Original paper

Folia Neuropathologica

Exercise attenuates mitochondrial autophagy and neuronal degeneration in MPTP induced Parkinson's disease by regulating inflammatory pathway

Zhiwei Li[1], Hua Lv[1], Xiaoli Cui[1], Wei Di[1], Xiansong Cheng[1], Jun Liu[1], Alok Shiomurti Tripathi[2]

[1]Department of Neurology, Shaanxi Provincial People's Hospital, Xi'an, China, [2]Department of Pharmacology, Era College of Pharmacy, Era University, Lucknow (UP), India

Folia Neuropathol 2023; 61 (4): 426-432 *DOI: https://doi.org/10.5114/fn.2023.132424*

Abstract

Parkinson's disease (PD) is a chronic neuronal loss of dopamine and drugs used for its management has several limitations. The present report determines the effect of exercise on mitochondrial autophagy against PD. Parkinson's disease was induced by 15 doses of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 30 mg/kg, i.p.) for 3 weeks, on five consecutive days in a week. Exposure of exercise was provided for 40 min for a period of 2 weeks after PD confirmation. Assessment of behaviour was performed to evaluate the effect of exercise on motor function and cognitive function in PD rats. Levels of reactive oxygen species (ROS) and inflammatory cytokines were assessed in PD rats using enzyme linked immunosorbent assay (ELISA). Expression of myocyte-specific enhancer factor 2D (MEF2D) and NADH dehydrogenase 6 (ND6) was estimated in PD rats. Exposure to exercise ameliorates the altered motor function and cognitive function in PD rats. There was a reduction in ROS and cytokine levels in the brain tissue of the exercise group compared to the negative control group. Exercise ameliorates the altered expression of apoptotic proteins and mRNA expression of MEF2D and ND6 in the brain tissue of MPTP induced PD rats. In conclusion, data of study reveal that exercise protects the mitochondrial autophagy in PD rats by reducing inflammatory cytokines and oxidative stress.

Key words: *exercise, Parkinson's disease, autophagy, inflammation.*

Introduction

Parkinson's disease (PD) is one of the major progressive disorders of neuronal loss commonly occurring above 65 years of age [14]. Degeneration of dopaminergic neuron in the substantia nigra region of brain, alters the non-motor and motor function in PD patients [1]. Phosphorylation of α-Synuclein lead to its deposition which causes increase in oxidative stress and inflammation, alters cell membrane as it activates mitochondrial autophagy that leads to dysfunction [7]. Inflammatory cytokine release enhances due to microglia activation and contributes to neuroinflammation. Moreover, neuroinflammation is the major cause of activation of apoptosis and dysregulation of autophagy, which develops into neurodegenerative disorder [2]. Inflammasomes activate due to over production of reactive oxygen species (ROS) and contribute to activation of the mitochondrial caspase pathway, which alters the cellular functioning and activates cellular apoptosis [12].

Management of chronic disorders needs a multiple approach including lifestyle change, and exercise is an essential requirement of healthy life. The literature reveals that physical activity or regular exercise gives symptomatic relief to the patient suffering from PD [9]. Moreover, exercise is reported to improve mitochondri-

Communicating author:
Jun Liu, Department of Neurology, Shaanxi Provincial People's Hospital, Xi'an, 710068, China, phone: +86-13279292639, e-mail: liujunshennei@sina.com

al function, reduces neuroinflammation, and promotes neurogenesis by enhancing the neurotropic factor [21]. Physical activity improves motor function like balancing and muscle strength in PD as well [3]. The inflammatory pathway is also regulated by inflammasomes, and Toll-like receptor 4 (TLR4) expression and physical activity regulates the inflammatory pathway by reducing these factors to manage PD [4]. Thus, the present report determines the effect of exercise on PD.

