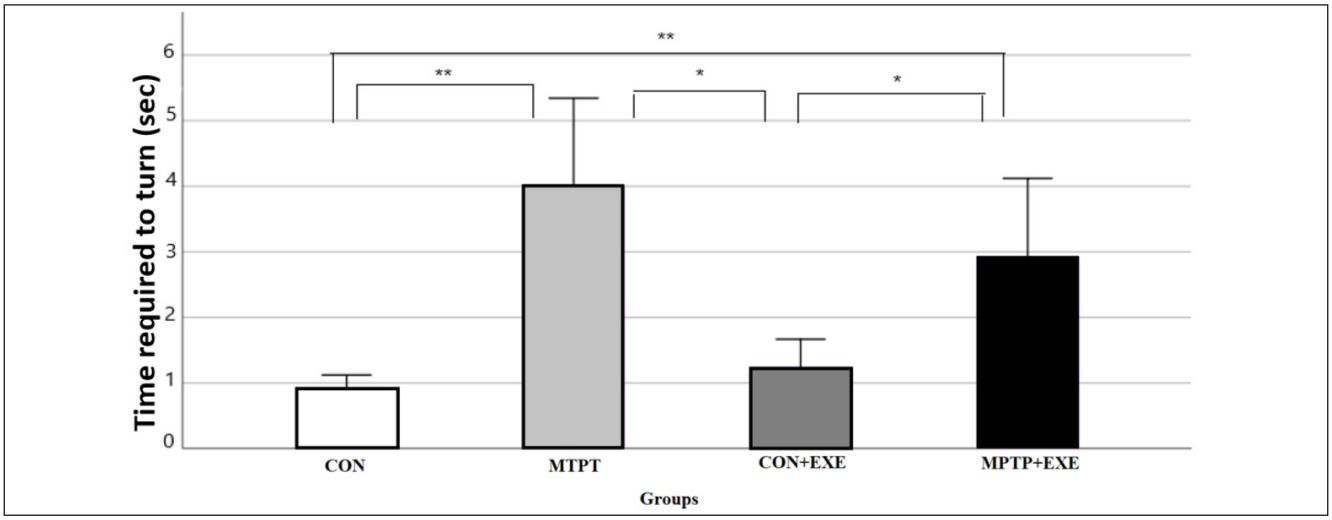

Figure 1. Tturn means the time it takes the animal to turn its head down after placing it on the pole. (n=6, *p < 0.05 vs. MPTP and CON+EXE groups, CON+EXE and MPTP+EXE groups, **p < 0.01 vs. CON and MPTP groups, CON and MPTP+EXE groups)

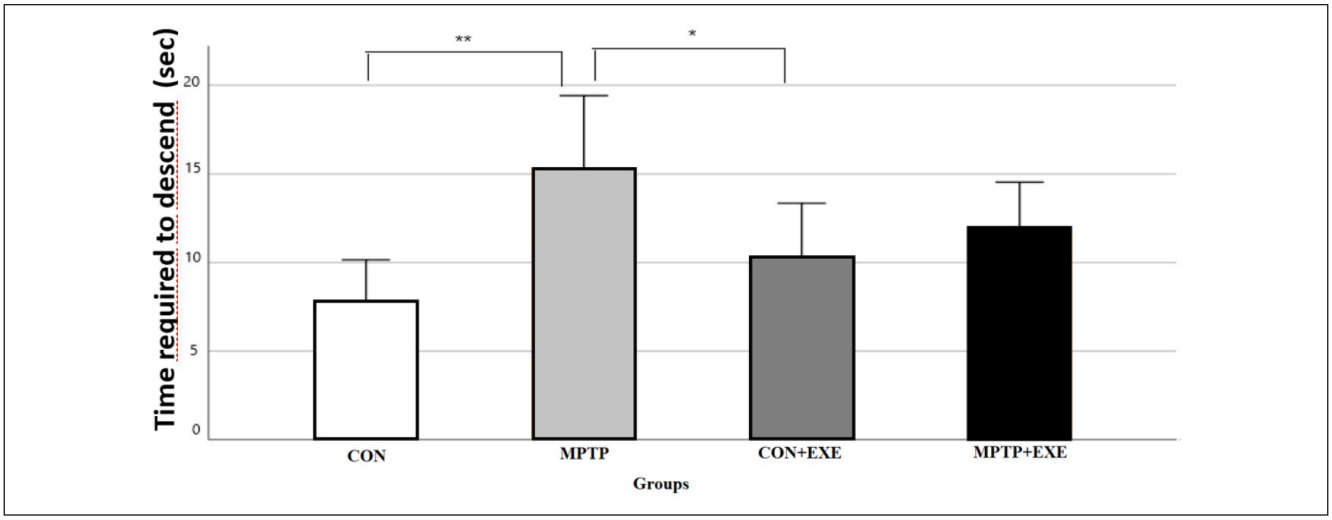

Figure 2. Total shows the time it takes to start and maintain movement. (n=6, *p < 0.05 vs. MPTP and CON+EXE groups, **p < 0.01 vs. CON and MPTP groups)

