Title: Synergistic Effects of Cerebrolysin and Aerobic Exercise on 6-Hydroxydopamine-Induced Model of Parkinson's Disease in Adult Male Rats

Running Title: Combination of Exercise and Cerebrolysin Improved PD-associated Motor Deficits

Authors: Seyed Zanyar Athari[1], Fereshteh Farajdokht[1], Daryoush Mohajeri[2], Mir Alireza Nourazar[3,*]

1. Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran.
3. Department of Basic Sciences, Faculty of Veterinary Medicine, Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran.

***Corresponding Author**: Mir Alireza Nourazar, Department of Basic Sciences, Faculty of Veterinary Medicine, Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran. Email: Noura347@yahoo.com; Nourazar@iaut.ac.ir

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Abstract

Neurotrophic factors and physical activities have beneficial effects on neurodegenerative disorders. This study assessed the effect of physical activity (EXE) and Cerebrolysin (CBL), individually or in conjunction, in a hemiparkinsonian model (PD) caused by 6-hydroxydopamine (6-OHDA). The study utilized seventy-two male Wistar rats, which were distributed into six groups (n=12): Sham (received intra substantia nigra (SN) injection of normal saline), PD (underwent 6-OHDA (12.5 μg) injection 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/day for three weeks), and PD+CBL+EXE. Rotation with apomorphine and Murprogo's test were 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. 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 decreased malondialdehyde levels and α-synuclein protein levels, increased TAC level and GPx activity, as well as a greater neuronal count in the SN. Notably, the combination effects were greater than single therapy and levodopa treatment. Our findings indicated 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.

Keywords: Cerebrolysin, Parkinson's disease, Exercise, Oxidative Stress, α-synuclein

Introduction

The destruction of dopaminergic (DAergic) neurons in Substantia Nigra pars compacta (SNpc) and Ventral Tegmental Area (VTA) causes Parkinson's disease (PD) (Balestrino & Schapira, 2020; Wright Willis, Evanoff, Lian, Criswell, & Racette, 2010). The number of patients suffer from PD from 2.5 million in 1990 reached to 6.1 million in 2016, and it is estimated that it will be 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 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 (Tue 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, Kurup, Mathew, & Baby, 2015; Warnecke et al., 2016). On the other hand, in advanced PD 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 comprising approximately 15% tiny peptides and 85% amino acids (Mahmoudi et al., 2018; Plosker & Gauthier, 2009), exhibiting neurological effects similar to endogenous neuronal factors. The pharmacodynamics effects of CBL include neuronal survival (Plosker & Gauthier, 2009), neuroprotection (Álvarez

et al., 2000; Rockenstein et al., 2006; Ubhi et al., 2009; Veinbergs, Mante, Mallory, & Masliah, 2000), neuroplasticity, and neurogenesis (Hartbauer, Hutter-Paier, & Windisch, 2001). Hartbauer et al. showed that CBL degrades malondialdehyde (MDA) and nitric oxide (NO) and improves oxidative damage by enhancing endogenous antioxidant enzyme activity such as glutathione peroxidase (GPx) (Ardjmand, Shahaboddin, Mazoochi, & Ghavipanjeh, 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, Mohammed, Mourad, Khadrawy, & Aboul Ezz, 2016; Requejo et al., 2018; Rockenstein et al., 2015).

Physical activity has been shown to increase neurotrophic factors, antioxidant capacity and decrease inflammation, which can be proposed as a non-invasive intervention to minimize neuronal damage and improve PD symptoms (Osali, 2020; Yau, Gil-Mohapel, Christie, & So, 2014). The best exercise regimen for people with PD, according to major health organizations, is moderate-intensity aerobic activity, which involves maintaining the heart rate at 64-76% of its maximal reserve (Piercy et al., 2018; World Health Organization, 2018). Moderate aerobic exercise has been proven to enhance balance and gait in PD patients (Li, Song, Shen, & Wang, 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, Aparicio, Aranda, & Radak, 2013; Cerqueira, Marinho, Neiva, & Lourenço, 2020; Takehara et al., 2017).

Considering that both CBL and moderate exercise independently enhance neuronal health and functional improvement in PD models, we suggest that 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.

Material 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

The Ethics Committee at the Tabriz branch of Islamic Azad University confirmed that all experimental procedures adhered to established ethical standards for the treatment and use of animals (IR.IAU.TABRIZ.REC.1401.082). In this study, 72 adult male Wistar rats weighing 250 ± 20 grams (8–10 weeks) were used. The rodents were maintained in a carefully regulated setting involving a 12-hour cycle of illumination 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.

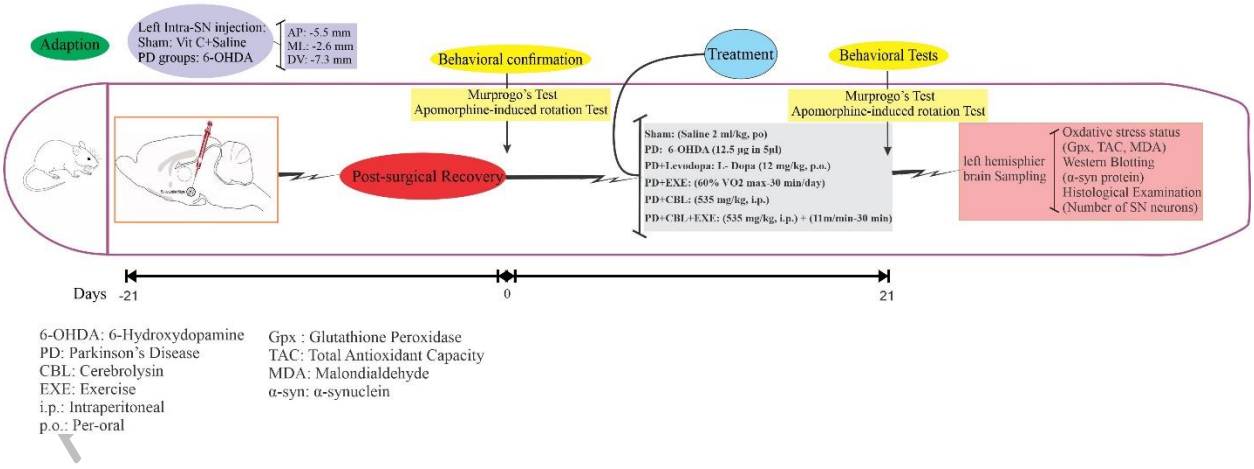

Figure 1. Timeline of this investigation

Grouping

The rats were arbitrarily allocated into six groups (n=12/group): I) sham group, administered an SN injection of the 6-OHDA vehicle and received normal saline treatment (i.p) for three weeks, II) PD group (PD), received a SN injection of 6-OHDA toxin and subjected to normal saline treatment (i.p.) for 21 days, III) PD+Levodopa group, PD animals 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 (i.p) 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 the course of 21 days, and VI) PD+CBL+EXE group, PD animals were received a combination of CBL and treadmill exercise for 21 days.