Material and methods

Animals

Sprague Dawley rats (either sex, age: 8 weeks, 250-300 gm) were housed as per the conditions given in the guidelines (humidity: 55 ±5%, temperature: 22 ±2°C, light-dark cycle of 12 h each). All the animals were fed with water and standard pellet diet. The protocol was reviewed and approved by the ethical committee of Shaanxi Provincial People's Hospital, China (650/02/C/CPCSEA/12/2018).

Experimental protocol

The protocol was designed for three groups (n = 6) such as control group; the negative control group receives 15 doses of MPTP 30 mg/kg, i.p. for 3 weeks, on five consecutive days in a week. The exercise treated group receives exercise for two weeks after the induction of PD.

Treadmill exercise

Exercise training was provided for 14 days to each rat after induction of PD using rodent treadmill. Every day 40-min training was given at 8 m/min with inclination of 0 degree, which includes 5 min at 2 m/min speed, then 2 m/min speed for 5 min and at the end 8 m/min for 30 min. Biochemical, neuronal and behaviour activities were estimated within 48 h after the last session of exercise.

Estimation of motor function

Motor function and muscle balance was estimated using rotarod apparatus. All the animals were mounted on the apparatus which rotates and the time to fall was recorded as the latency period.

Behavioural studies

Apomorphine-induced rotation behaviour was assessed to determine the changes over the dopaminergic system in PD rats. Apomorphine reduces the dopamine level which alters the number of rotations in PD rats. Contralateral rotation was induced by the administration of 3 mg/kg, s.c. and changes in behaviour were monitored for the duration of 1 h.

Morris Water Maze test

Cognitive function in rats was estimated using Morris Water Maze (MWM) test as per previously published reports [22]. The MWM apparatus dimensions are as follows: circular pool: 120 cm, height: 50 cm, depth: 30 cm. The pool was separated into four different quadrants and a platform was placed in one quadrant, trials were given on 5 consecutive days. The platform was removed on day 6 and the time spent in the target quadrant was observed.

Preparation of brain tissue homogenate and estimation of cytokines

All the animals were sacrificed to isolate brain and homogenize it in phosphate buffer, which was centrifuged at 3000 rpm for the duration of 15 min. Supernatant solution was used to determine biochemicals. ELISA method was used to estimate the level of interleukin (IL)-1β, IL-10, IL-6 and tumour necrosis factor α (TNF-α) in the tissue.

Assessment of ROS

MitoSOX red mitochondrial superoxide indicator was used to assess the production of ROS. MitoSOX red (5 µM) staining was used to stain the tissue homogenate in the dark at 37°C for 30 min. The intracellular level of ROS was estimated at wavelength of 510 nm and 580 nm for excitation and remission using a fluorescence plate reader.

qRT-PCR

qRT-PCR method was used for the estimation the mRNA expression of MEF2D and ND6. The TRIzol kit was used to isolate the total RNA from brain tissue homogenate as per the manufacturer's directions. Single-strand cDNA synthesis was performed and qRT-PCR was performed as per the Fast Start Universal SYBR Green Master; analysis was executed with ABI 7300 Real Time PCR System. Below are the details of primers used in the study (Table I).

Western blot assay

In brain tissue homogenate, extraction of total protein was performed with RIPA lysis buffer, a commercial kit was used for its extraction and separation was attained through sodium dodecyl sulfate-polyacrylamide gels (12% w/v). Thereafter separated protein was fil-

Table I. Details of primers

No.	Primer	Forward	Reverse
1	ME2FD	5'-ATGGCAACAGCCTAAACAAAGT-3'	5'-GTGGTGAGCGAGTGGGTAGA-3'
2	ND6	5'-ATTAAACAACCAACAAACCCAC-3'	5'TTTGGTTGGTTGTCTTGGGTT-3'
3	β-actin	5'-AAGGACTCCTATAGTGGGTGACGA-3'	5'-ATCTTCTCCATGTCGTCCCAGTTG-3'

tered through filter paper and incubated with primary antibodies like caspase 3, Bcl2 and TLR4 for overnight at 4°C, which was later incubated with IIry antibody. Densitometry of blots was assessed with the image lab software after development of blot with ChemiDoc MP Imaging System.