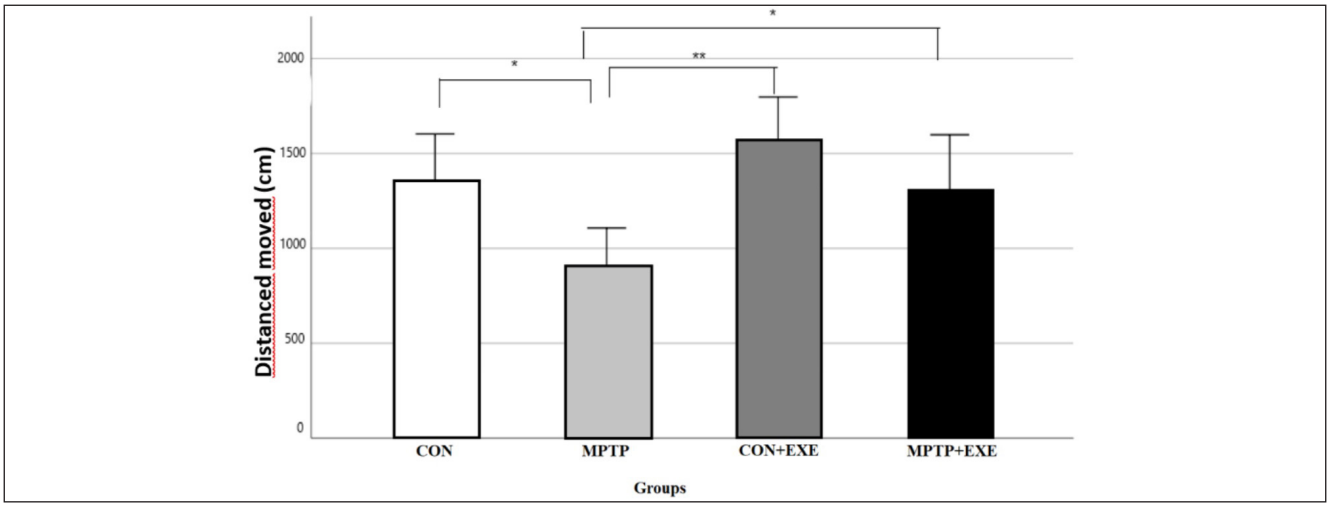

Figure 3. The mean distance moved in the EPM test (n=6, *p<0.05 vs. MPTP and CON groups, MPTP and MPTP+EXE groups, **p<0.01 vs. MPTP and CON+EXE groups)

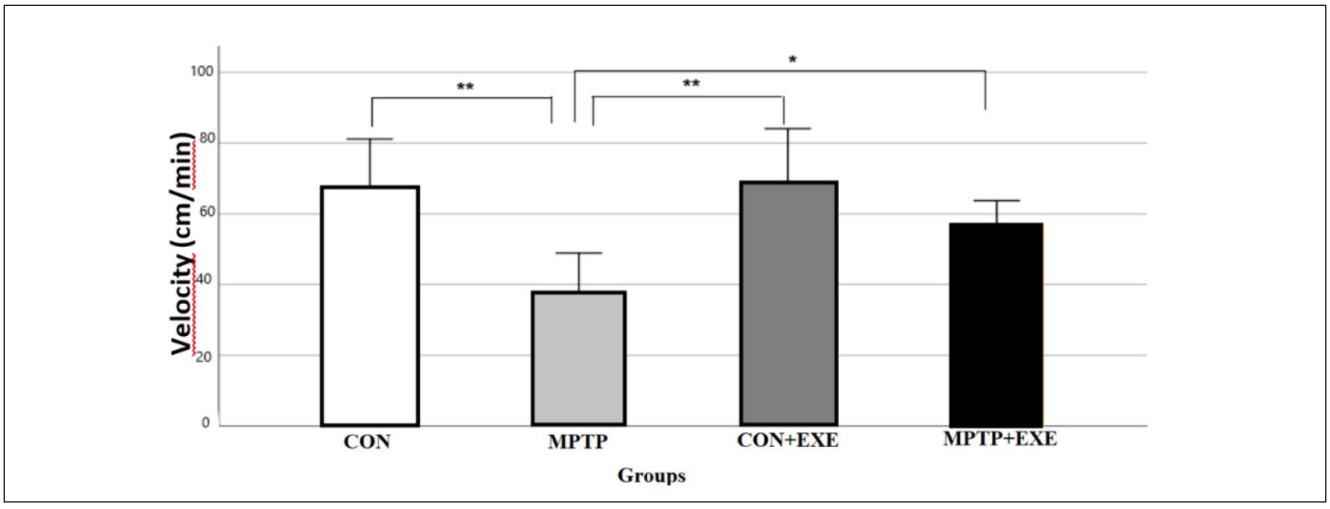

Figure 4. The velocity in the OFT test (n=6, *p<0.05 vs. MPTP and CON groups, MPTP and MPTP+EXE groups, **p<0.01 vs. MPTP and CON+EXE groups)