Induction of PD model

The stereotaxic equipment was used to mount the head of the anesthetized rat. In accordance with 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, Behzadi, & Baluchnejadmojarad, 2002). The sham group received the same amount of normal saline and vitamin C as the vehicle of 6-OHDA. Both saline and the toxin were given in a gradually manner, with a flow speed of 1 µl/min, and the needle (No. 27) was withdrawn 5 min subsequent to 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 (prior to treatment) and twenty-one days subsequent to 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 a 30-min period at 10-min 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, while 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 high 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. The identical test was conducted for the contralateral forelimb. Subsequently, the rat's right forelimb was situated on a 9-cm 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

In order to identify the maximum oxygen consumption (VO_2 max) in the studied rats, it was done as follows: 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) where the animals exercised with an intensity of approximately 60% VO2 max for three weeks, 6 days a week, and 30 minutes each

day. At the conclusion of each week, 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 subsequent to the final behavioral assessment, all rats were euthanized by under a 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 in order to histologically evaluate the quantity of SN neurons.

Evaluation of oxidative stress levels

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

Malondialdehyde (MDA) levels

The concentration of MDA, indicative of lipid peroxidation, was assessed by Thiobarbituric acid reaction (TBAR) colorimetric assay kit according to the instructions of the manufacturer. A plate reader measured the absorbance at 540 nm, and the result was shown as nmol/mg protein (Ozdemir, Cetinkaya, Ersan, Kucukosman, & Ersan, 2009).

Glutathione peroxidase (GPx)

The RANSEL kit (Randox Laboratories Ltd.) was used to estimate 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 was conducted utilizing the 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methodology. Absorbance measurements were conducted at a wavelength of 600 nm utilizing a spectrophotometer, with the findings expressed in µmol/mg protein.

Western blotting

In order 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 12,000 g for a duration of 10 minutes at a temperature of 4 °C. The protein concentrations in the resulting supernatant were assessed applying the Bradford method. Following electrophoresis on a 12.5% SDS-polyacrylamide gel, 40 µg of protein was subsequently transferred onto a PVDF membrane (Roche, UK). Subsequently, the membrane underwent an overnight incubation at 4 °C with primary antibodies (Santa Cruz, CA, USA) specifically aimed at α-syn (sc-12767) and β-actin (sc-47778), which served as the loading control. Following three washes with TBST, the membranes underwent a treatment for two hours with HRP-conjugated goat anti-rabbit IgG secondary antibody at a dilution of 1:5000 (sc-2004). Following the washing process with PBS, the membranes were immersed in 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 Image J software (Jin, Wu, Lu, Gong, & Shi, 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 ± standard error of the mean (S.E.M). GraphPad Prism 6.01 software (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-value of less than 0.05.

Results

Cerebrolysin and treadmill exercise, alone or in combination, decreased contralateral rotations in the PD animals

As Figure 2A shows, injection of 6-OHDA obviously 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 PD model. Moreover, normal saline-treated PD animals showed higher contralateral rotations than the sham group at the end of the treatment (Fig. 2B). However, 21 days 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 ($p<0.001$ for

both). Combination therapy demonstrated greater efficacy than levodopa treatment in reducing apomorphine-induced rotations ($p<0.001$).

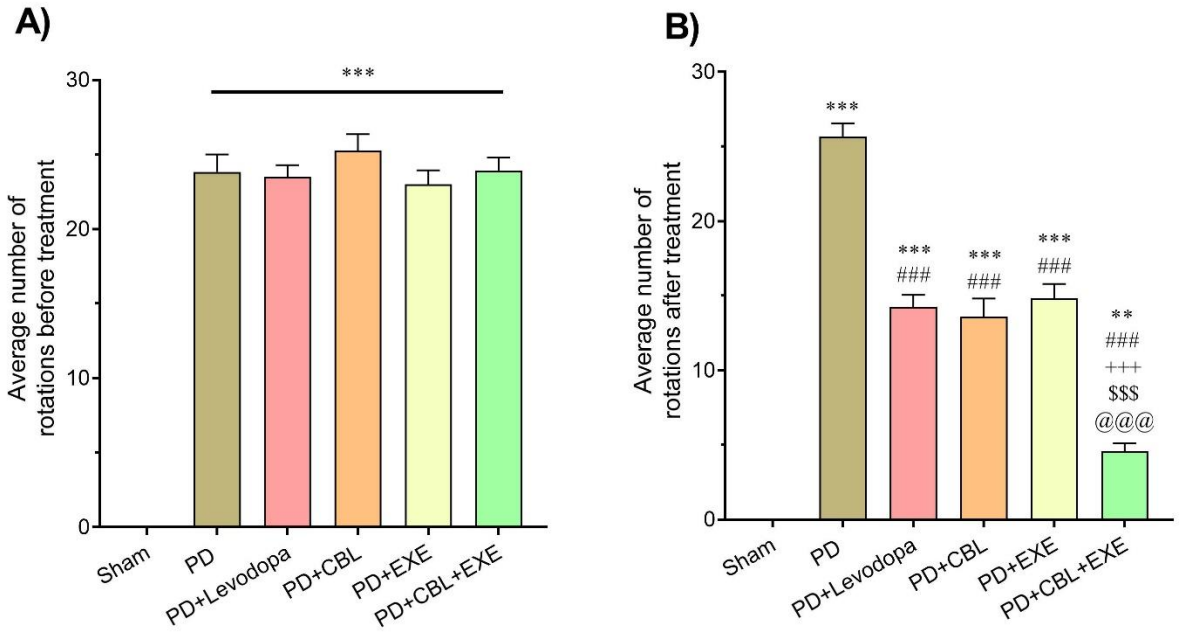

Figure 2. Effect of CBL and EXE on the average number of rotations: A) before treatment and B) after treatment in the PD rats. Data are expressed as mean ± SEM (n=12). **p<0.01, ***p<0.001 versus Sham group; $^{###}$p<0.001 versus PD animals; $^{+++}$ p<0.001 versus PD+Levodopa; $^{\$\$\$}$ p<0.001 versus PD+CBL; $^{@@@}$ p<0.001 versus PD+ EXE. [PD, Parkinson's disease; CBL, Cerebrolysin; EXE, Exercise].

Cerebrolysin and treadmill exercise, alone or in combination, attenuated muscle rigidity in the PD rats

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, Fig. 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 was observed between the PD+EXE and PD+Levodopa groups ($p<0.05$). Interestingly, combination therapy had greater effect in decreasing Murprogo's score than single treatment with CBL ($p<0.001$) or treadmill exercise ($p<0.01$).