Statistical analysis

Data of the report are represented as mean ±SEM; the study compared all the data statistically with one way ANOVA, and further Dunnett Post hoc test (GraphPad Prism software, ver. 6.1; USA).

Results

Assessment of the effect of exercise on motor function

The exercise effect was observed on the motor function in MPTP induced PD rat model using apomorphine-induced rotation behaviour and rotarod apparatus. The negative control group shows a significant increase in the total number of rotation/40 min and number of fall/min compared to the control group. Exercise reduces the number of rotation/40 min (apomorphine-induced rotation behaviour) and fall/min in MPTP induced PD rats (Fig. 1A, B).

Assessment of the effect of exercise on cognitive function

Cognitive function was assessed in exercise exposed MPTP induced PD rats using MWM apparatus as shown in Figure 2. Escape latency was enhanced in the negative control group during the 5 days' training period compared to the control group. The negative control group shows a reduction in the percentage of time spent in the target quadrant and number of crossings compared to the control group of rats. However, exercise exposure ameliorates the altered cognitive function in MPTP induced PD rats.

Assessment of the effect of exercise on the level of cytokines

The level of cytokines was assessed in the brain tissue of exercise exposed MPTP induced PD rats using ELISA as shown in Figure 3. The negative control group shows an increase in IL-1β, IL-6 and TNF-α levels and reduction in IL-10 compared to the control group of

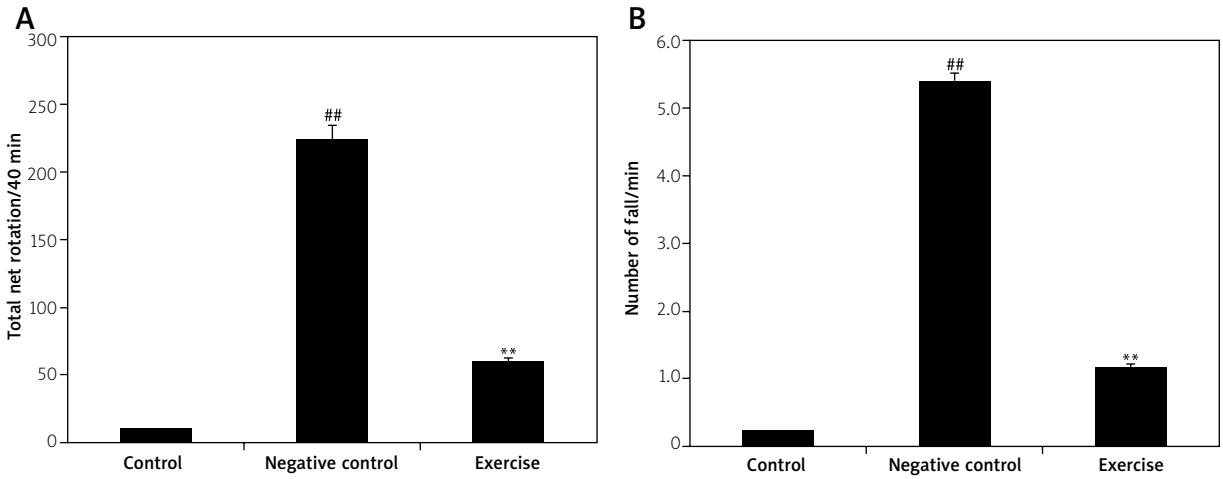


Fig. 1. Assessment of the effect of exercise on the motor function in MPTP induced Parkinson's disease rat model. **A**) Assessment of apomorphine-induced rotation behaviour; **B**) Assessment of the number of fall/min using rotarod apparatus. Mean ±SEM (n = 6); $^{\#\#}p < 0.01$ vs. the control group, $^{**}p < 0.01$ vs. the negative control group.