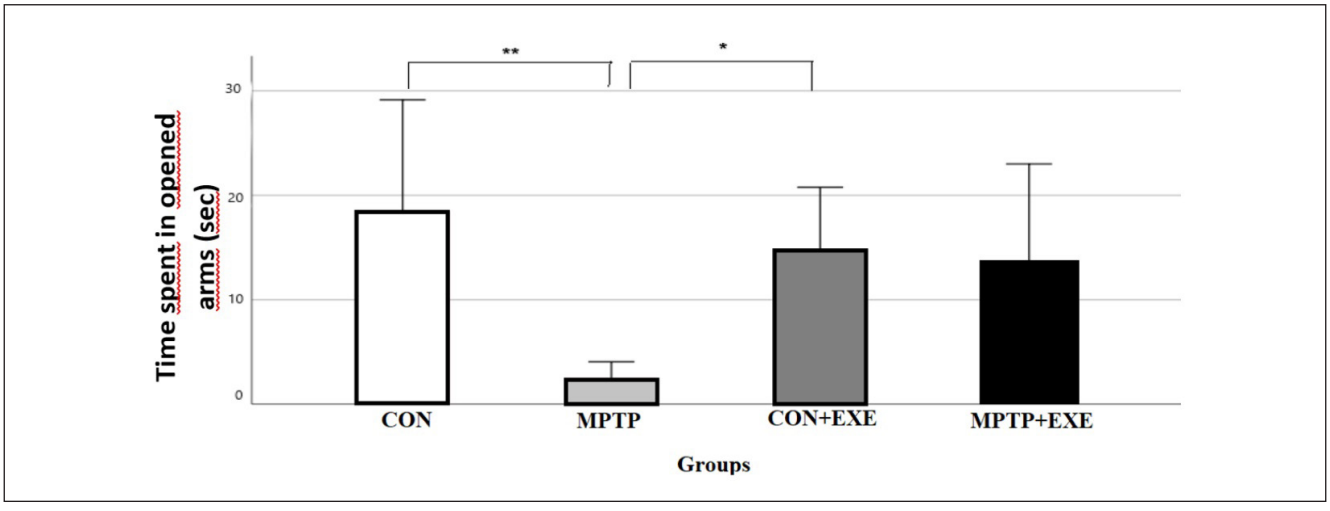

Figure 5. The spent time in the opened arms in the EPM test (n=6, *p < 0.05 vs. MPTP and CON+EXE groups, **p<0.01 vs. CON and CON+EXE groups)

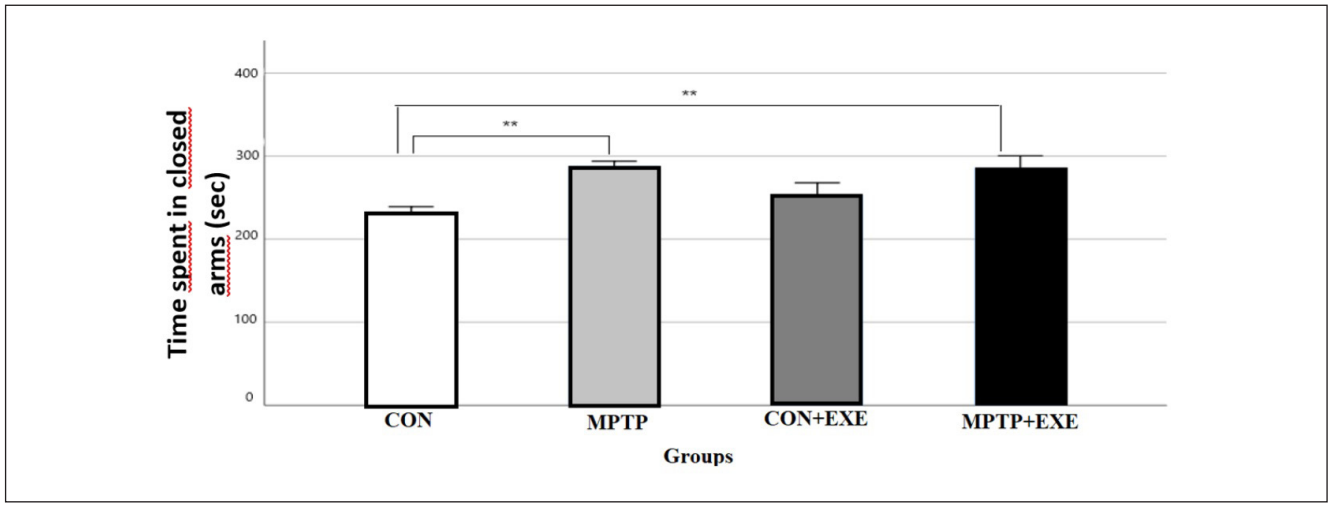

Figure 6. The spent time in the closed arms in the EPM test (n=6, **p<0.01 vs. CON and MPTP groups, CON and MPTP+EXE groups)