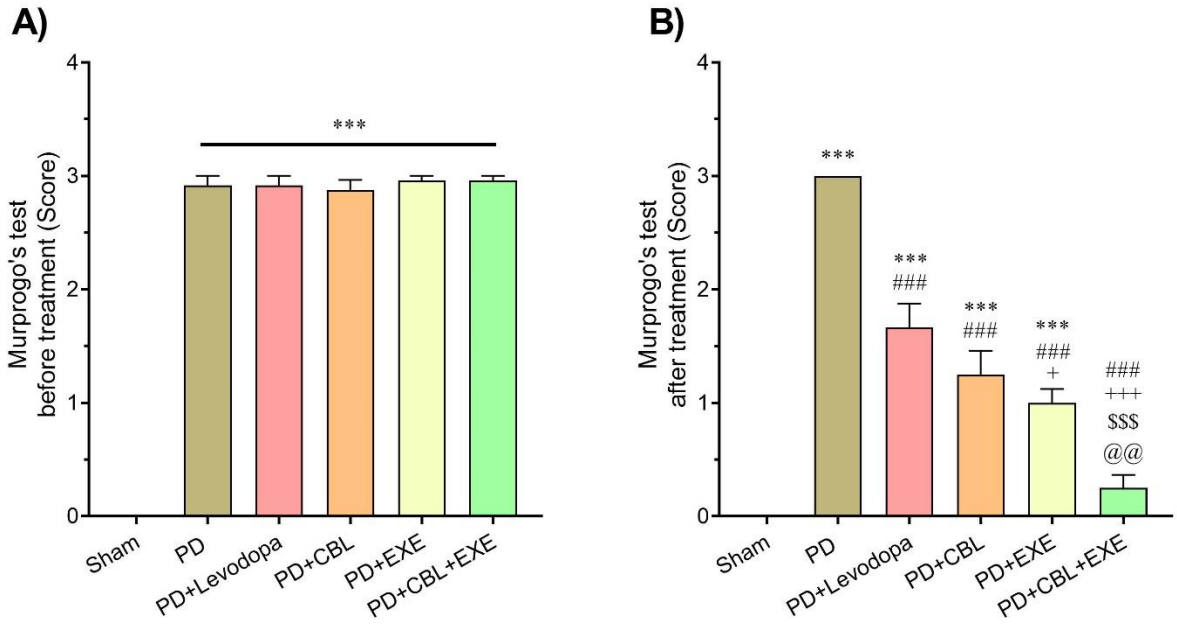

Figure 3. Effect of CBL and EXE on the average marks acquired from the Murprogo's test, A) prior to treatment and B) subsequent to treatment in the PD animals. The mean ± SEM is used to represent the data (n=12). ***$p<0.001$ versus Sham group; $^{###}p<0.001$ versus PD animals; $^{+}p<0.05$, $^{+++}p<0.001$ versus PD+Levodopa; $^{\$\$\$}$ $p<0.001$ versus PD+CBL; $^{@@}$ $p<0.01$ versus PD+EXE. [PD, Parkinson's disease; CBL, Cerebrolysin; EXE, Exercise].

Cerebrolysin and treadmill exercise, alone or in combination, decreased lipid peroxidation and enhanced enzymatic antioxidant activity in PD rats

We also found that 6-OHDA injection significantly ($p<0.001$, Fig 4A) increased brain MDA levels in the PD groups, except for the PD+CBL+EXE group, when compared to the Sham animals. Nevertheless, treatment with Levodopa, CBL, EXE, or 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 ($p<0.001$).

Moreover, 6-OHDA injection markedly ($p<0.001$) decreased enzyme activity of GPx in the brain of PD animals as compared to the Sham group (Fig. 4B). Nevertheless, the PD+CBL+EXE and Sham groups did not differ significantly. The results also demonstrated that administration of Levodopa and CBL, and treadmill exercise, as well as 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, Fig. 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 ($p<0.001$).