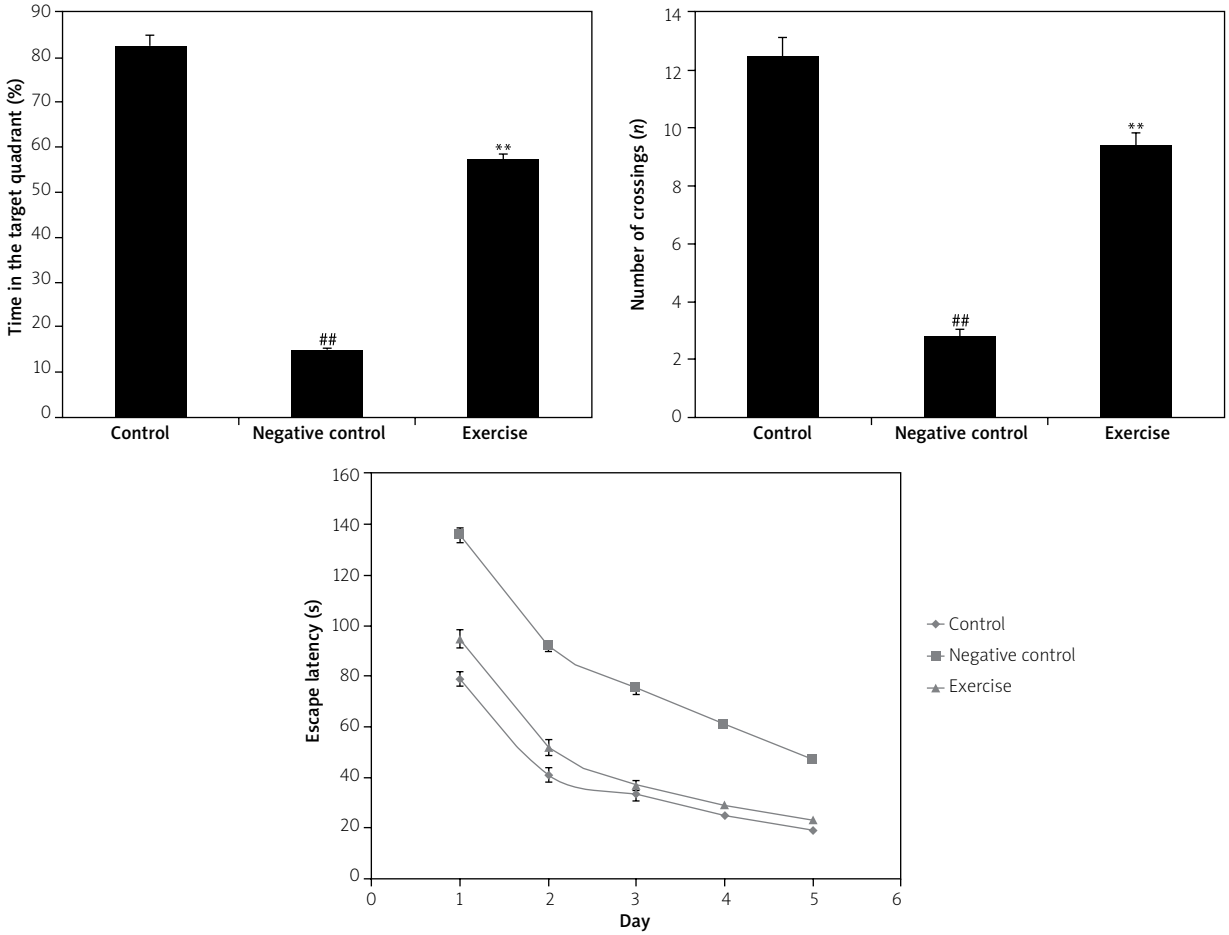


Fig. 2. Exercise ameliorates cognitive behaviour in MPTP induced Parkinson's disease rats. Mean ±SEM (n = 6); $^{\#\#}p < 0.01$ vs. the control group, $^{**}p < 0.01$ vs. the negative control group.

rats. Exposure to exercise attenuates the level of cytokines in brain tissue of MPTP induced PD rats.