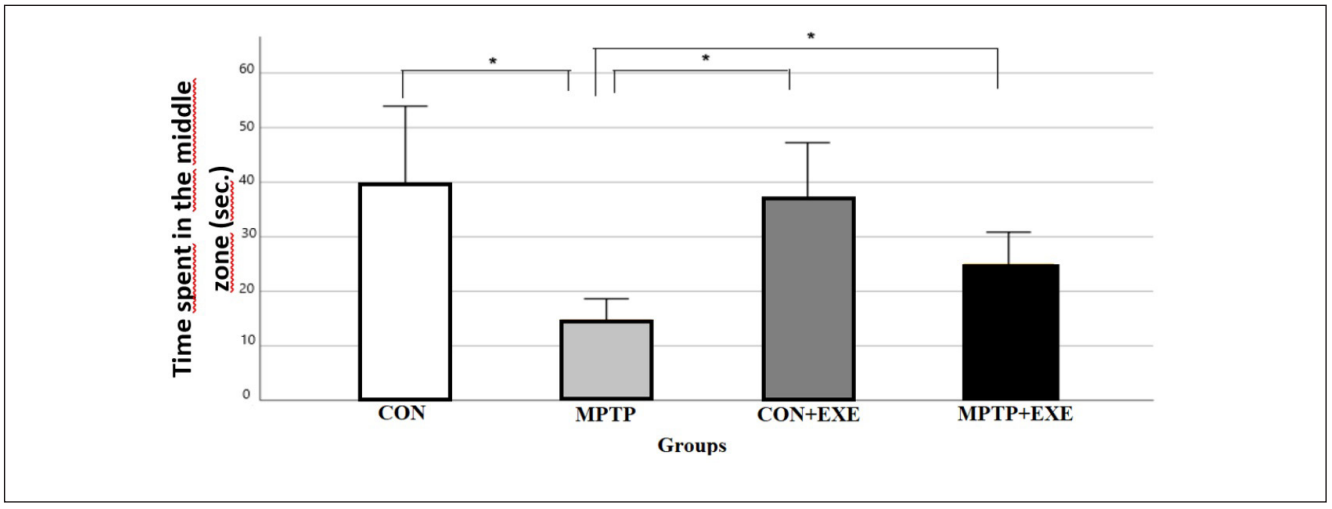

Figure 7. The spent time in the middle zone in the OFT test (n=6, *p < 0.05 vs. CON and MPTP groups, MPTP and CON+EXE groups, MPTP and MPTP+EXE groups)

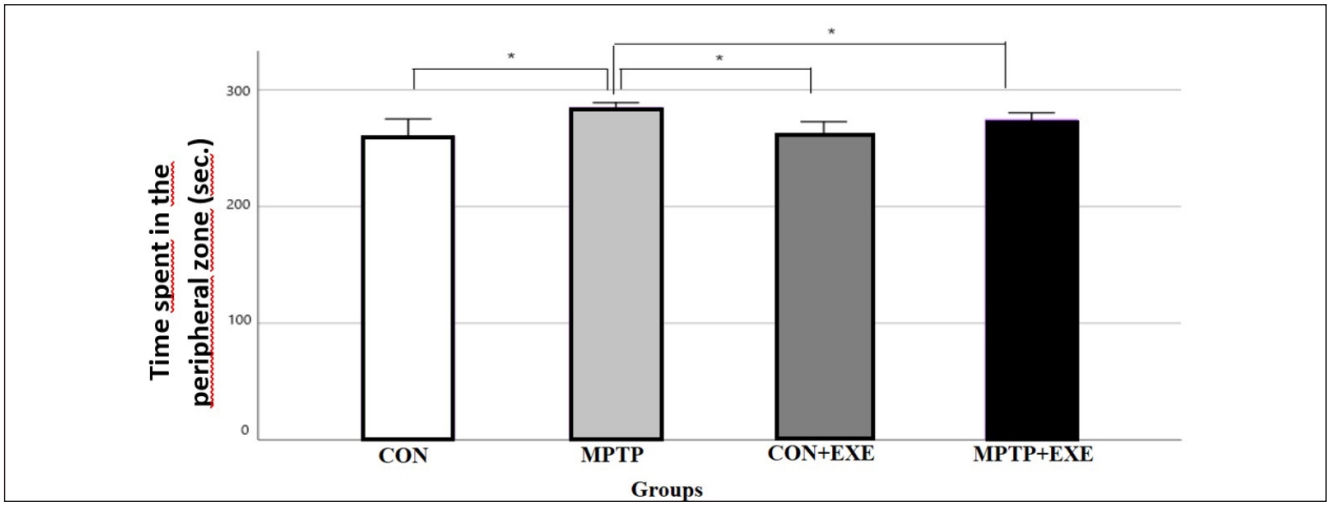

Figure 8. The spent time in the peripheral zone in the OFT test (n=6, *p<0.05 vs. CON and MPTP groups, MPTP and CON+EXE groups, MPTP and MPTP+EXE groups

