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Research Paper

Synergistic Effects of Cerebrolysin and Aerobic Exercise on 6-hydroxydopamine-induced Model of Parkinson Disease in Adult Male Rats

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ABSTRACT

Introduction: Neurotrophic factors and physical activities have beneficial effects on neurodegenerative disorders. This study assessed the impact of physical exercise (EXE) and cerebrolysin (CBL), individually or in combination, in a Parkinson's disease (PD) model caused by 6-hydroxydopamine (6-OHDA).

Methods: The study utilized 62 male Wistar rats, which were divided into six groups (n=12): Sham (received intra substantia nigra [SN] injection of normal saline), PD (underwent 12.5 µg injection of 6-OHDA into the left SN), PD+levodopa (treated with levodopa; 12 mg/kg, gavage, for three weeks), PD+CBL (treated with intraperitoneal injection of CBL 2.5 mL/kg, for three weeks), PD+EXE (exercised 30 min/d for three weeks), and PD+CBL+EXE. Rotation with apomorphine and Murprogo's test was assessed 21 days after PD induction and after treatments. Ultimately, the levels of lipid peroxidation marker and total antioxidant capacity (TAC), glutathione peroxidase (GPx) activity, α-synuclein protein expression, and histopathological changes of the SN were evaluated ipsilateral to the lesioned side.

Results: The results showed that CBL and exercise, alone or in combination, decreased ipsilateral apomorphine rotation and muscle rigidity in the PD animals. Moreover, these behavioral changes were associated with reduced malondialdehyde (MDA) levels and α-synuclein protein levels, increased TAC level and GPx activity, and a greater neuronal count in the SN. Notably, the combination effects were greater than single therapy and levodopa treatment.

Conclusion: Our findings indicate that the combination of exercise and CBL ameliorated 6-OHDA-induced motor impairments by attenuating oxidative stress and protein expression of α-synuclein and preserving neurons in the SN.

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Highlights

- Cerebrolysin combined with exercise significantly reduced apomorphine-induced rotations in Parkinsonian rats.
- The combined therapy improved muscle rigidity more effectively than levodopa or monotherapies.
- Treatment with Cerebrolysin and exercise significantly enhanced antioxidant defenses, including TAC and GPx activity.
- Neuronal preservation in the substantia nigra of rats was most pronounced with the combined therapy.

Plain Language Summary

Parkinson's disease (PD) is a progressive brain disorder that leads to movement difficulties, such as stiffness, tremors, and trouble walking. These symptoms occur because of the gradual loss of certain brain cells that control movement. While current treatments such as levodopa drug use can help reduce symptoms, they cannot stop the disease from progressing or protect the brain cells from further damage. In this study, we examined whether combining exercise with cerebrolysin use (a drug known for its brain cell-supporting properties) can be more effective than cerebrolysin use or exercise alone in rats with PD. The results showed that rats receiving the combined therapy demonstrated better motor performance and reduced muscle stiffness compared to those treated with only cerebrolysin use or exercise. The combination therapy reduced markers of oxidative stress and the harmful α-synuclein protein level, and preserved more brain cells in the affected region. These findings suggest that combining lifestyle strategies (exercise) with neuroprotective drugs (cerebrolysin) may lead to more effective treatment in Parkinson's disease, although further research is needed to confirm these results in humans.

1. Introduction

The destruction of dopaminergic (DAergic) neurons in substantia nigra pars compacta (SNpc), and ventral tegmental area causes Parkinson disease (PD) (Balestrino & Schapira, 2020; Wright et al., 2010). The number of patients suffering from PD from 2.5 million in 1990 reached 6.1 million in 2016, and it is estimated that it will double by 2040 (Dorsey et al., 2018).

Several etiologies are involved in this disease, including genetic factors, increased intracellular calcium, oxidative stress, mitochondrial dysfunction, and cytotoxicity (Spatola & Wider, 2014; Surmeier, 2007). Pathologically, the hallmarks of PD consist of the death of DAergic neurons in substantia nigra (SN), dopamine depletion of the striatum, and cytoplasmic inclusions of proteins, known as Lewy bodies that primarily contain α-synuclein (α-syn) protein (Balestrino & Schapira, 2020). High levels of α-syn contribute to neurotoxicity through multiple mechanisms, including mitochondrial dysfunction, impaired autophagy, and increased oxidative stress (Tu et al., 2021). Notably, oxidative stress can induce additional aggregation of α-syn, creating a harmful feedback loop that worsens neuronal damage and accelerates disease progression (Jansen van Rensburg et al., 2021).

The existing PD therapies comprise dopamine analogs like levodopa, which is considered the gold standard therapy, inhibitors of dopamine-degrading enzymes, and deep brain electrical stimulation (Kalia & Lang, 2015). These medications manage symptoms in the initial phases of the disease but do not prevent ongoing neuronal degeneration. Evidence shows that chronic levodopa therapy may result in falling and freezing, dysarthria, dysphagia, dementia, hallucinations, daytime drowsiness, and urinary incontinence, known as levodopa-resistant symptoms (Pulikkalpura et al., 2015; Warnecke et al., 2016). On the other hand, in advanced PD, a chronic and high dose of levodopa is needed, which may lead to neuronal death (Müller, 2013). Therefore, alternative therapies are highly demanded.

Cerebrolysin (CBL), a pure peptide produced from porcine brain, comprises approximately 15% tiny peptides and 85% amino acids (Mahmoudi et al., 2018; Plosker & Gauthier, 2009), exhibiting neurological effects similar

to endogenous neuronal factors. The pharmacodynamic effects of CBL include neuronal survival (Plosker & Gauthier, 2009), neuroprotection (Álvarez et al., 2000; Rockenstein et al., 2006; Ubhi et al., 2009; Veinbergs et al., 2000), neuroplasticity, and neurogenesis (Hartbauer et al., 2001). Hartbauer et al. (2001) showed that CBL degrades malondialdehyde (MDA) and nitric oxide and improves oxidative damage by enhancing endogenous antioxidant enzyme activity such as glutathione peroxidase (GPx) (Ardjmand et al., 2019). Previous studies also supported the beneficial effects of CBL in improving behavioral and biochemical symptoms of PD in rodent models by decreasing oxidative damage and apoptosis and protecting nigrostriatal DAergic neurons (Noor et al., 2016; Requejo et al., 2018; Rockenstein et al., 2015).