Assessment of the effect of exercise on reactive oxygen species

The effect of exercise was observed on the production of ROS in the brain tissue of MPTP induced PD rats. There was a significant increase in ROS production in the negative control group compared to the control group of rats. Exposure to exercise reduces significantly the ROS production in brain tissue of MPTP induced PD rats (Fig. 4).

Assessment of the effect of exercise on mRNA expression of MEF2D and ND6

The effect of exercise was estimated on the relative mRNA expression of MEF2D and ND6 in the brain tissue of MPTP induced PD rats using qRT-PCR method

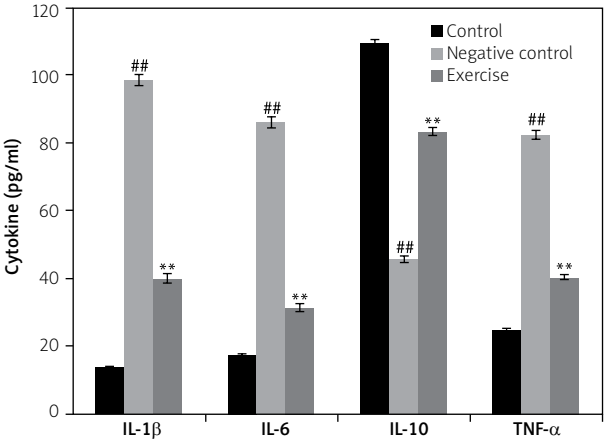


Fig. 3. Exercise ameliorates the cytokine level in brain tissue of MPTP induced Parkinson's disease rats. Mean ±SEM (n = 6); $^{\#\#}p < 0.01$ vs. the control group, $^{**}p < 0.01$ vs. the negative control group.

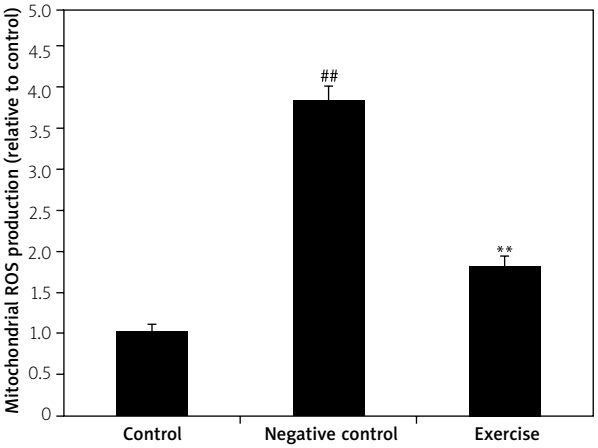


Fig. 4. Exercise reduces ROS production in the brain tissue of MPTP induced Parkinson's disease rats. Mean ±SEM (n = 6); $^{\#\#}p < 0.01$ vs. the control group, $^{**}p < 0.01$ vs. the negative control group.

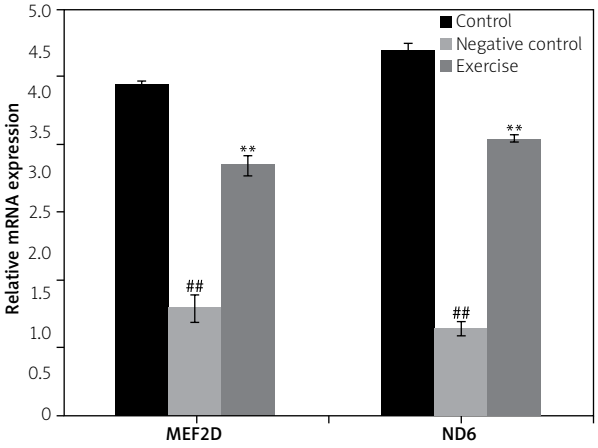


Fig. 5. Exercise improves mRNA expression of MEF2D and ND6 in the brain tissue of MPTP induced Parkinson's disease rats. Mean ±SEM (n = 6); $^{\#\#}p < 0.01$ vs. the control group, $^{**}p < 0.01$ vs. the negative control group.

