Title: Deep Brain Stimulation of the Subthalamic Nucleus Ameliorates Behavioral Deficits and Modulates Inflammatory and Oxidative Stress Biomarkers in Experimental Models of Parkinson's Disease

Running Title: DBS of the Subthalamic Nucleus

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ABSTRACT

Purpose: Parkinson's disease (PD) is a progressive neurodegenerative disorder and the second most prevalent neurological condition worldwide. Deep Brain Stimulation (DBS) targeting the subthalamic nucleus (STN) has emerged as a contemporary therapeutic approach, demonstrating efficacy in alleviating PD symptoms. This study aimed to examine the effects of STN-DBS on behavioral abnormalities and serum levels of inflammatory and oxidative stress biomarkers in a rat model of PD.

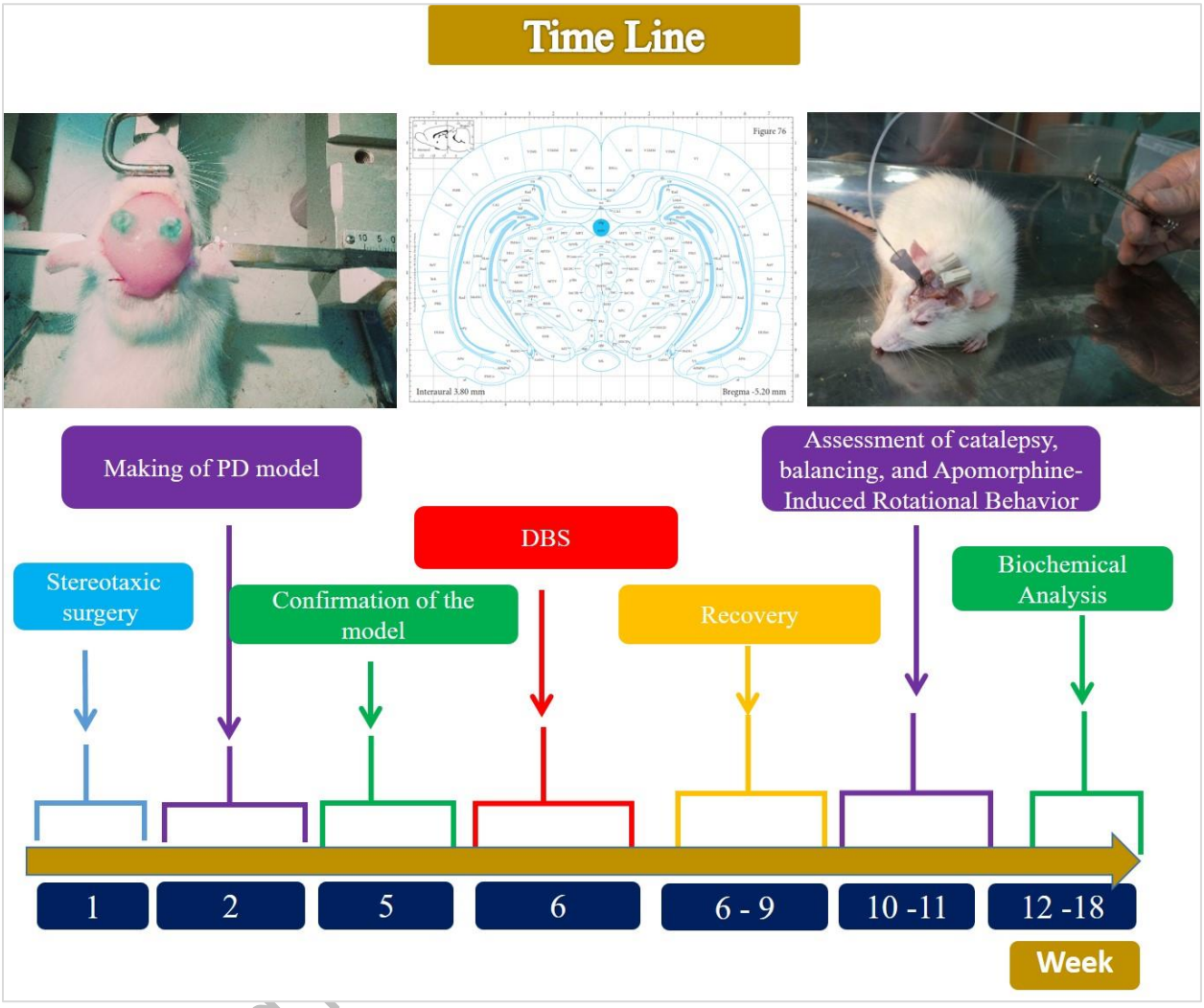
Methods: PD was induced in Wistar rats through administration of the neurotoxin 6-hydroxydopamine (6-OHDA). The animals were then randomly assigned to five groups, each consisting of eight rats: Group 1 (Normal) served as healthy controls without any intervention; Group 2 (PD group) included PD model rats implanted with electrodes but not subjected to DBS; Groups 3 to 5 comprised PD model rats receiving one hour of subthalamic nucleus DBS (STN-DBS) daily for three consecutive days, targeting the right hemisphere, left hemisphere, and bilateral hemispheres, respectively. Three weeks after 6-OHDA treatment, DBS was administered to Groups 3 through 5, followed by behavioral assessments using the Bar test for catalepsy and the Rotarod test for motor coordination. Upon completion of behavioral testing, serum samples were collected to quantify inflammatory markers and oxidative stress parameters via enzyme-linked immunosorbent assay (ELISA) and photometric assays, respectively.