Physical activity increases neurotrophic factors antioxidant capacity and decreases inflammation, which can be proposed as a non-invasive intervention to minimize neuronal damage and improve PD symptoms (Osali, 2020; Yau et al., 2014). According to major health organizations, moderate-intensity aerobic activity is the best exercise regimen for people with PD, which involves maintaining the heart rate at 64%-76% of its maximal reserve (Piercy et al., 2018; Riebe et al., 2018). Moderate aerobic exercise has been proven to enhance balance and gait in PD patients (Li et al., 2021). Research indicates that engaging in moderate aerobic exercise augments the antioxidant power of the brain and increases the neural activity of the motor cortex. However, intense aerobic exercise diminishes brain antioxidant capacity and may lead to chronic inflammation and neural damage (Camiletti-Moirón et al., 2013; Cerqueira et al., 2020; Takehara et al., 2017).

Considering that both CBL and moderate exercise independently enhance neuronal health and functional improvement in PD models, we suggest their combined application could produce a synergistic effect. This study, therefore, examined the impacts of the concurrent administration of CBL and aerobic exercise on motor performance, oxidative stress indicators, α-syn protein expression, and the quantity of SN neurons in a PD model.

2. Materials and Methods

Materials

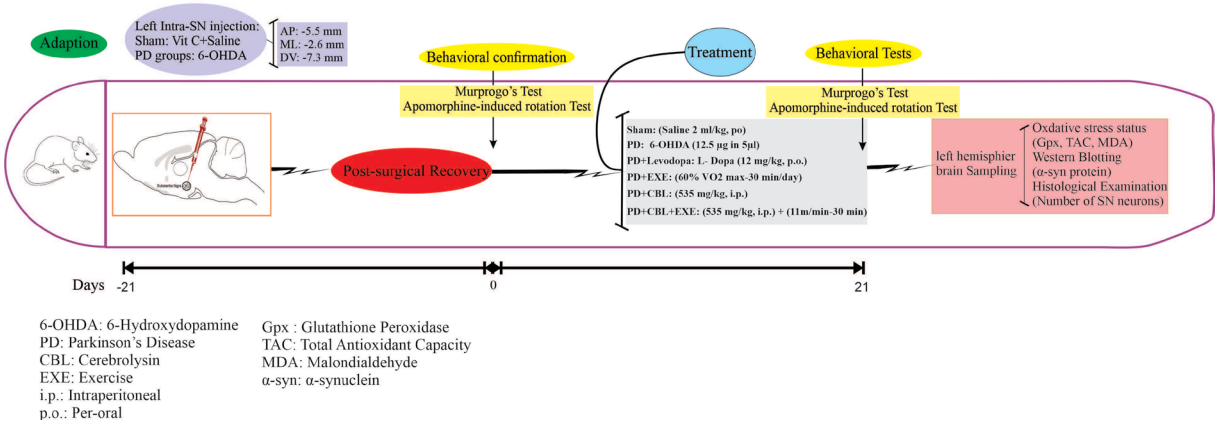
CBL was obtained from Neropharma Corporation (Austria), while Benzedrine (Lot: SLBF5500V), ascorbic acid (Lot: MKCM8021), and 6-OHDA (Lot: STBH3207V) were all purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

Animals

In this study, 72 adult male Wistar rats weighing 250±20 g (8–10 weeks) were used. The rodents were maintained in a carefully regulated setting involving a 12-hour illumination cycle and darkness alongside a stable temperature of 24±2 ˚C. The same diet and water were freely available, and after a week of getting used to the new condition, the experiment was started (Figure 1).

Grouping

The rats were randomly allocated into six groups (n=12/group): I) Sham group, administered an SN injection of the 6-OHDA vehicle and received normal saline treatment (IP) for three weeks; II) PD group (PD), received an SN injection of 6-OHDA toxin and subjected to normal saline treatment (IP) for 21 days, III) PD+levodopa group, PD animals



6-OHDA: 6-Hydroxydopamine
PD: Parkinson's Disease
CBL: Cerebrolysin
EXE: Exercise
i.p.: Intraperitoneal
p.o.: Per-oral

Gpx : Glutathione Peroxidase
TAC: Total Antioxidant Capacity
MDA: Malondialdehyde
α-syn: α-synuclein

Figure 1. Timeline of this investigation

were treated with levodopa 12 mg/kg by gavage twice daily (Carvalho et al., 2017), IV) PD+CBL group, PD rats were received daily injection of CBL 2.5 ml/kg (IP) for 21 days (Noor et al., 2016), V) PD + exercise (EXE) group, PD rats underwent a schedule of treadmill exercise at a velocity of 11 m/min over 21 days, and VI) PD+CBL+EXE group, PD animals were received a combination of CBL and treadmill exercise for 21 days.

Induction of the PD model

The stereotaxic equipment was used to mount the head of the anesthetized rat. Following the coordinates obtained from the Paxinos atlas (anteroposterior=-5.5 mm, dorsoventral=-7.3 mm, mediolateral=-2.6 mm), the left SNpc of each anesthetized rat was injected with 12.5 µg of 6-OHDA toxin dissolved in 5 µL of a mixture of normal saline and 0.2% ascorbic acid solution (Roghani et al., 2002) normal saline and vitamin C as the vehicle of 6-OHDA. Both saline and the toxin were given gradually, with a flow speed of 1 µL/min, and the needle (No. 27) was withdrawn 5 min after the injection (Roghani et al., 2010).

Behavioral tests

All behavioral tests were done by a blinded experimenter to the animal treatments three weeks after 6-OHDA injection to confirm the model (before treatment) and 21 days after the lesion (after treatment).

Rotational behavior

The rotation induced by apomorphine serves as a standard assay for evaluating the extent of motor dysfunction produced by 6-OHDA. The animals were individually situated within a circular Plexiglas enclosure measuring 30 cm in diameter. Following an initial habituation period of 10 min, a subcutaneous injection of 0.5 mg/kg apomorphine hydrochloride was administered. Contralateral turns toward the lesion side were recorded over 30 minutes at 10-minute intervals (Wei et al., 2014).