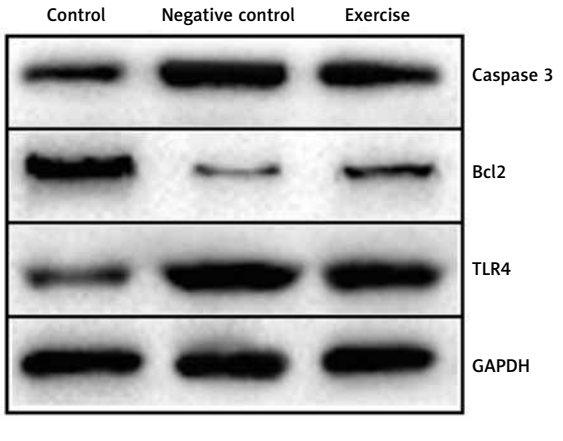
as shown in Figure 5. There was a significant reduction in relative mRNA expression of MEF2D and ND6 in the negative control group compared to the control group. Moreover, relative mRNA expression of MEF2D and ND6 was significantly enhanced in exercise exposed MPTP induced PD rats.

Assessment of the effect of exercise on the expression of protein

Expression of proteins such as caspase 3, Bcl2 and TLR4 was estimated in the brain tissue of exercise exposed MPTP induced PD rats. The negative control group shows alteration in expression of TLR4, caspase 3 and Bcl2 protein compared to the control group. However, exposure to exercise reduces the expression of caspase3 and TLR4 protein and increases Bcl2 protein in the brain tissue of MPTP induced PD rats.

Discussion

Parkinson's disease is one of the major chronic neurodegenerative disorders in which alteration in motor function occurs. Mitochondrial autophagy is involved in the development of PD [13]. There are several conventional drugs available for the management of PD, however for the effective management of PD, there is a need of assessment of an alternative approach.



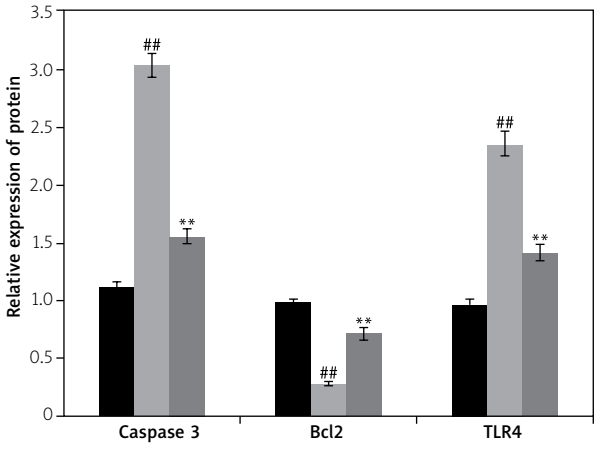


Fig. 6. Exercise ameliorates expression of proteins in the brain tissue of MPTP induced Parkinson's disease rats. Mean ±SEM (n = 6); $^{\#\#}p < 0.01$ vs. the control group, $^{**}p < 0.01$ vs. the negative control group.

The motor nervous system controls the voluntary movement of skeletal muscle and exercise involve movement of skeletal muscle, which contributes to the promotion of motor function [16]. There are several reports revealing that exercise shows the beneficial effect in the management of PD [18], however exact molecular pathway involved in the beneficial effect of exercise on PD. Thus, the present study evaluates the beneficial effect of exercise on PD by regulating mitochondrial autophagy through the inflammatory pathway.