Results: The 6-OHDA lesion induced significant behavioral deficits, which were ameliorated by STN-DBS. Compared to the normal group, the PD group exhibited significantly elevated serum levels of malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and catalase (CAT) activity ($p < 0.001$), alongside reductions in reduced glutathione (GSH) and superoxide dismutase (SOD) levels ($p < 0.001$). In the DBS-treated groups, MDA, TNF-α, and IL-6 concentrations were significantly decreased relative to the PD group ($p < 0.001$), while GSH levels were significantly increased ($p < 0.05$ to $p < 0.01$). Additionally, CAT activity was significantly reduced in all DBS groups ($p < 0.01$), and SOD activity was significantly elevated in the left hemisphere and bilateral DBS groups compared to the PD group ($p < 0.001$ and $p < 0.05$, respectively). Bilateral stimulation had a greater effect on motor coordination and catalepsy than stimulation of the right or left hemisphere. In contrast, the impact of bilateral stimulation on inflammatory factors and oxidative stress markers was not greater than that of unilateral stimulation.

Conclusion: These findings indicate that STN-DBS mitigates behavioral impairments and modulates markers of inflammation and oxidative stress in the 6-OHDA-induced rat model of PD, suggesting its therapeutic potential for managing PD-related pathophysiology.

Keywords: Parkinson's Disease; 6-OHDA; Deep Brain Stimulation; Inflammatory Biomarkers; Oxidative Stress

Graphical Abstract



Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder and the second most common age-related neurodegenerative disease after Alzheimer's disease. This condition disrupts the function of brain regions known as the basal ganglia (Liu & Dluzen, 2007). The incidence of PD is approximately 0.3 percent in the general population and affects about 1 percent of individuals over the age of 60 (Roohani et al., 2011). The primary pathophysiological factor in PD is the degeneration of dopaminergic neurons in the nigrostriatal pathway, leading to dopamine depletion and a relative increase in acetylcholine. Specifically, the neurons projecting from the substantia nigra pars compacta (SNc) to the striatum, which constitute the nigrostriatal pathway, are destroyed (Scholtissen et al., 2006). Bustelli et al. (2024) have shown that 6-OHDA induced impairment to nigrostriatal pathway and locus coeruleus (Bustelli et al., 2024). Injecting 6-OHDA into the striatum replicates the gradual and progressive loss of SNc neurons, while directly injecting 6-OHDA into the locus coeruleus causes a more rapid degeneration of its cell bodies (Bustelli et al., 2024). Clinical symptoms of PD include resting tremor, rigidity, bradykinesia, and loss of postural reflexes. Secondary motor symptoms such as hypomimia, freezing, dysphagia, dysarthria, sialorrhea, and dystonia, as well as non-motor symptoms including cognitive or neurobehavioral abnormalities, sleep disorders, and autonomic dysfunction, are also characteristic features of PD (Jankovic, 2008). Risk factors for PD include age, male gender, certain environmental exposures such as specific pesticides, substances including 1-methyl-4-phenyl tetrahydropyridine (MPTP) and annonacin, β2-adrenoreceptor antagonists, and family history (Balestrino & Schapira, 2020).