Murprogo's test

In this examination, a score of zero was assigned to the rat if it stood or walked ordinarily on a flat surface. In contrast, a score of 0.5 was given to the animal if it remained stationary or exhibited movement disorders in its limbs. Next, we utilized a 3-cm height platform and placed the animals' right forelimb upon it. If the animal's forelimb remains on the platform for 10 seconds or longer, it receives 0.5 points. An identical test was conducted for the contralateral forelimb. Subsequently, the rat's right forelimb was situated on a 9-cm height platform; if the forelimb remained on the platform for 10 seconds or longer, a score of 1 was awarded. The test was repeated with the opposite forelimb, and the rat received an extra point if the hand was kept steady. We classified animals as PD animals if they had a cumulative score of 3.5 (Morpurgo, 1962).

Moderate aerobic exercise

The following was done to identify the maximum oxygen consumption (VO_2 max) in the studied rats: The treadmill was initially calibrated to a speed of 0.3 km/h. Then, every 3 minutes, the velocity was raised by 0.3 km/h until the rat could run at the same speed for at least 1.3 minutes and could not continue at a higher speed. VO_2 max value was then used in the moderate-intensity exercise program. The exercise protocol in this study was a type of forced exercise using a treadmill (Azma Technik Co., Iran). The animals exercised with an intensity of approximately 60% VO_2 max for three weeks, 6 days a week, and 30 minutes daily. After each week, the animals were tested for maximal effort to determine VO_2max for the intensity of the following week's exercise program (Belotto et al., 2010).

Tissue collection

The day after the final behavioral assessment, all rats were euthanized under deep anesthesia. The left brain hemisphere (ipsilateral to the lesioned side) was extracted on a cool surface and frozen at -80 ˚C. The brain was placed in a 4% buffered paraformaldehyde fixation solution to histologically evaluate the quantity of SN neurons.

Evaluation of oxidative stress levels

For this purpose, frozen brain tissue was suspended in chilled 1.15% potassium chloride solution and subsequently centrifuged for 15 min at a speed of 12000 rpm and a temperature of 4 ˚C. The quantity of protein in the supernatant was determined using the Bradford method.

MDA levels

The concentration of MDA, indicative of lipid peroxidation, was assessed using a thiobarbituric acid reaction (TBAR) colorimetric assay kit according to the manufacturer's instructions. A plate reader measured the absorbance at 540 nm, and the result was shown as nmol/mg protein (Ozdemir et al., 2009).

GPx

The RANSEL kit (Randox Laboratories Ltd.) was used to estimate the enzyme activity of GPx in the brain samples. The absorbance was determined at 340 nm, and the results were expressed as nmol/mg protein.

Total antioxidant capacity (TAC) levels

A Randox kit (Randox Laboratories Ltd, Crumlin, United Kingdom) was utilized to assess brain TAC levels. This evaluation used the 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) methodology. Absorbance measurements were conducted at a wavelength of 600 nm using a spectrophotometer, with the findings expressed in µmol/mg protein.

Western blotting

To obtain the supernatant, the samples underwent homogenization at a ratio of 1:10 (w:v) in Tris–HCl buffer containing a protease inhibitor cocktail, followed by centrifugation at 12000 g for 10 minutes at a temperature of 4 ˚C. The protein concentrations in the resulting supernatant were assessed by applying the Bradford method. Following electrophoresis on a 12.5% SDS-polyacrylamide gel, 40 µg of protein was transferred onto a PVDF (polyvinylidene difluoride) membrane (Roche, UK). Subsequently, the membrane underwent an overnight incubation at 4 ˚C with primary antibodies (Santa Cruz, CA, USA) aimed explicitly at α-syn (sc-12767) and β-actin (sc-47778), which served as the loading control. Following three washes with TBST (tris-buffered saline tween-20), the membranes underwent treatment for two hours with HRP-conjugated goat anti-rabbit IgG secondary antibody at a 1:5000 (sc-2004) dilution. Following the washing process with PBS, the membranes were immersed in an ECL detection solution (Amersham, UK) and subsequently exposed to x-ray film to confirm the presence of signals. The assessment of protein band density was conducted utilizing ImageJ software, version 1.52 (Jin et al., 2008).

Histological examination

Tissues fixed with paraformaldehyde were embedded in paraffin, and coronal slices measuring 40 µm were obtained from the midbrain (-4.52 to -6.04 mm from bregma) (Paxinos & Watson, 2014) utilizing a microtome. These slices were subsequently stained with 0.1% cresyl violet and Nissl-stained cells in the SNpc were quantified by light microscopy (magnification ×200) across three brain sections in a blind manner.

Data analysis

Values were reported as Mean±SEM. GraphPad Prism software, version 6.01 (GraphPad Software Inc., La Jolla, CA, USA) was used to analyze the statistics. A one-way analysis of variance (ANOVA) with a Tukey post-hoc test was used to find group differences. The threshold for determining statistical significance was established at a $P<0.05$.

3. Results

Decreased contralateral rotations in PD animals after CBL and treadmill exercise, alone or in combination

As Figure 2A shows, the injection of 6-OHDA increased the mean number of contralateral rotations to the injection side relative to the sham group ($P<0.001$) (before treatment), confirming the induction of the PD model. Moreover, normal saline-treated PD animals showed higher contralateral rotations than the sham group at the end of the treatment (Figure 2B). However, 21 days of treatment with levodopa, CBL, treadmill exercise ($P<0.001$ for all comparisons), or a combination of exercise and CBL significantly decreased the number of contralateral rotations in PD rats ($P<0.01$). The PD+CBL+EXE group also performed considerably fewer contralateral rotations than the PD+CBL and PD+EXE groups ($P<0.001$). Combination therapy demonstrated greater efficacy than levodopa treatment in reducing apomorphine-induced rotations ($P<0.001$).