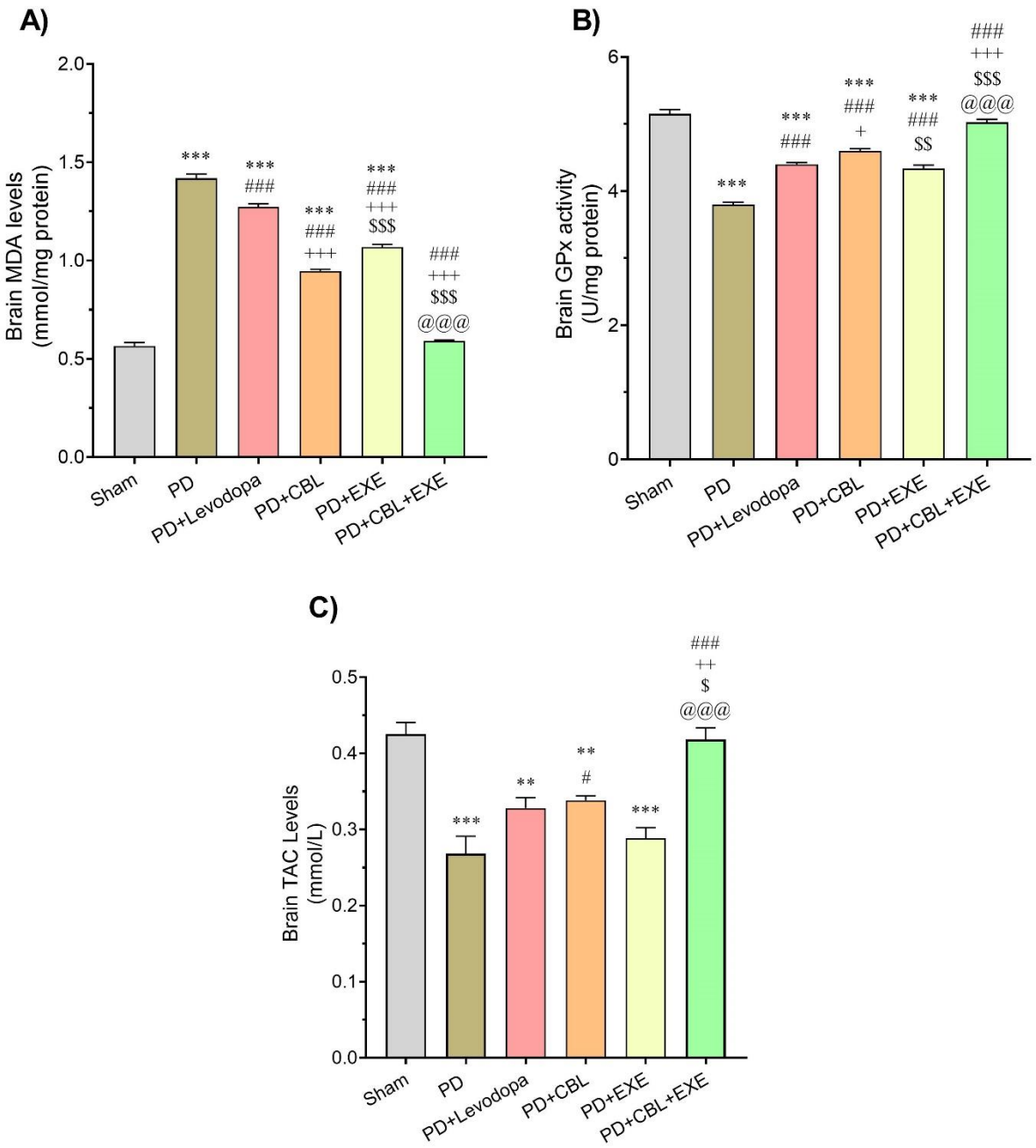

Figure 4. Effect of CBL and EXE on brain A) MDA levels, B) GPx activity, and C) TAC levels in the PD animals. Data are expressed as mean ± SEM (n=6). *p<0.05 and ***p<0.001 versus Sham group; ##p<0.01 and ###p<0.001 versus PD animals; +++p<0.001 versus PD+Levodopa; $^{\$\$\$}$p<0.001 versus PD+CBL; $^{@@@}$p<0.001 versus PD+EXE. [PD, Parkinson's disease; CBL, Cerebrolysin; EXE, Exercise; GPx: Glutathione Peroxidase; MDA: Malondialdehyde; TAC: Total Antioxidant Capacity].

Cerebrolysin and treadmill exercise, alone or in combination, decreased α-syn protein levels in the PD rats

In Figure 5, the result of immunoblotting showed that α-syn protein levels in the PD groups was significantly higher than the Sham group ($p<0.001$). However, treatment with Levodopa, CBL, and EXE, as well as 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), though combination therapy was more effective than the effect of Levodopa ($p<0.05$). Interestingly, the effect 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.

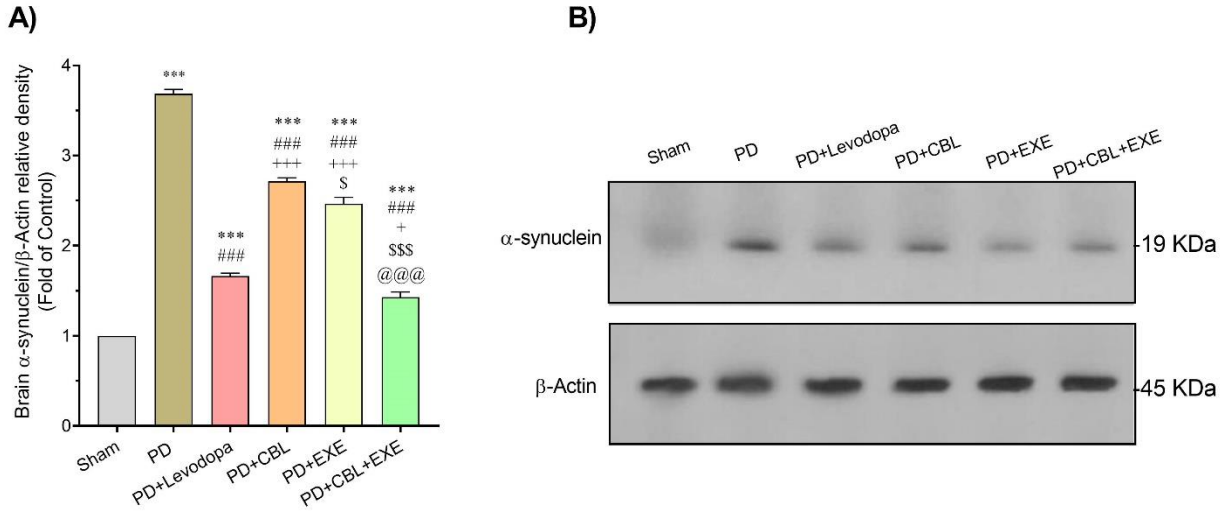

Figure 5. Effect of CBL and EXE on α-synuclein protein expression in the 6-OHDA-lesioned rats. A) Quantitative densitometric evaluation of α-synuclein protein in the brain. B) Representative images of the protein bands assessed by Western blotting. Data are expressed as mean±SEM (n=4). $^{***}p<0.001$ versus Sham group; $^{###}p<0.001$ versus PD animals; $^{+++}p<0.001$ versus Levodopa; $^{\$\$\$}$ $p<0.001$ versus CBL; $^{@@@}$ $p<0.001$ versus EXE. [PD, Parkinson's disease; CBL, Cerebrolysin; EXE, Exercise; SNpc: Substantia nigra].

Cerebrolysin and treadmill exercise, alone or in combination, protected neurons in the left SNpc

Figure 6 illustrates that the quantity of SNpc neurons in the PD groups, with the exception of 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 and/or treadmill exercise markedly augmented the quantity of SNpc neurons in contrast 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.