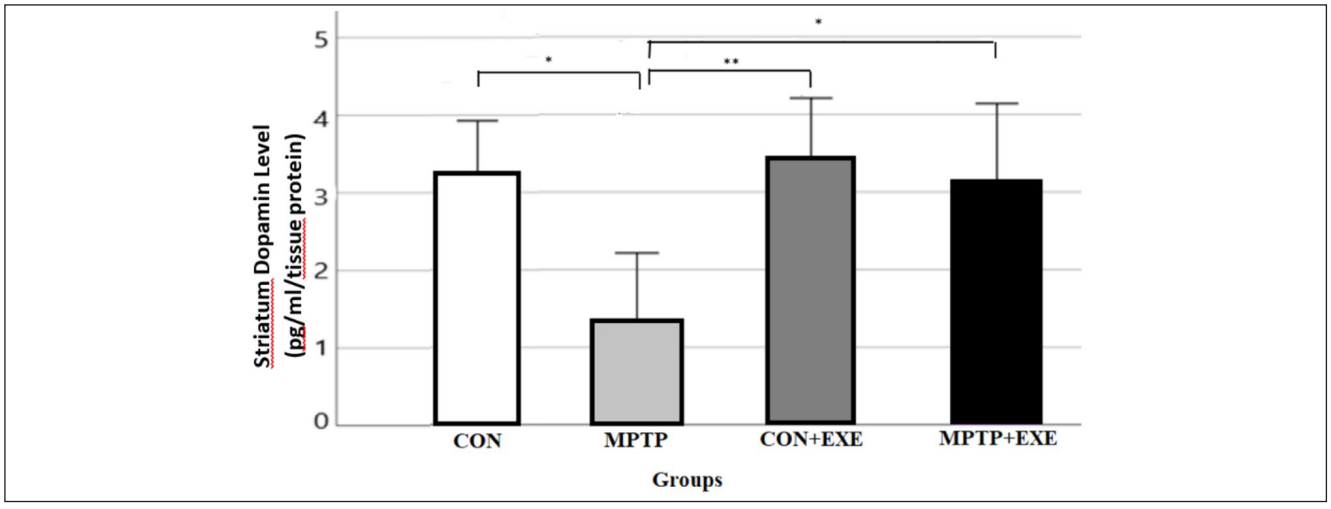

Figure 9. The striatal dopamine levels (n=6, *p<0.05 vs. CON and MPTP groups, MPTP and MPTP+EXE groups, **p<0.01 vs. MPTP and CON+EXE groups)

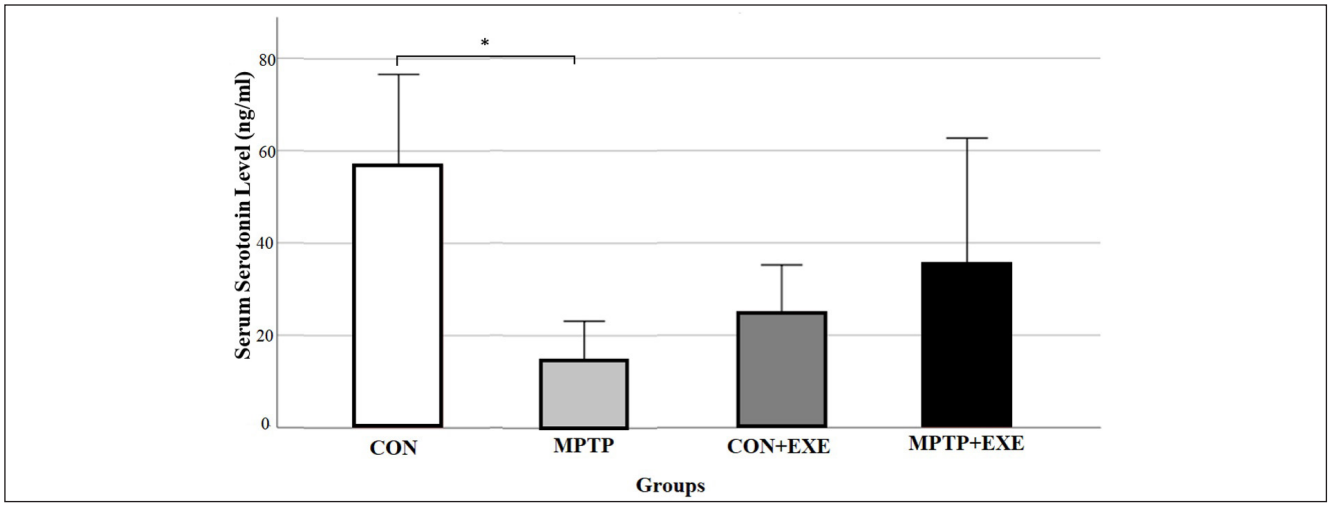

Figure 10. Serum serotonin levels (n=6, *p<0.05 vs. CON and MPTP groups)

DISCUSSION

PD is the second most common neurodegenerative disease [3,21]. The priority as a treatment approach is the treatment of motor symptoms, as motor symptoms further prevent the patient from continuing his daily life. However, it is known that non-motor symptoms occur long before the motor symptoms of the disease appear [6]. Treating non-motor symptoms together with motor symptoms will increase the patient's quality of life [7]. It is known that the main reason for the emergence of motor symptoms is dopamine deficiency. However, there is no definite information about the cause of non-motor symptoms. Studies show changes not only in the dopaminergic system but also in the cholinergic and noradrenergic systems in Parkinson's patients [8].

Parkinson's model with MPTP injection is accepted as the "gold standard" in the development of symptomatic treatment methods, evaluation of pharmacological agents, and development of new strategies. The advantages of the model created with MPTP are that mice cost less to purchase and house than other animals, MPTP does not require stereotaxic surgery, causes bilateral lesions, and shows most of the known biochemical features of PD [13].

In our pole test results, the time to start movement was extended in the MPTP group. However, exercising in the Parkinson model group did not significantly reduce the time to start movement (Tturn). Exercise does not improve the bradykinesia in PD. However, the time required to descend was prolonged only in the MPTP group. However, distance moved and velocity values decreased significantly in the solely Parkinson model group compared to others. Although the forced treadmill exercise did not shorten the starting time to move, it improved the ability to maintain movement by healing motor coordination. We reported in our previous study that moderate-intensity forced treadmill exercise provided restorative effects on motor coordination [22].