Attenuated muscle rigidity in the PD rats after CBL and treadmill exercise, alone or in combination

Figure 3A illustrates that three weeks post-6-OHDA injection, the average scores in the Murprogo's test increased substantially compared to the sham group ($P<0.001$), thereby confirming the successful induction of the PD model. After 21 days of treatment, the PD, PD+levodopa, PD+CBL, and PD+EXE groups exhibited significantly higher scores compared to the sham group ($P<0.001$ for all comparisons, Figure 3B). Nonetheless, no substantial difference was observed between the PD+CBL+EXE and the sham groups. Besides, administration of levodopa, CBL, treadmill exercise, and a combination of exercise and CBL significantly decreased Murprogo's scores compared to the normal saline-treated PD group ($P<0.001$). Furthermore, noteworthy differences were observed between the PD+EXE and PD+levodopa groups ($P<0.05$). Interestingly, combination therapy had a greater effect in decreasing Murprogo's score than single treatment with CBL ($P<0.001$) or treadmill exercise ($P<0.01$).

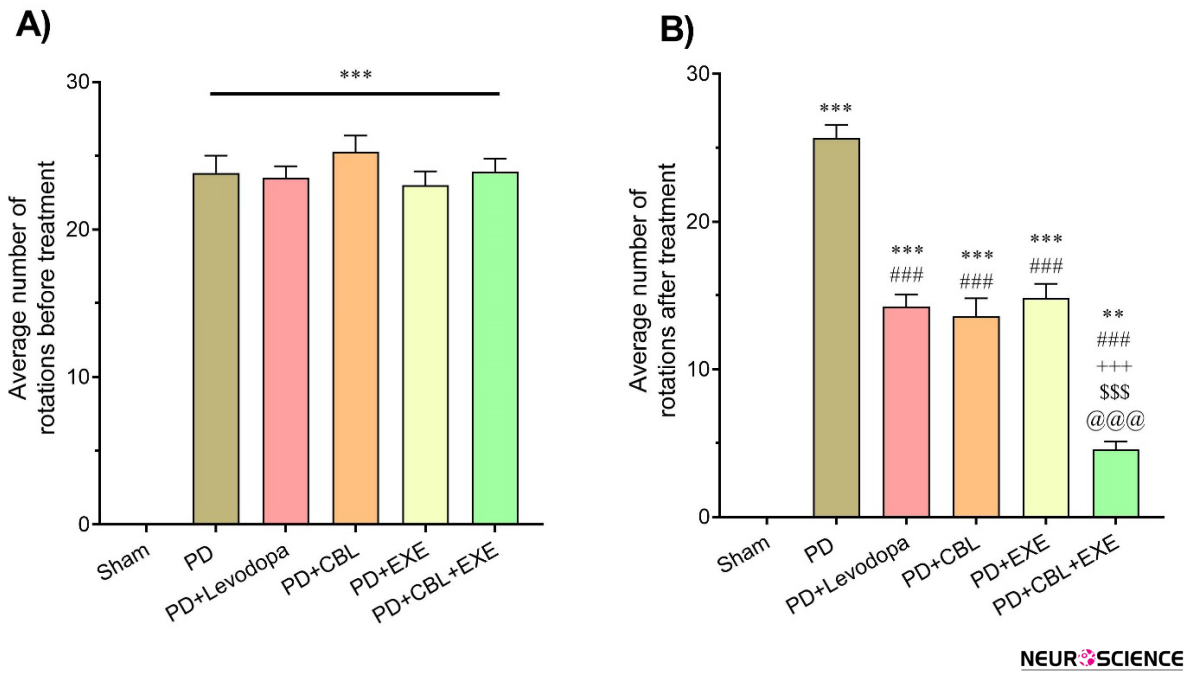


Figure 2. Effect of CBL and EXE on the average number of rotations before (A) and after treatment (B) in the PD rats

Abbreviations: PD: Parkinson disease; CBL: Cerebrolysin; EXE: Exercise.

Note: Data are expressed as Mean±SEM (n=12). **P<0.01, ***P<0.001 versus sham group; ###P<0.001 versus PD animals; +++P<0.001 versus PD+levodopa; $$$P<0.001 versus PD+CBL; @@@P<0.001 versus PD+ EXE.

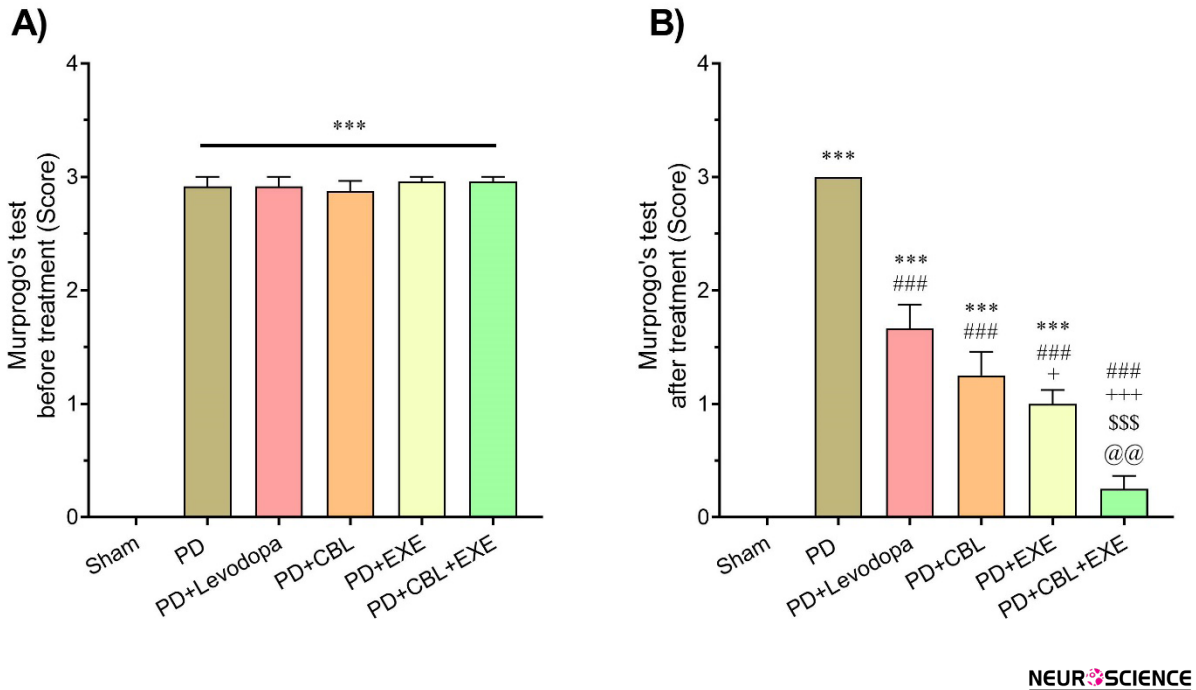


Figure 3. Effect of CBL and EXE on the average marks acquired from the Murprogo's test, before (A) and after treatment (B) in the PD animals

Abbreviations: PD: Parkinson disease; CBL: Cerebrolysin; EXE: Exercise.