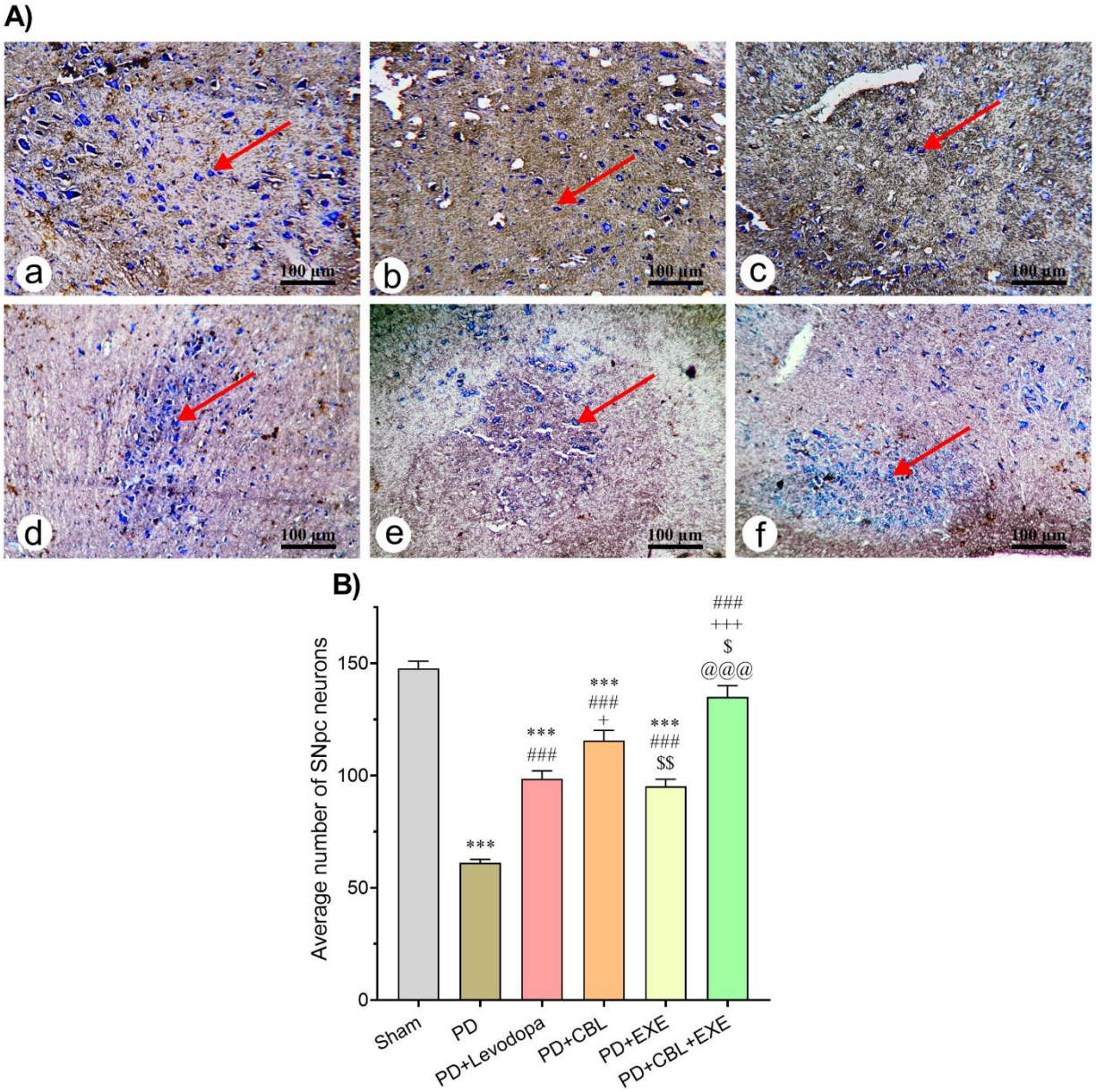

Figure 6. Effect of CBL and EXE on the number of SNpc in the PD rats. A) Cresyl violet-stained microscopic images of SNpc in different groups (a: Sham group; b: PD group; c: PD+Levodopa; d: PD+CBL; e: PD+EXE; and f: PD+CBL+EXE), Scale bar= 100 µm. B) The mean number of neurons in the SNpc (mean ± SEM, n=5). Red arrows indicate representative neurons. [PD, Parkinson's disease; CBL, Cerebrolysin; EXE, Exercise; SNpc: Substantia nigra pars compacta].

Discussion

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

Injection of 6-OHDA into different nigrostriatal pathway results in asymmetric motor behaviors that may indicate the quality of the lesion and the severity of the injury (Deumens, Blokland, & Prickaerts, 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 an increased brain lipid peroxidation and decreased GPx activity.

However, CBL treatment and EXE markedly improved these motor impairments. Of note, the combination of CBL and moderate-intensity exercise (PD+CBL+EXE group) was more effective than single therapy and even than Levodopa administration. In line with our findings, single therapy 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 reported that intravenous injection of CBL (3 ml/kg) or encapsulated CBL for 5 days increased 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, Abbas, & Tan, 2018; Lauzé, Daneault, & Duval, 2016; Svensson, Lexell, & Deierborg, 2015). Nadeau et al. found that chronic treadmill training (24 weeks) improved walking speed and endurance in PD participants (Nadeau, Pourcher, & Corbeil, 2014). Chuang et al. also demonstrated that 4 weeks 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 fall 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, Stratman, & Lardy, 1988). 6-OHDA injection into SNpc mimics PD symptoms by extensive degeneration of DAergic neurons mainly through overproduction of free radicals and disruption of mitochondrial electron transfer chain (C. Gonçalves et al., 2020; Mazzio, Reams, & Soliman, 2004; Yin, Geng, & Zhu, 2011). The impact of antioxidants on the prevention or mitigation of damage induced by free radicals in PD has been examined (Peerapatdit, Patchanans, Likidlilid, Poldee, & Sriratanasathavorn, 2006). Our investigation demonstrated that 6-OHDA injection into the SN resulted in a substantial raise 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, decreased lipid peroxidation and improved antioxidant status resulting in a protection of striatal neurons in PD rats. Omar 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 nanodelivery 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, 2007). Of note, we found that 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. The reduction of 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 up-regulation and activates the Nrf2 pathway, promoting the expression of antioxidant genes such as SOD 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 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, Gleichmann, & Cheng, 2008; Mocchetti, Bachis, Nosheny, & Tanda, 2007). It has been also reported that exercise improved motor deficits and protects DAergic neurons in SNpc in 6-OHDA-lesioned animals by suppression of oxidative stress and neuroinflammatory responses, and up-regulation of BDNF in the striatum (Costa et al., 2017; Crowley, Nolan, & Sullivan, 2019; Dias, Junn, & Mouradian, 2013; Dutra et al., 2012; Lau, Patki, Das-Panja, Le, & Ahmad, 2011; M. Kokaia, 2009; 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 the 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, thereby enhancing the quality of life for patients with PD.

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 and decreasing oxidative stress, and protecting striatal neurons.

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Availability of data and materials

Upon reasonable request, the corresponding author can provide the data that substantiates the findings of this study.

Authors' contributions

SZA, Investigation, Writing the original draft; FF, Writing, review & editing the manuscript; MAN, Investigation, Methodology. DM, Histopathological Study, Statistical analysis.

Ethics approval and consent to participate

This research received approval from the ethics committee at the Tabriz branch of Islamic Azad University (IR.IAU.TABRIZ.REC.1401.082), and all experimental procedures adhered to established guidelines for the ethical treatment of animals in research.

Patient consent for publication

N/A

Competing interests

N/A

References

Abdel-Salam, O., Mohammed, N., Youness, E., Khadrawy, Y., Omara, E., & Sleem, A. (2014). Cerebrolysin protects against rotenone-induced oxidative stress and neurodegeneration. *Journal of Neurorestoratology*, *2*, 47. https://doi.org/10.2147/JN.S50114

Álvarez, X. A., Lombardi, V. R. M., Fernández-Novoa, L., García, M., Sampedro, C., Cagiao, A., … Windisch, M. (2000). Cerebrolysin® reduces microglial activation in vivo and in vitro: a potential mechanism of neuroprotection. In *Advances in Dementia Research* (pp. 281–292). Vienna: Springer Vienna. https://doi.org/10.1007/978-3-7091-6781-6_30

Ardjmand, A., Shahaboddin, M. E., Mazoochi, T., & Ghavipanjeh, G. (2019). Ameliorative effects of cerebrolysin against isoproterenol-induced myocardial injury in male rats. *Life Sciences*, *227*, 187–192. https://doi.org/10.1016/j.lfs.2019.04.056

Balestrino, R., & Schapira, A. H. V. (2020). Parkinson disease. *European Journal of Neurology*, *27*(1), 27–42. https://doi.org/10.1111/ene.14108

Belotto, M. F., Magdalon, J., Rodrigues, H. G., Vinolo, M. A. R., Curi, R., Pithon-Curi, T. C., & Hatanaka, E. (2010). Moderate exercise improves leucocyte function and decreases inflammation in diabetes. *Clinical and Experimental Immunology*, *162*(2), 237–243. https://doi.org/10.1111/j.1365-2249.2010.04240.x