There are studies showing that exercise improves mood [23,41], and it even reduces anxiety in humans [24]. However, there is conflicting information regarding the alteration of anxiety in

exercising animals. In other words, many studies report that exercise decreases anxiety, while other researches report that exercise increases anxiety [25,26]. The study by Pietrelli et al. reported that moderate-intensity treadmill exercise reduced anxiety in rodents [11]. However, the study by Burghardt et al. reported that exercise had no effect on anxiety [27]. In our study, we found that moderate-intensity forced treadmill exercise reduces anxiety, a symptom of PD. Because parameters for locomotor activity [total distance and velocity of movement] decreased in animals with the MPTP injection in OFT [28] and spent more time in the peripheral area than in the middle region [29] are findings showing that anxiety develops. However, the results of the EPM test showed that anxiety did not decrease in the Parkinson's model group that exercised. Because the MPTP+EXE group spent more time in closed arms. It was known that the EPM test causes more anxiety in mice [30]. In other words, it is thought that the curative effect of forced exercise is relatively less at higher anxiety levels. In one study, the level of corticosterone, known as the stress hormone, was lower in the group that volunteered for 10 days compared to the groups that did the forced treadmill or forced spinning wheel exercise. Researchers have stated that voluntary exercise works like a reward system and increases motivation, and accordingly, corticosterone levels do not increase [31].

The effect of different types of exercise on brain neurotransmitters has been investigated for many years. It is known in the literature that both central dopaminergic and serotonergic activities change depending on exercise [32]. A deficiency of the dopamine neurotransmitter is accountable for the clinical symptoms of PD [33]. There is conflicting information in the published literature regarding the effect of treadmill exercise on dopamine levels in the striatum. Many scientific researches have reported that treadmill exercise increases dopamine levels in the striatum [32,34], while others do not [32,35]. We found that striatal dopamine levels were significantly decreased in the MPTP group compared to the control group after MPTP-induced degeneration, while no difference was found in the other groups in this research. It has been thought that the improvement in motor coordination may be related to the increased dopamine level with exercise.

There are also contradictions in the literature regarding the striatal levels of serotonin. Many scientific researches have reported that treadmill exercise elevates the level of serotonin in the striatum [36], while others have verified that there is no effect of exercise on the level of serotonin [37]. Also, other studies have stated that exercise reduces the level of serotonin [35,38]. Chaouloff et al. have done many studies examining the relationship between exercise and serotonin. These scientists gave rodents substances that affect dopamine activity and examined the relationship between exercise and serotonin levels in their study. As a result of this study, they found that dopamine metabolism increased in areas rich in serotonin [34]. In fact, except for one study [39], Chaouloff et al. reported that they could not find any change in serotonin levels in their other studies [32,40]. It has been reported that while physical exercise increases serotonin synthesis and metabolism in the midbrain, it does not change serotonin synthesis in the striatum [32]. We did not find a significant difference between the groups in the level of striatal serotonin in our study (Figure not seen). However, serum serotonin levels were significantly reduced in only the Parkinson's model group compared with the healthy control group. Again, no significant change was found in the exercise groups.

CONCLUSION

In conclusion, while moderate-forced treadmill exercise increased dopamine levels in the striatum in the Parkinson's mouse model, it did not cause a change in serotonin levels in the striatum. However, the decrease in serum serotonin level was determined only in the MPTP group. During exercise, different neurotransmitters are secreted in many parts of the brain, and these neurotransmitters exert different effects in different regions. Further studies at the molecular level are needed to present the activities of all neurotransmitters in different brain regions and their interactions with each other.

Peer-review: Externally peer-reviewed.

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

Financial Disclosure: This study has received financial support from Gaziantep University Scientific Research Support Fund (Project No: TF.YLT.21.29).

Author's Contributions: Conception; NO, Design; NO, HKY, ABY, HU, MO, EY, Supervision; NO, HC, Funding; ABY, HU, MO, Materials; NO, HKY, ABY, HU, MO, EY, HC, Data Collection and/or Processing; NO, HKY, Analysis and/or interpretation; NO, ABY, HU, MO, EY, Literature; NO, HKY, Review; NO, HKY, Writing; NO, Critical Review; NO.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University (no: 2023/11, protocol no:304 date: 11.05.2023).

REFERENCES

1- Damier P, Hirsch EC, Agid Y, & Graybiel AM (1999) The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. Brain: a journal of neurology. 122 (Pt 8), 1437–1448. https://doi.org/10.1093/brain/122.8.1437

2- Ibraheem A, Ogeleyinbo I, Emmanuel K, Idar E, Oyewale J, Kunlere O, Olabiyi A.A., Adeniyi P.A (2019) Assessment of the Impacts of Selenocysteine on the Dopaminergic System in the Substantia Nigra and Striatum of 6-OHDA Parkinson's Disease Rat Model. IBRO Reports. 7:25. https://doi.org/10.1016/j.ibror.2019.09.053

3- Ito H (2015) Symptoms and signs of Parkinson's disease and other movement disorders. Deep Brain Stimulation for Neurological Disorders: Theoretical Background and Clinical Application. 21-37. ISBN: 978-3-319-08476-3