Cellular energy production primarily occurs in the mitochondria, while production of energy cell generates H_2O_2, which contributes to the oxidative stress [11]. Production of ROS gets enhanced due to generation of oxidative stress. Accumulation of ROS in an excess amount activates the mitochondrial apoptotic pathway [17]. Moreover, oxidative stress has higher sensitivity towards the DA neurons which are involved in the pathogenesis of PD. Production of ROS is enhanced in the negative control group compared to the control group of rat and exposure to exercise reverses it. Pathogenesis of PD also reveals the involvement of the autoimmunity and inflammatory pathway, as TLR4 is reported to be involved in the activation of microglia and activates the immune system, which leads to neuronal injury and neuroinflammation [10]. It also enhances the production of cytokines in the neuronal tissue which stimulates the deposition of α-synuclein. Moreover, TLR4 is reported to be upregulated in MPTP treated rats [15] as data of the present study also support. There was a reduction in the level of cytokines and TLR4 expression in brain tissue of the exercise exposed group compared to the negative control group of rats.

Neuronal injury and neuronal inflammation alter the normal neurological functioning of other neuronal cells due to release of a number of mediators of inflammation, which leads to alteration in the protein synthesis in neuron [8]. These changes contribute to the activation of the apoptosis pathway and also autophagy, which further leads to progression of neurodegeneration. Autophagy terminology is used in the context of cell damage in which lysosomal component breaks down the cellular components [6]. Mitochondria is majorly involved in the generation of energy, which is utilized by the cell for normal functioning, due to stress function of mitochondrial function alters due to stress that activates autophagy by lysosomal dysfunction [23]. Several proteins such as caspase and Bcl2 family are involved in the cellular injury and data of the study reveal that administration of MPTP alters the expression of caspase 3 and Bcl2 in the brain tissue, however exposure to exercise ameliorates this altered expression of protein in MPTP induced PD rats.

Cellular nuclei contain DNA, mitochondrial DNA (MtDNA) is the only DNA available outside the nucleus. ND6 is an essential component of mitochondrial complex 1 [5]. The literature suggests that myocyte enhancer factor-2 (MEF2) protein contributes to the proliferation and survival of neuronal cells and alteration in its expression involved in the deposition of α-synuclein and neuronal injury, develops into PD [19]. MEF2 binds to mitochondrial complex 1 at the site coded with ND6 [20]. Moreover, it is well documented that MPTP induces PD in rats by dysregulating MEF2D and ND6 expression [24] and data of the presented report also reveal it. The exercise exposed group shows a significant increase in the expression of MEF2D and ND6 protein. These data support that exercise is involved in the prevention of PD development and also show the exercise beneficial effect against PD.

Conclusions

In conclusion, data of the study reveal that exercise prevents neuronal degeneration in MPTP induced PD rats, as it reduces production of ROS which leads to activation of inflammatory cytokines and activates the autophagy reaction by promoting the expression of MEF2D and ND6 protein. These data support that exercise reduces the inflammatory pathway, which reduces neuronal degeneration in MPTP induced PD rats.

Acknowledgements

All the authors of the presented report are thankful to the Shaanxi Natural Science Basic Research Program (2021JM-556) for providing necessary funds to conduct the experimental work (650/02/C/CPCSEA/12/2018).

Disclosure

The authors report no conflict of interest.