Bhalsing, K. S., Abbas, M. M., & Tan, L. C. S. (2018). Role of Physical Activity in Parkinson's Disease. *Annals of Indian Academy of Neurology*, *21*(4), 242–249. https://doi.org/10.4103/aian.AIAN_169_18

Blum, D., Torch, S., Lambeng, N., Nissou, M.-F., Benabid, A.-L., Sadoul, R., & Verna, J.-M. (2001). Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. *Progress in Neurobiology*, *65*(2), 135–172. https://doi.org/10.1016/S0301-0082(01)00003-X

C. Gonçalves, V., J. L. L. Pinheiro, D., de la Rosa, T., G. de Almeida, A.-C., A. Scorza, F., & A. Scorza, C. (2020). Propolis as a Potential Disease-Modifying Strategy in Parkinson's disease: Cardioprotective and Neuroprotective Effects in the 6-OHDA Rat Model. *Nutrients*, *12*(6), 1551. https://doi.org/10.3390/nu12061551

Camiletti-Moirón, D., Aparicio, V. A., Aranda, P., & Radak, Z. (2013). Does exercise reduce brain oxidative stress? A systematic review. *Scandinavian Journal of Medicine & Science in*

Sports, *23*(4), e202–e212. https://doi.org/10.1111/sms.12065

Carvalho, M. M., Campos, F. L., Marques, M., Soares-Cunha, C., Kokras, N., Dalla, C., … Salgado, A. J. (2017). Effect of Levodopa on Reward and Impulsivity in a Rat Model of Parkinson's Disease. *Frontiers in Behavioral Neuroscience*, *11*, 145. https://doi.org/10.3389/fnbeh.2017.00145

Cerqueira, É., Marinho, D. A., Neiva, H. P., & Lourenço, O. (2020). Inflammatory Effects of High and Moderate Intensity Exercise—A Systematic Review. *Frontiers in Physiology*, *10*. https://doi.org/10.3389/fphys.2019.01550

Chakkittukandiyil, A., Sajini, D. V., Karuppaiah, A., & Selvaraj, D. (2022). The principal molecular mechanisms behind the activation of Keap1/Nrf2/ARE pathway leading to neuroprotective action in Parkinson's disease. *Neurochemistry International*, *156*, 105325. https://doi.org/10.1016/j.neuint.2022.105325

Chuang, C.-S., Su, H.-L., Cheng, F.-C., Hsu, S., Chuang, C.-F., & Liu, C.-S. (2010). Quantitative evaluation of motor function before and after engraftment of dopaminergic neurons in a rat model of Parkinson's disease. *Journal of Biomedical Science*, *17*(1), 9. https://doi.org/10.1186/1423-0127-17-9

Costa, R. O. da, Gadelha-Filho, C. V. J., Costa, A. E. M. da, Feitosa, M. L., Araújo, D. P. de, Lucena, J. D. de, … Barros Viana, G. S. de. (2017). The Treadmill Exercise Protects against Dopaminergic Neuron Loss and Brain Oxidative Stress in Parkinsonian Rats. *Oxidative Medicine and Cellular Longevity*, *2017*, 1–10. https://doi.org/10.1155/2017/2138169

Crowley, E. K., Nolan, Y. M., & Sullivan, A. M. (2019). Exercise as a therapeutic intervention for motor and non-motor symptoms in Parkinson's disease: Evidence from rodent models. *Progress in Neurobiology*, *172*, 2–22. https://doi.org/10.1016/j.pneurobio.2018.11.003

Deumens, R., Blokland, A., & Prickaerts, J. (2002). Modeling Parkinson's Disease in Rats: An Evaluation of 6-OHDA Lesions of the Nigrostriatal Pathway. *Experimental Neurology*, *175*(2), 303–317. https://doi.org/10.1006/exnr.2002.7891

Delic, V., Griffin, J. W., Zivkovic, S., Zhang, Y., Phan, T. A., Gong, H., ... & Bradshaw, P. C. (2017). Individual amino acid supplementation can improve energy metabolism and decrease ROS production in neuronal cells overexpressing alpha-synuclein. *Neuromolecular Medicine*, *19*, 322-344. https://doi.org/10.1007/s12017-017-8448-8

Devi, S. A., & Kiran, T. R. (2004). Regional responses in antioxidant system to exercise

training and dietary Vitamin E in aging rat brain. *Neurobiology of Aging*, *25*(4), 501–508. https://doi.org/10.1016/S0197-4580(03)00112-X

Dias, V., Junn, E., & Mouradian, M. M. (2013). The Role of Oxidative Stress in Parkinson's Disease. *Journal of Parkinson's Disease*, *3*(4), 461–491. https://doi.org/10.3233/JPD-130230

Dorsey, E. R., Elbaz, A., Nichols, E., Abd-Allah, F., Abdelalim, A., Adsuar, J. C., … Murray, C. J. L. (2018). Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology*, *17*(11), 939–953. https://doi.org/10.1016/S1474-4422(18)30295-3

Dutra, M. F., Jaeger, M., Ilha, J., Kalil-Gaspar, P. I., Marcuzzo, S., & Achaval, M. (2012). Exercise improves motor deficits and alters striatal GFAP expression in a 6-OHDA-induced rat model of Parkinson's disease. *Neurological Sciences*, *33*(5), 1137–1144. https://doi.org/10.1007/s10072-011-0925-5

Hartbauer, M., Hutter-Paier, B., & Windisch, M. (2001). Effects of Cerebrolysin on the outgrowth and protection of processes of cultured brain neurons. *Journal of Neural Transmission*, *108*(5), 581–592. https://doi.org/10.1007/s007020170058

Hsueh, S.-C., Chen, K.-Y., Lai, J.-H., Wu, C.-C., Yu, Y.-W., Luo, Y., … Chiang, Y.-H. (2018). Voluntary Physical Exercise Improves Subsequent Motor and Cognitive Impairments in a Rat Model of Parkinson's Disease. *International Journal of Molecular Sciences*, *19*(2), 508. https://doi.org/10.3390/ijms19020508

Jansen van Rensburg, Z., Abrahams, S., Bardien, S., & Kenyon, C. (2021). Toxic feedback loop involving iron, reactive oxygen species, α-synuclein and neuromelanin in Parkinson's disease and intervention with turmeric. *Molecular neurobiology*, *58*(11), 5920-5936. https://doi.org/10.1007/s12035-021-02516-5

Ji, L. L., Stratman, F. W., & Lardy, H. A. (1988). Antioxidant enzyme systems in rat liver and skeletal muscle. *Archives of Biochemistry and Biophysics*, *263*(1), 150–160. https://doi.org/10.1016/0003-9861(88)90623-6

Jin, F., Wu, Q., Lu, Y.-F., Gong, Q.-H., & Shi, J.-S. (2008). Neuroprotective effect of resveratrol on 6-OHDA-induced Parkinson's disease in rats. *European Journal of Pharmacology*, *600*(1–3), 78–82. https://doi.org/10.1016/j.ejphar.2008.10.005