Note: Data are presented by the Mean±SEM (n=12). ***P<0.001 versus sham group; ###P<0.001 versus PD animals; +P<0.05, +++P<0.001 versus PD+levodopa; $$$P<0.001 versus PD+CBL; @@P<0.01 versus PD+EXE.

Decreased lipid peroxidation and enhanced enzymatic antioxidant activity in PD rats after CBL and treadmill exercise, alone or in combination

We also found that 6-OHDA injection significantly (P<0.001, Figure 4A) increased brain MDA levels in the PD groups, except for the PD+CBL+EXE group, compared to the sham animals. Nevertheless, treatment with levodopa, CBL, EXE, or a combination of CBL+EXE significantly decreased brain lipid peroxidation compared to the PD group (P<0.001 for all comparisons). Of note, the effects of CBL and EXE, alone or in combination, were greater than levodopa therapy in decreasing MDA levels (P<0.001 for all comparisons). Furthermore, MDA levels were reduced more effectively by CBL therapy than by EXE alone (P<0.001). Likewise, combination therapy was more effective than single therapy (P<0.001).

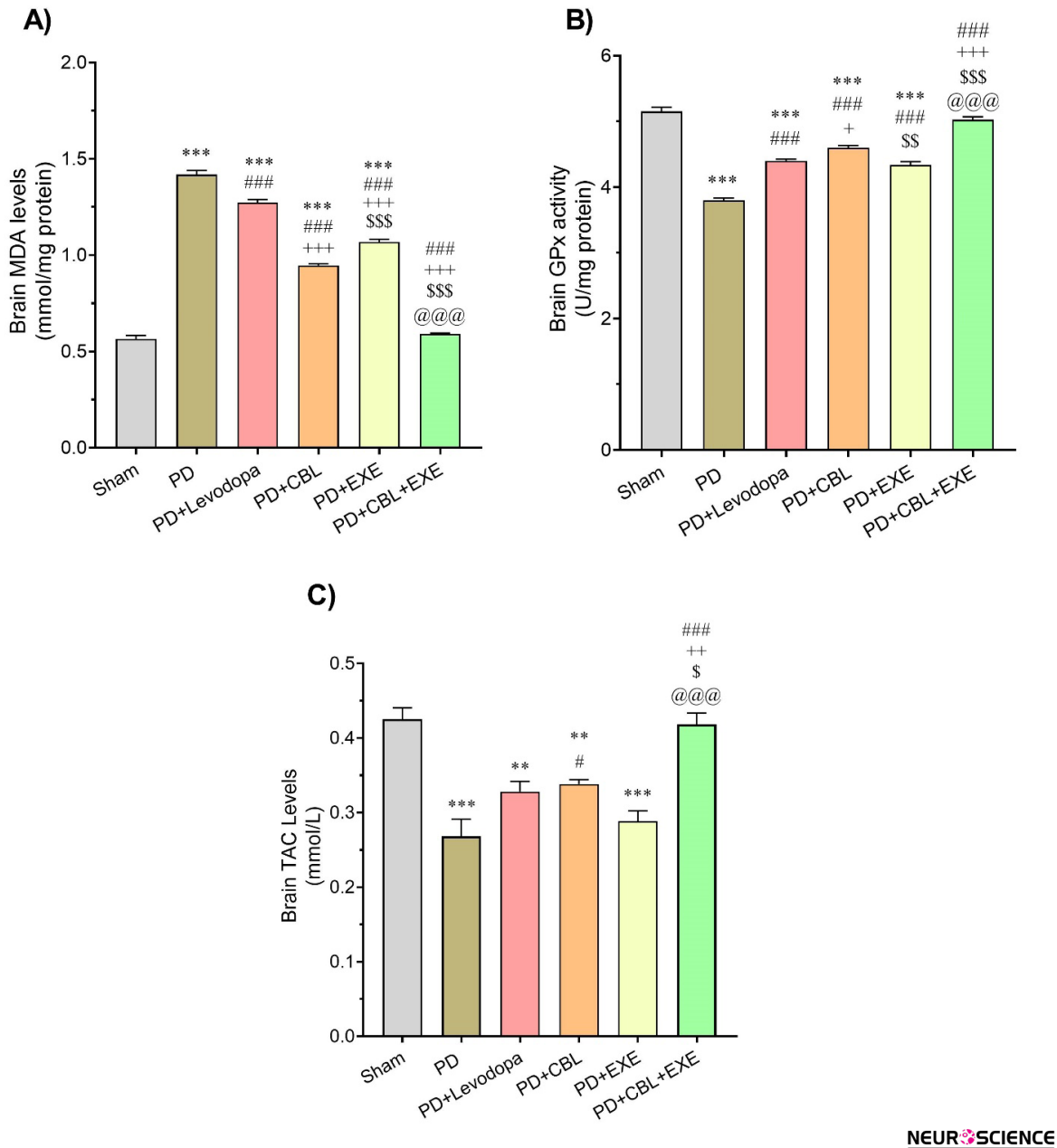


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Figure 4. Effect of CBL and EXE on brain A) MDA levels, B) GPx activity, and C) TAC levels in the PD animals

Abbreviations: PD: Parkinson disease; CBL: Cerebrolysin; EXE: Exercise; GPx: Glutathione peroxidase; MDA: Malondialdehyde; TAC: Total antioxidant capacity.