References

1. Alexander GE. Biology of Parkinson's disease: pathogenesis and pathophysiology of a multisystem neurodegenerative disorder. Dialogues Clin Neurosci 2004; 6: 259-280.
2. Alirezaei M, Kemball CC, Whitton JL. Autophagy, inflammation and neurodegenerative disease. Eur J Neurosci 2011; 33: 197-204.
3. Bhalsing KS, Abbas MM, Tan LCS. Role of physical activity in Parkinson's disease. Ann Indian Acad Neurol 2018; 21: 242-249.
4. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 2017; 9: 7204-7218.
5. Chinnery PF, Hudson G. Mitochondrial genetics. Br Med Bull 2013; 106: 135-159.
6. Das G, Shravage BV, Baehrecke EH. Regulation and function of autophagy during cell survival and cell death. Cold Spring Harb Perspect Biol 2012; 4: a008813.

7. Dias V, Junn E, Mouradian MM. The role of oxidative stress in Parkinson's disease. J Parkinsons Dis 2013; 3: 461-491.
8. DiSabato DJ, Quan N, Godbout JP. Neuroinflammation: the devil is in the details. J Neurochem 2016; 139: 136-153.
9. Fayyaz M, Jaffery SS, Anwer F, Zil-E-Ali A, Anjum I. The effect of physical activity in Parkinson's disease: A mini-review. Cureus 2018; 10: e2995.
10. Fiebich BL, Batista CRA, Saliba SW, Yousif NM, de Oliveira ACP. Role of microglia TLRs in neurodegeneration. Front Cell Neurosci 2018; 12: 329.
11. Guo C, Sun L, Chen X, Zhang D. Oxidative stress, mitochondrial damage and neurodegenerative diseases. Neural Regen Res 2013; 8: 2003-2014.
12. Harijith A, Ebenezer DL, Natarajan V. Reactive oxygen species at the crossroads of inflammasome and inflammation. Front Physiol 2014; 5: 352.
13. Lynch-Day MA, Mao K, Wang K, Zhao M, Klionsky DJ. The role of autophagy in Parkinson's disease. Cold Spring Harb Perspect Med 2012; 2: a009357.
14. Mhyre TR, Boyd JT, Hamill RW, Maguire-Zeiss KA. Parkinson's disease. Subcell Biochem 2012; 65: 389-455.
15. Noelker C, Morel L, Lescot T, Osterloh A, Alvarez-Fischer D, Breloer M, Henze C, Depboylu C, Skrzydelski D, Michel PP, Dodel RC, Lu L, Hirsch EC, Hunot S, Hartmann A. Toll like receptor 4 mediates cell death in a mouse MPTP model of Parkinson disease. Sci Rep 2013; 3: 1393.
16. Perrey S. Promoting motor function by exercising the brain. Brain Sci 2013; 3: 101-122.
17. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta 2016; 1863: 2977-2992.
18. Rosenthal LS, Dorsey ER. The benefits of exercise in Parkinson disease. JAMA Neurol 2013; 70: 156-157.
19. She H, Mao Z. Regulation of myocyte enhancer factor-2 transcription factors by neurotoxins. Neurotoxicology 2011; 32: 563-566.
20. She H, Yang Q, Shepherd K, Smith Y, Miller G, Testa C, Mao Z. Direct regulation of complex I by mitochondrial MEF2D is disrupted in a mouse model of Parkinson disease and in human patients. J Clin Invest 2011; 121: 930-940.
21. Vecchio LM, Meng Y, Xhima K, Lipsman N, Hamani C, Aubert I. The neuroprotective effects of exercise: Maintaining a healthy brain throughout aging. Brain Plast 2018; 4: 17-52.
22. Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 2006; 1: 848-858.
23. Wang C, Youle RJ. The role of mitochondria in apoptosis. Annu Rev Genet 2009; 43: 95-118.
24. Yao L, Li W, She H, Dou J, Jia L, He Y, Yang Q, Zhu J, Cápiro NL, Walker DI, Pennell KD, Pang Y, Liu Y, Han Y, Mao Z. Activation of transcription factor MEF2D by bis(3)-cognitin protects dopaminergic neurons and ameliorates Parkinsonian motor defects. J Biol Chem 2012; 287: 34246-34255.