Kalia, L. V., & Lang, A. E. (2015). Parkinson's disease. *The Lancet*, *386*(9996), 896–912. https://doi.org/10.1016/S0140-6736(14)61393-3

Lau, Y.-S., Patki, G., Das-Panja, K., Le, W.-D., & Ahmad, S. O. (2011). Neuroprotective effects and mechanisms of exercise in a chronic mouse model of Parkinson's disease with moderate neurodegeneration. *European Journal of Neuroscience*, *33*(7), 1264–1274. https://doi.org/10.1111/j.1460-9568.2011.07626.x

Lauzé, M., Daneault, J.-F., & Duval, C. (2016). The Effects of Physical Activity in Parkinson's Disease: A Review. *Journal of Parkinson's Disease*, *6*(4), 685–698. https://doi.org/10.3233/JPD-160790

Li, Y., Song, H., Shen, L., & Wang, Y. (2021). The efficacy and safety of moderate aerobic exercise for patients with Parkinson's disease: a systematic review and meta-analysis of randomized controlled trials. *Annals of Palliative Medicine*, *10*(3), 2638–2649. https://doi.org/10.21037/apm-20-1661

Lin, K. J., Lin, K. L., Chen, S. D., Liou, C. W., Chuang, Y. C., Lin, H. Y., & Lin, T. K. (2019). The overcrowded crossroads: mitochondria, alpha-synuclein, and the endo-lysosomal system interaction in Parkinson's disease. *International journal of molecular sciences*, *20*(21), 5312. https://doi.org/10.3390/ijms20215312

M. Kokaia. (2009). Glial Cell Line-Derived Neurotrophic Factor - an overview | ScienceDirect Topics. *Encyclopedia of Basic Epilepsy Research*. Retrieved from https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/glial-cell-line-derived-neurotrophic-factor

Mahmoudi, J., Mohaddes, G., Erfani, M., Sadigh-Eteghad, S., Karimi, P., Rajabi, M., … Farajdokht, F. (2018). Cerebrolysin attenuates hyperalgesia, photophobia, and neuroinflammation in a nitroglycerin-induced migraine model in rats. *Brain Research Bulletin*, *140*, 197–204. https://doi.org/10.1016/j.brainresbull.2018.05.008

Mattson, M. P., Gleichmann, M., & Cheng, A. (2008). Mitochondria in Neuroplasticity and Neurological Disorders. *Neuron*, *60*(5), 748–766. https://doi.org/10.1016/j.neuron.2008.10.010

Mazzio, E. A., Reams, R. R., & Soliman, K. F. A. (2004). The role of oxidative stress, impaired glycolysis and mitochondrial respiratory redox failure in the cytotoxic effects of 6-hydroxydopamine in vitro. *Brain Research*, *1004*(1–2), 29–44. https://doi.org/10.1016/j.brainres.2003.12.034

Mocchetti, I., Bachis, A., Nosheny, R. L., & Tanda, G. (2007). Brain-derived neurotrophic factor expression in the substantia nigra does not change after lesions of dopaminergic neurons.

Neurotoxicity Research, *12*(2), 135–143. https://doi.org/10.1007/BF03033922

Morpurgo, C. (1962). Effects of antiparkinson drugs on a phenothiazine-induced catatonic reaction. *Archives Internationales de Pharmacodynamie et de Therapie*, *137*, 84–90. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14476206

Müller, T. (2013). Detoxification and antioxidative therapy for levodopa-induced neurodegeneration in Parkinson's disease. *Expert Review of Neurotherapeutics*, *13*(6), 707–718. https://doi.org/10.1586/ern.13.50

Nadeau, A., Pourcher, E., & Corbeil, P. (2014). Effects of 24 wk of Treadmill Training on Gait Performance in Parkinson's Disease. *Medicine & Science in Sports & Exercise*, *46*(4), 645–655. https://doi.org/10.1249/MSS.0000000000000144

Noor, N. A., Mohammed, H. S., Mourad, I. M., Khadrawy, Y. A., & Aboul Ezz, H. S. (2016). A promising therapeutic potential of cerebrolysin in 6-OHDA rat model of Parkinson's disease. *Life Sciences*, *155*, 174–179. https://doi.org/10.1016/j.lfs.2016.05.022

Ogonovszky, H., Berkes, I., Kumagai, S., Kaneko, T., Tahara, S., Goto, S., & Radák, Z. (2005). The effects of moderate-, strenuous- and over-training on oxidative stress markers, DNA repair, and memory, in rat brain. *Neurochemistry International*, *46*(8), 635–640. https://doi.org/10.1016/j.neuint.2005.02.009

Osali, A. (2020). Aerobic exercise and nano-curcumin supplementation improve inflammation in elderly females with metabolic syndrome. *Diabetology & Metabolic Syndrome*, *12*(1), 26. https://doi.org/10.1186/s13098-020-00532-4

Ozdemir, E., Cetinkaya, S., Ersan, S., Kucukosman, S., & Ersan, E. E. (2009). Serum selenium and plasma malondialdehyde levels and antioxidant enzyme activities in patients with obsessive–compulsive disorder. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *33*(1), 62–65. https://doi.org/10.1016/j.pnpbp.2008.10.004

Ozkizilcik, A., Sharma, A., Lafuente, J. V., Muresanu, D. F., Castellani, R. J., Nozari, A., … Sharma, H. S. (2019). Nanodelivery of cerebrolysin reduces pathophysiology of Parkinson's disease. In *Progress in Brain Research* (pp. 201–246). https://doi.org/10.1016/bs.pbr.2019.03.014

Paxinos, G., & Watson, C. (2014). The Rat Brain in Stereotaxic Coordinates Seventh Edition. *Elsevier Academic Press*.

Peerapatdit, T., Patchanans, N., Likidlilid, A., Poldee, S., & Sriratanasathavorn, C. (2006).

Plasma lipid peroxidation and antioxidiant nutrients in type 2 diabetic patients. *Journal of the Medical Association of Thailand = Chotmaihet Thangphaet*, *89*(5), 147–155.