Note: Data are presented as Mean±SEM (n=6). *P<0.05 and ***P<0.001 versus sham group; $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 versus PD animals; $^{+++}$P<0.001 versus PD+levodopa; $^{\$\$\$}$P<0.001 versus PD+CBL; $^{@@@}$P<0.001 versus PD+EXE.

Moreover, 6-OHDA injection markedly (P<0.001) decreased the enzyme activity of GPx in the brain of PD animals as compared to the sham group (Figure 4B). Nevertheless, the PD+CBL+EXE and sham groups did not differ significantly. The results also demonstrated that the administration of levodopa and CBL, treadmill exercise, and a combination of CBL+EXE significantly (P<0.001 for all comparisons) increased brain GPx activity in PD animals. Furthermore, the effects of CBL alone (P<0.05) or in combination with EXE (P<0.001) were greater than levodopa therapy in increasing GPx activity. Additionally, the effect of CBL treatment was greater than EXE training in increasing GPx activity (P<0.01). Furthermore, in the PD+CBL+EXE group, GPx activity was higher than the PD+CBL and the PD+EXE groups (P<0.001 for both).

Likewise, brain TAC levels in the PD animals, except for the PD+CBL+EXE group, were significantly lower than the sham group (P<0.001 for PD and PD+EXE and P<0.01 for PD+levodopa and PD+CBL, Figure 4C). Although levodopa and treadmill exercise could not significantly increase TAC levels, CBL alone (P<0.05) or in combination with treadmill training (P<0.001) significantly increased TAC levels as compared to the PD rats. Interestingly, the effect of combination therapy in increasing TAC levels was greater than the treatments with levodopa and CBL (P<0.05) or EXE alone (P<0.001).

Decreased α-syn protein levels in the PD rats after CBL and treadmill exercise, alone or in combination

In Figure 5, immunoblotting results show that α-syn protein levels in the PD groups are significantly higher than in the sham group (P<0.001). However, treatment with levodopa, CBL, and EXE and a combination of CBL and EXE markedly decreased protein expression of α-synuclein in the PD animals (P<0.001 for all comparisons). Moreover, the effect of levodopa on α-syn protein expression was more effective than single treatments with CBL or EXE (P<0.001 for both comparisons). However, combination therapy was more effective than the effect of levodopa (P<0.05). Interestingly, the impact of treadmill exercise was greater than CBL (P<0.001) in down-regulating α-syn protein expression. Furthermore, combination therapy was more effective than single therapy with CBL or EXE (P<0.001) in diminishing α-syn protein expression.

Protected neurons in the left SNpc after CBL and treadmill exercise, alone or in combination

Figure 6 illustrates that the quantity of SNpc neurons in the PD groups, except for the PD+CBL+EXE group, was markedly lower than that in the sham group (P<0.001 for all comparisons). Nonetheless, treatment with levodopa and CBL or treadmill exercise markedly augmented the quantity of SNpc neurons compared

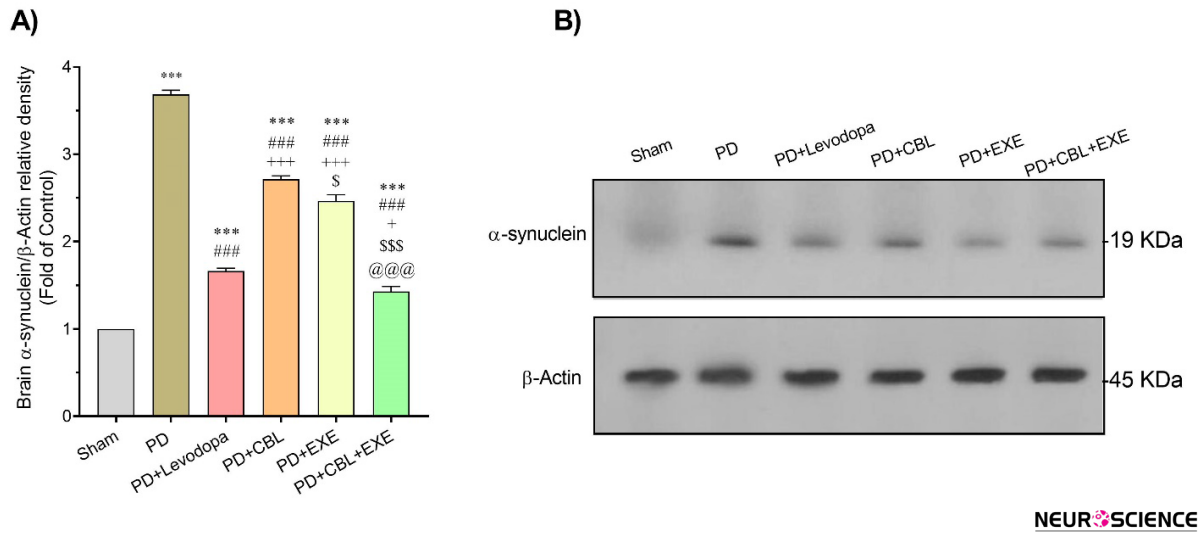

Figure 5. Effect of CBL and EXE on α-synuclein protein expression in the 6-OHDA-lesioned rats

A) Quantitative densitometric evaluation of α-synuclein protein in the brain, B) Representative images of the protein bands assessed by Western blotting

Abbreviations: PD: Parkinson disease; CBL: Cerebrolysin; EXE: Exercise; SNpc: Substantia nigra.

Note: Data are expressed as Mean±SEM (n=4). ***P<0.001 versus the sham group; $^{###}$P<0.001 versus PD animals; $^{+++}$P<0.001 versus levodopa; $^{\$\$\$}$P<0.001 versus CBL; $^{@@@}$P<0.001 versus EXE.

to the PD rats (P<0.001 for all comparisons). Furthermore, the impact of CBL alone (P<0.05) and in conjunction with EXE (P<0.001) surpassed that of levodopa therapy. Interestingly, CBL administration was significantly (P<0.01) more effective than treadmill training in protecting the SNpc neurons in PD animals. Moreover, substantial differences were observed between the PD+CBL+EXE group and the PD+CBL (P<0.05) and PD+EXE (P<0.001) groups.