4- Gorton LM, Vuckovic MG, Vertelkina N, Petzinger GM, Jakowec MW, & Wood RI (2010) Exercise effects on motor and affective behavior and catecholamine neurochemistry in the MPTP-lesioned mouse. Behavioural brain research. 213(2):253–262. https://doi.org/10.1016/j.bbr.2010.05.009

5- Walsh,K, & Bennett G (2001) Parkinson's disease and anxiety. Postgraduate medical journal. 77(904), 89–93. https://doi.org/10.1136/pmj.77.904.89

6- Schuurman AG, van den Akker M, Ensinck KT, Metsemakers JF, Knottnerus JA, Leentjens AF, & Buntinx, F (2002) Increased risk of Parkinson's disease after depression: a retrospective cohort study. Neurology. 58(10):1501–1504. https://doi.org/10.1212/WNL.58.10.1501

7- Chaudhuri KR, Healy DG, Schapira AH & National Institute for Clinical Excellence (2006) Non-motor symptoms of Parkinson's disease: diagnosis and management. The Lancet. 5(3):235–245. https://doi.org/10.1016/S1474-4422(06)70373-8

8- Laux G. (2022) Parkinson and depression: review and outlook. Journal of neural transmission. 129(5-6):601–608. https://doi.org/10.1007/s00702-021-02456-3

9- Frouni I, Kwan C, Belliveau S, Huot P (2022) Cognition and serotonin in Parkinson's disease. Progress in brain research. 269(1):373–403. https://doi.org/10.1016/bs.pbr.2022.01.013

10- Pedersen BK, Saltin B. (2006) Evidence for prescribing exercise as therapy in chronic disease. Scandinavian journal of medicine & science in sports. 16(Suppl 1):3–63. https://doi.org/10.1111/j.1600-0838.2006.00520.x

11- Pietrelli A, Lopez-Costa J, Goñi R, Brusco A, Basso N (2012) Aerobic exercise prevents age-dependent cognitive decline and reduces anxiety-related behaviors in middle-aged and old rats. Neuroscience. 202:252–266. https://doi.org/10.1016/j.neuroscience.2011.11.054

12- Jackson-Lewis V, Przedborski S (2007) Protocol for the MPTP mouse model of Parkinson's disease. Nature protocols. 2(1):141–151. https://doi.org/10.1038/nprot.2006.342

13- Duty S, Jenner P (2011) Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. British journal of pharmacology. 164(4):1357–1391. https://doi.org/10.1111/j.1476-5381.2011.01426.x

14- Tuon T, Valvassori SS, Lopes-Borges J, Luciano T, Trom CB, Silva LA, Quevedo J, Souza CT, Lira FS, Pinh, RA (2012) Physical training exerts neuroprotective effects in the regulation of neurochemical factors in an animal model of Parkinson's disease. Neuroscience. 227:305–312. https://doi.org/10.1016/j.neuroscience.2012.09.063

15- Lau YS, Patki G, Das-Panja K, Le WD, Ahmad SO (2011) Neuroprotective effects and mechanisms of exercise in a chronic mouse model of Parkinson's disease with moderate neurodegeneration. The European journal of neuroscience. 33(7):1264–1274. https://doi.org/10.1111/j.1460-9568.2011.07626.x

16- Kobayashi T, Araki T, Itoyama Y, Takeshita M, Ohta T, Oshima Y (1997) Effects of L-dopa and bromocriptine on haloperidol-induced motor deficits in mice. Life sciences. 61(26):2529–2538. https://doi.org/10.1016/s0024-3205(97)01007-2

17- Ogawa N, Mizukawa K, Hirose Y, Kajita S, Ohara S, Watanabe Y (1987) MPTP-induced parkinsonian model in mice: biochemistry, pharmacology and behavior. European neurology. 26(Suppl 1):16–23. https://doi.org/10.1159/000116351

18- Kayir H, Uzbay IT (2004) Evidence for the role of nitric oxide in caffeine-induced locomotor activity in mice. Psychopharmacology. 172(1):11–15. https://doi.org/10.1007/s00213-003-1625-5

19- Rozas G, Guerra MJ, Labandeira-García JL (1997) An automated rotarod method for quantitative drug-free evaluation of overall motor deficits in rat models of parkinsonism. Brain research. Brain research protocols. 2(1):75–84. https://doi.org/10.1016/s1385-299x(97)00034-2

20- Rozas G, López-Martín E, Guerra MJ, Labandeira-García JL (1998) The overall rod performance test in the MPTP-treated-mouse model of Parkinsonism. Journal of neuroscience methods. 83(2):165–175. https://doi.org/10.1016/s0165-0270(98)00078-8

21- Simon DK, Tanner CM, Brundin P (2020) Parkinson Disease Epidemiology, Pathology, Genetics, and Pathophysiology. Clinics in geriatric medicine. 36(1):1–12. https://doi.org/10.1016/j.cger.2019.08.002

22- Doğru NÖ, Bal R (2019) Effects of Voluntary and forced exercise on anxiety-related behaviours and motor activity in Parkinson mouse model. Eur J Ther. 25(2):97-103. https://doi.org/10.5152/EurJTher.2018.18011

23- Dunn AL, Reigle TG, Youngstedt SD, Armstrong RB, Dishman RK (1996) Brain norepinephrine and metabolites after treadmill training and wheel running in rats. Medicine and science in sports and exercise. 28(2): 204–209. https://doi.org/10.1097/00005768-199602000-00008