Piercy, K. L., Troiano, R. P., Ballard, R. M., Carlson, S. A., Fulton, J. E., Galuska, D. A., … Olson, R. D. (2018). The Physical Activity Guidelines for Americans. *JAMA*, *320*(19), 2020. https://doi.org/10.1001/jama.2018.14854

Plosker, G. L., & Gauthier, S. (2009). Cerebrolysin. *Drugs & Aging*, *26*(11), 893–915. https://doi.org/10.2165/11203320-000000000-00000

Pulikkalpura, H., Kurup, R., Mathew, P. J., & Baby, S. (2015). Levodopa in Mucuna pruriens and its degradation. *Scientific Reports*. https://doi.org/10.1038/srep11078

Requejo, C., Ruiz-Ortega, J. A., Cepeda, H., Sharma, A., Sharma, H. S., Ozkizilcik, A., … Lafuente, J. V. (2018). Nanodelivery of Cerebrolysin and Rearing in Enriched Environment Induce Neuroprotective Effects in a Preclinical Rat Model of Parkinson's Disease. *Molecular Neurobiology*, *55*(1), 286–299. https://doi.org/10.1007/s12035-017-0741-x

Rockenstein, E., Desplats, P., Ubhi, K., Mante, M., Florio, J., Adame, A., … Masliah, E. (2015). Neuropeptide Treatment with Cerebrolysin Enhances the Survival of Grafted Neural Stem Cell in an α-Synuclein Transgenic Model of Parkinson's Disease. *Journal of Experimental Neuroscience*, *9s2*(2), JEN.S25521. https://doi.org/10.4137/JEN.S25521

Rockenstein, E., Mante, M., Adame, A., Crews, L., Moessler, H., & Masliah, E. (2007). Effects of Cerebrolysin™ on neurogenesis in an APP transgenic model of Alzheimer's disease. *Acta Neuropathologica*, *113*(3), 265–275. https://doi.org/10.1007/s00401-006-0166-5

Rockenstein, E., Torrance, M., Mante, M., Adame, A., Paulino, A., Rose, J. B., … Masliah, E. (2006). Cerebrolysin decreases amyloid-β production by regulating amyloid protein precursor maturation in a transgenic model of Alzheimer's disease. *Journal of Neuroscience Research*, *83*(7), 1252–1261. https://doi.org/10.1002/jnr.20818

Roghani, M., Behzadi, G., & Baluchnejadmojarad, T. (2002). Efficacy of elevated body swing test in the early model of Parkinson's disease in rat. *Physiology & Behavior*, *76*(4–5), 507–510. https://doi.org/10.1016/S0031-9384(02)00753-9

Roghani, M., Niknam, A., Jalali-Nadoushan, M.-R., Kiasalari, Z., Khalili, M., & Baluchnejadmojarad, T. (2010). Oral pelargonidin exerts dose-dependent neuroprotection in 6-hydroxydopamine rat model of hemi-parkinsonism. *Brain Research Bulletin*, *82*(5–6), 279–283. https://doi.org/10.1016/j.brainresbull.2010.06.004

Spatola, M., & Wider, C. (2014). Genetics of Parkinson's disease: the yield. *Parkinsonism & Related Disorders*, *20*, S35--S38. https://doi.org/10.1016/S1353-8020(13)70011-7

Surmeier, D. J. (2007). Calcium, ageing, and neuronal vulnerability in Parkinson's disease. *The Lancet Neurology*, *6*(10), 933–938. https://doi.org/10.1016/S1474-4422(07)70246-6

Svensson, M., Lexell, J., & Deierborg, T. (2015). Effects of Physical Exercise on Neuroinflammation, Neuroplasticity, Neurodegeneration, and Behavior: What We Can Learn From Animal Models in Clinical Settings. *Neurorehabilitation and Neural Repair*, *29*(6), 577–589. https://doi.org/10.1177/1545968314562108

Takehara, N., Tsubaki, A., Yamazaki, Y., Kanaya, C., Sato, D., Morishita, S., & Onishi, H. (2017). Changes in Oxyhemoglobin Concentration in the Prefrontal Cortex and Primary Motor Cortex During Low- and Moderate-Intensity Exercise on a Cycle Ergometer. In *Advances in Experimental Medicine and Biology* (pp. 241–247). https://doi.org/10.1007/978-3-319-55231-6_33

Tu, H. Y., Yuan, B. S., Hou, X. O., Zhang, X. J., Pei, C. S., Ma, Y. T., ... & Hu, L. F. (2021). α-synuclein suppresses microglial autophagy and promotes neurodegeneration in a mouse model of Parkinson's disease. *Aging cell*, *20*(12), e13522. https://doi.org/10.1111/acel.13522

Ubhi, K., Rockenstein, E., Doppler, E., Mante, M., Adame, A., Patrick, C., … Masliah, E. (2009). Neurofibrillary and neurodegenerative pathology in APP-transgenic mice injected with AAV2-mutant TAU: neuroprotective effects of Cerebrolysin. *Acta Neuropathologica*, *117*(6), 699–712. https://doi.org/10.1007/s00401-009-0505-4

Veinbergs, I., Mante, M., Mallory, M., & Masliah, E. (2000). Neurotrophic effects of Cerebrolysin® in animal models of excitotoxicity. In *Advances in Dementia Research* (pp. 273–280). Vienna: Springer Vienna. https://doi.org/10.1007/978-3-7091-6781-6_29

Warnecke, T., Suttrup, I., Schröder, J. B., Osada, N., Oelenberg, S., Hamacher, C., … Dziewas, R. (2016). Levodopa responsiveness of dysphagia in advanced Parkinson's disease and reliability testing of the FEES-Levodopa-test. *Parkinsonism & Related Disorders*, *28*, 100–106. https://doi.org/10.1016/j.parkreldis.2016.04.034

Wei, X., He, S., Wang, Z., Wu, J., Zhang, J., Cheng, Y., … Xiao, J. (2014). Fibroblast growth factor 1attenuates 6-hydroxydopamine-induced neurotoxicity: an in vitro and in vivo investigation in experimental models of parkinson's disease. *American Journal of Translational Research*, *6*(6), 664–677. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25628778

World Health Organization. (2018). Global action plan on physical activity 2018–2030. In *Journal of Policy Modeling*.

Wright Willis, A., Evanoff, B. A., Lian, M., Criswell, S. R., & Racette, B. A. (2010). Geographic and Ethnic Variation in Parkinson Disease: A Population-Based Study of US Medicare Beneficiaries. *Neuroepidemiology*, *34*(3), 143–151. https://doi.org/10.1159/000275491

Wu, S.-Y., Wang, T.-F., Yu, L., Jen, C. J., Chuang, J.-I., Wu, F.-S., … Kuo, Y.-M. (2011). Running exercise protects the substantia nigra dopaminergic neurons against inflammation-induced degeneration via the activation of BDNF signaling pathway. *Brain, Behavior, and Immunity*, *25*(1), 135–146. https://doi.org/10.1016/j.bbi.2010.09.006

Yau, S., Gil-Mohapel, J., Christie, B. R., & So, K. (2014). Physical Exercise-Induced Adult Neurogenesis: A Good Strategy to Prevent Cognitive Decline in Neurodegenerative Diseases? *BioMed Research International*, 1–20. https://doi.org/10.1155/2014/403120

Yin, L.-L., Geng, X.-C., & Zhu, X.-Z. (2011). The involvement of RGS9 in l-3,4-dihydroxyphenylalanine-induced dyskinesias in unilateral 6-OHDA lesion rat model. *Brain Research Bulletin*, *86*(5–6), 367–372. https://doi.org/10.1016/j.brainresbull.2011.09.016