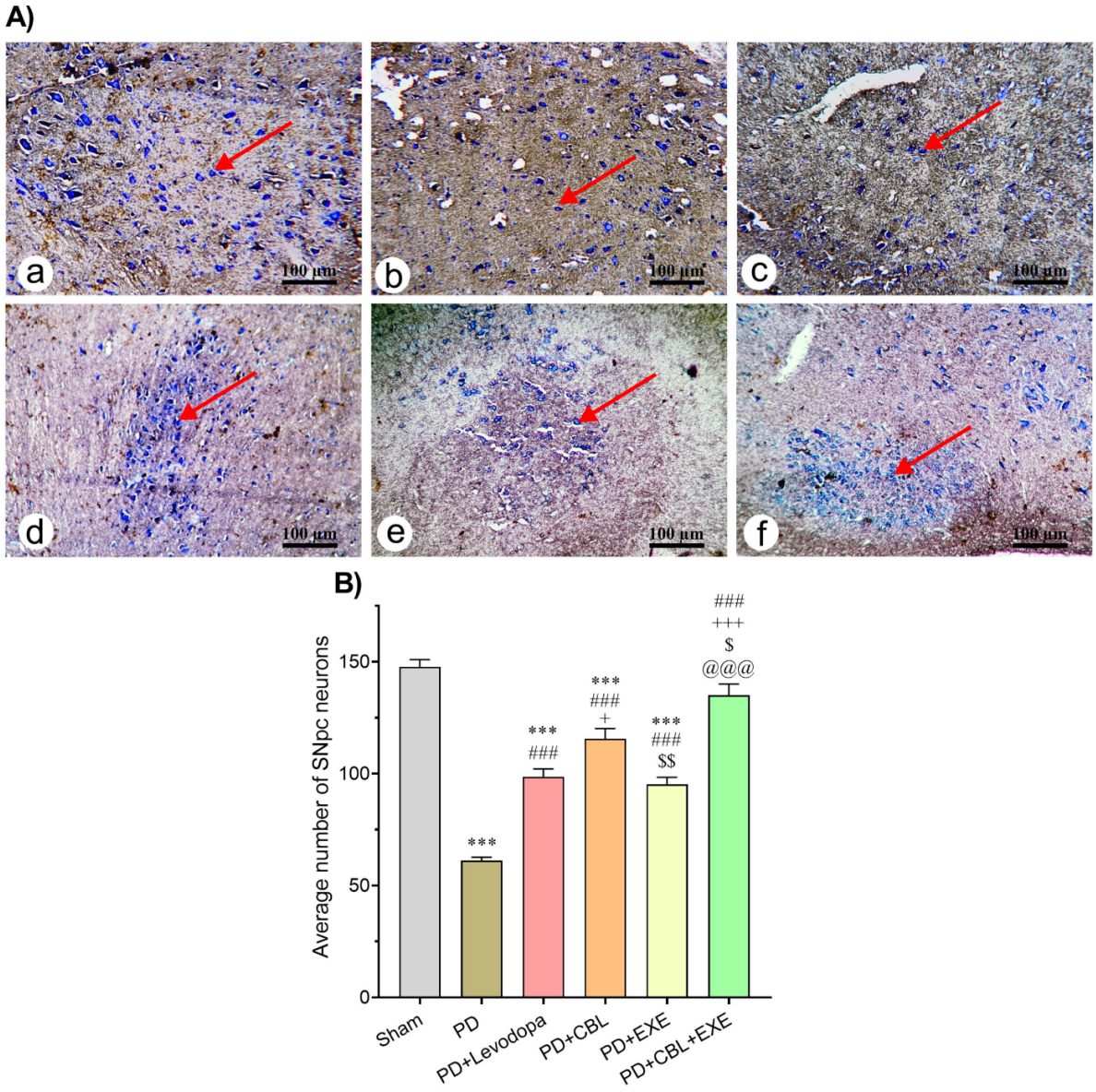

Figure 6. Effect of CBL and EXE on the number of SNpc in the PD rats

A) Cresyl violet-stained microscopic images of SNpc in different groups (a: Sham group; b: PD group; c: PD+levodopa; d: PD+CBL; e: PD+EXE; and f: PD+CBL+EXE, scale bar=100 µm)

B) The number of neurons in the SNpc (Mean±SEM, n=5)

Abbreviations: PD: Parkinson disease; CBL: Cerebrolysin; EXE: Exercise; SNpc: Substantia nigra pars compacta.

Note: Red arrows indicate representative neurons.

4. Discussion

The current investigation demonstrates that CBL and aerobic exercise, separately or together, promote motor function in 6-OHDA-lesioned rats, as indicated by reduced muscular rigidity and rotational movements. These behavioral changes are accompanied by decreased brain lipid peroxidation, enhanced GPx enzyme activity, and reduced α-syn protein expression. Besides, CBL and EXE, alone or in combination, protect the SNpc neurons in the PD animals.

Injection of 6-OHDA into different nigrostriatal pathways results in asymmetric motor behaviors that may indicate the lesion's quality and the injury's severity (Deumens et al., 2002). Noor et al. showed that bilateral 6-OHDA injection into SN significantly reduced midbrain and striatal dopamine, increased MDA and nitric oxide, and decreased glutathione levels (Noor et al., 2016). Likewise, the findings of this study demonstrated that 6-OHDA caused muscular rigidity and heightened contralateral spins towards the lesioned side in PD rats. These behavioral changes were accompanied by increased brain lipid peroxidation and decreased GPx activity.

However, CBL treatment and EXE markedly improved these motor impairments. Of note is that combining CBL and moderate-intensity exercise (PD+CBL+EXE group) was more effective than single therapy and even levodopa administration. In line with our findings, a single treatment with CBL at the dose of 2.5 mL/kg for 21 has been shown to reduce MDA levels and increase glutathione levels in the midbrain and striatum of 6-OHDA-lesioned rats (Noor et al., 2016). Another study reports that intravenous injection of CBL (3 mL/kg) or encapsulated CBL for 5 days increases latency to fall in the rotarod test along with an increase in dopamine and its metabolites in the SNpc of MPTP-induced PD mice model (Ozkizilcik et al., 2019).