24- Carek PJ, Laibstain SE, Carek SM (2011) Exercise for the treatment of depression and anxiety. International journal of psychiatry in medicine. 41(1):15–28. https://doi.org/10.2190/PM.41.1.c

25- Ozbeyli D, Gokalp AG, Koral T, Ocal OY, Dogan B, Akakin D, Yuksel M, Kasimay O (2015) Protective effect of exercise and sildenafil on acute stress and cognitive function. Physiology & behavior. 151:230–237. https://doi.org/10.1016/j.physbeh.2015.07.030

26- Motaghinejad M, Fatima S, Karimian M, Ganji S (2016) Protective effects of forced exercise against nicotine-

induced anxiety, depression and cognition impairment in rat. Journal of basic and clinical physiology and pharmacology. 27(1):19–27. https://doi.org/10.1515/jbcpp-2014-0128

27- Burghardt PR, Fulk LJ, Hand GA, Wilson MA (2004) The effects of chronic treadmill and wheel running on behavior in rats. Brain research. 1019(1-2):84–96. https://doi.org/10.1016/j.brainres.2004.05.086

28- Taylor TN, Greene JG, Miller GW (2010) Behavioral phenotyping of mouse models of Parkinson's disease. Behavioural brain research. 211(1):1–10. https://doi.org/10.1016/j.bbr.2010.03.004

29- Johnson RA (2016) Voluntary Running-Wheel Activity, Arterial Blood Gases, and Thermal Antinociception in Rats after 3 Buprenorphine Formulations. J Am Assoc Lab Anim Sci. 55(3):306–311.PMCID: 27177564

30- Erdogan F, Kucuk A, Gölgeli A, Liman N, Sagsoz H (2007) Assesment of The Effect of Pentylenetetrazole-induced Kindling on Behavior and Emotional Learning in Rats. EPILEPSI. 13(2):66-72.

31- Sasaki H, Hattori Y, Ikeda Y, Kamagata M, Iwami S, Yasuda S, Tahara Y, Shibata S (2016) Forced rather than voluntary exercise entrains peripheral clocks via a corticosterone/noradrenaline increase in PER2:LUC mice. Scientific reports. 6:27607. https://doi.org/10.1038/srep27607

32- Meeusen, R., & De Meirleir, K. (1995). Exercise and brain neurotransmission. Sports medicine (Auckland, N.Z.), 20(3), 160–188. https://doi.org/10.2165/00007256-199520030-00004

33- Tang C, Liu M, Zhou Z, Li H, Yang C, Yang L, Xiang J (2023) Treadmill Exercise Alleviates Cognition Disorder by Activating the FNDC5: Dual Role of Integrin αV/β5 in Parkinson's Disease. International journal of molecular sciences. 24(9):7830. https://doi.org/10.3390/ijms24097830

34- Chaouloff F, Laude D, Merino D, Serrurrier B, Guezennec Y, Elghozi JL (1987) Amphetamine and alpha-methyl-p-tyrosine affect the exercise-induced imbalance between the availability of tryptophan and synthesis of serotonin in the brain of the rat. Neuropharmacology. 26(8):1099–1106. https://doi.org/10.1016/0028-3908(87)90254-1

35- Lukaszyk A, Buczko W, Wiśniewski K (1983) The effect of strenuous exercise on the reactivity of the central dopaminergic system in the rat. Polish journal of pharmacology and pharmacy. 35(1):29–36. PMID: 6684280

36- Bailey SP, Davis JM, Ahlborn EN (1993) Neuroendocrine and substrate responses to altered brain 5-HT activity during prolonged exercise to fatigue. Journal of applied physiology. 74(6):3006–3012. https://doi.org/10.1152/jappl.1993.74.6.3006

37- Chaouloff F, Laude D, Elghozi JL (1989) PHysical exercise: evidence for differential consequences of tryptophan on 5-HT synthesis and metabolism in central serotonergic cell bodies and terminals. Journal of neural transmission. 78(2):121–130. https://doi.org/10.1007/BF01252498

38- Hellhammer DH, Hingtgen JN, Wade SE, Shea PA, Aprison MH (1983) Serotonergic changes in specific areas of rat brain associated with activity--stress gastric lesions. Psychosomatic medicine. 45(2):115–122. https://doi.org/10.1097/00006842-198305000-00004

39- Cicardo VH, Carbone SE, de Rondina DC, Mastronardi IO (1986) Stress by forced swimming in the rat: effects of mianserin and moclobemide on GABAergic-monoaminergic systems in the brain. Comparative biochemistry and physiology. C, Comparative pharmacology and toxicology. 83(1):133–135. https://doi.org/10.1016/0742-8413(86)90025-3

40- Acworth I, Nicholass J, Morgan B, Newsholme EA (1986) Effect of sustained exercise on concentrations of plasma aromatic and branched-chain amino acids and brain amines. Biochemical and biophysical research communications. 137(1):149–153. https://doi.org/10.1016/0006-291x(86)91188-5

41- Kaplan DS, Bozkurt M (2018) Investigating the most commonly applied lactate recovery method according to the positions in football. Eur J Ther. 24(4):224-229. https://doi.org/10.5152/EurJTher.2018.463