Likewise, several preclinical and clinical studies have proven the beneficial effects of physical training on motor dysfunction in PD (Bhalsing et al., 2018; Lauzé et al., 2016; Svensson et al., 2015). Nadeau et al. found that chronic treadmill training (24 weeks) improved walking speed and endurance in PD participants (Nadeau et al., 2014). Chuang et al. also demonstrated that 4 weeks of treadmill training improved gait performance and decreased methamphetamine-induced rotational behaviors in 6-OHDA-lesioned rats by protecting DAergic neurons of the SN against oxidative stress damage (Chuang et al., 2010). Another study showed that treadmill training for 14 days diminished apomorphine-induced rotation and decreased the number of falls in the rotarod test (Costa et al., 2017).

Evidence proves that excessive ROS generation is linked to the suppression of enzymatic antioxidant defenses, which may result in disruptions in cell architecture and metabolic activity, and, consequently, neuronal degradation (Blum et al., 2001; Ji et al., 1988). 6-OHDA injection into SNpc mimics PD symptoms by extensive degeneration of DAergic neurons mainly through overproduction of free radicals and disruption of the mitochondrial electron transfer chain (Gonçalves et al., 2020; Mazzio et al., 2004; Yin et al., 2011). The impact of antioxidants on preventing or mitigating damage induced by free radicals in PD has been examined (Peerapatdit et al, 2006). Our investigation demonstrates that 6-OHDA injection into the SN resulted in a substantial rise of brain MDA levels, accompanied by a drop in TAC levels and GPx activity, indicating the emergence of oxidative stress and a compromise of the antioxidant defense system.

Nevertheless, CBL and aerobic exercise, alone or simultaneously, decrease lipid peroxidation and improve antioxidant status, protecting striatal neurons in PD rats. Abdel-Salam et al., also demonstrated the protective properties of CBL in mitigating oxidative damage and neuronal death in a rotenone-induced PD model (Abdel-Salam et al., 2014). Requejo et al. also reported that nano delivery of CBL protected nigrostriatal DAergic neurons and improved motor impairments in 6-OHDA via the stimulation of the Akt signaling pathway (Requejo et al., 2018). Furthermore, evidence suggests that CBL enhances the survival and differentiation of transplanted neuronal stem cells into DAergic neurons within the striatum of an α-syn transgenic model of PD by increasing neurotrophic factors (Rockenstein et al., 2015; Rockenstein et al., 2007). Of note, we found that the antioxidant effect of combination therapy was better than monotherapy with CBL or EXE, indicating a synergistic effect of combined treatment.

Our study found that CBL and aerobic exercise, either alone or together, effectively reduced α-syn protein levels. Reducing α-syn levels is a key strategy for mitigating oxidative stress in PD (Delic et al., 2017). Aggregated α-syn disrupts mitochondrial function, resulting in increased ROS generation and subsequent cellular damage, including lipid peroxidation and DNA damage, potentially leading to apoptosis and neuroinflammation (Lin et al., 2019). However, lowering α-syn levels enhances mitochondrial biogenesis via PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha) up-regulation. It activates the Nrf2 pathway, promoting the expression of antioxidant genes such as superoxide dismutase and GPx. Additionally, augmented autophagy has been associated with decreased α-syn levels, which facilitates the removal of damaged proteins (Chakkittukandiyil et al., 2022).

Likewise, Hsueh et al. reported that short-term (one week) physical exercise ameliorated non-motor symptoms, and long-term exercise reduced motor dysfunction in 6-OHDA-induced PD animals, mainly by increasing tyrosine hydroxylase in SN and striatal BDNF (brain-derived neurotrophic factor) and bone marrow tyrosine kinase in chromosome X levels (Hsueh et al., 2018). Moreover, it has been well-accepted that exercise enhances endogenous antioxidant systems and improves brain neurotrophic factors and neurogenesis (Blum et al., 2001; Devi & Kiran, 2004; Mattson et al., 2008; Mocchetti et al., 2007). It has also been reported that exercise improves motor deficits and protects DAergic neurons in SNpc in 6-OHDA-lesioned animals by suppressing oxidative stress and neuroinflammatory responses and up-regulation of BDNF in the striatum (Costa et al., 2017; Crowley et al., 2019; Dias et al., 2013; Dutra et al., 2012; Lau et al., 2011; Palasz et al., 2019; Ogonovszky et al., 2005; Wu et al., 2011).

This study presents several limitations that warrant consideration in future research. While we assessed the impacts of CBL and aerobic exercise on motor function, oxidative stress, and α-syn expression in a PD model, BDNF levels and apoptotic markers in SN cells were not directly evaluated. Future research should examine these parameters to enhance understanding of the neuroprotective mechanisms associated with the observed benefits. Furthermore, prolonging the treatment duration and integrating more precise cellular analyses, such as differentiating between DAergic and non-DAergic neurons, may provide significant insights into the long-term effects and cell-specific responses to these interventions. Addressing these limitations may result in more effective and targeted treatment strategies, enhancing the quality of life for patients with PD.

5. Conclusion

According to the results of the present study, simultaneous moderate-intensity aerobic exercise and CBL administration synergistically attenuated 6-OHDA-induced motor dysfunction and muscle rigidity, possibly by enhancing antioxidant activity, decreasing oxidative stress, and protecting striatal neurons.

Ethical Considerations

Compliance with ethical guidelines

This research was approved by the Ethics Committee of Tabriz Medical Sciences Branch, Islamic Azad University, Tabriz, Iran (Code: IR.IAU.TABRIZ.REC.1401.082). All experimental procedures adhered to established guidelines for the ethical treatment of animals in research.

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Authors' contributions

Methodology: Mir Alireza Nourazar; Investigation: Seyed Zanyar Athari, and Mir Alireza Nourazar; Histopathological study, and statistical analysis: Daryoush Mohajeri; Writing the original draft: Seyed Zanyar Athari; Review, and editing: Fereshteh Farajdokht.

Conflict of interest

The authors declared no conflict of interest.

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