Levodopa (L-DOPA), which is converted to dopamine in the brain, is the most commonly used drug to treat PD. However, because it does not prevent the apoptosis of dopaminergic neurons, its effectiveness gradually diminishes, and the symptoms of the disease reemerge. In other words, a major therapeutic challenge with this drug is the changing response to treatment and the development of complications such as dyskinesia and the on-off phenomenon (DeMaagd & Philip, 2015). In patients with advanced PD, where oral medications like L-DOPA become ineffective or intolerable, intermittent subcutaneous injections of the dopamine receptor agonist Apomorphine can be highly effective. Long-term administration of Apomorphine for several hours daily is often required in patients experiencing significant motor fluctuations. However, similar to L-DOPA, prolonged use of Apomorphine may also lead to motor fluctuations characterized by the on-off phenomenon and dyskinesia (Frankel et al., 1990). Therefore, current pharmacological treatments

are not ideal, and the primary therapeutic challenges persist. Presently, existing medication strategies aimed at improving patients' functional capacity do not slow the progression of PD. Consequently, there is a pressing need for newer and more effective treatments. DBS is recognized as a safe and effective alternative for treating movement disorders, particularly PD. This stereotactic surgical procedure has been performed on more than 160,000 patients worldwide (Lozano & Lipsman, 2013).

In DBS, electrical pulses are continuously delivered at specific frequencies, amplitudes, and intensities to targeted brain regions via implanted electrodes. The STN and the internal globus pallidus (GPi) are the most common targets for DBS in the treatment of PD (Kalia et al., 2013). Although the precise mechanism of DBS remains unclear, its anti-inflammatory effects have been recognized. Inflammation following ischemia and brain injury involves the activation of platelets, endothelial cells, and localized microglia. Elevated levels of inflammatory cytokines and infiltration by neutrophils, macrophages, and lymphocytes exacerbate tissue damage. DBS, particularly when applied to the STN, has demonstrated protective effects on dopaminergic neurons in the nigrostriatal pathway in clinical models of PD (Fischer et al., 2017). Factors such as oxidative stress, decreased glutathione levels, DNA damage, and iron accumulation are primary contributors to the degeneration of dopaminergic neurons. Oxidative stress not only damages dopaminergic neurons but also induces cell death by disrupting phosphorylation-oxidative processes and reducing energy production (Dauer & Przedborski, 2003). Although several studies have investigated the mechanisms and effects of DBS, the impact of STN-DBS on serum levels of oxidative stress markers and inflammatory factors in PD model rats has not been explored. Since oxidative stress and inflammation play key roles in the development of PD, this study aims to evaluate serum levels of oxidative stress markers and inflammatory factors in rats with PD following STN-DBS.

Methods

Experimental Procedures

A total of 40 outbred male Wistar rats, weighing between 180 and 200 g, were used in this study conducted at the Neuroscience Research Center of Baqiyatallah University of Medical Sciences. The adult rats were from the Laboratory Animal Breeding Center of Baqiyatallah University of Medical Sciences. Rats The rats were under a light/dark cycle at a temperature of 23 ± 1 1 as and a humidity of 40–60%, with access to food and water. These conditions were maintained

consistently throughout the experiment. All animal experiment procedures were in accordance with the rules and guidelines of the Research Ethics Committee of Baqiyatallah University of Medical Sciences. Forty rats were randomly divided into five groups, each containing eight rats. Group 1 (Normal) consisted of healthy rats that did not receive any interventions. Group 2 (PD group) included Parkinson's rats with electrodes implanted in their brains; however, they did not receive any DBS. Group 3 (PD + right hemisphere STN-DBS) comprised Parkinson's rats that received one hour of DBS in the right hemisphere STN for three consecutive days (Gut & Winn, 2015). Group 4 (PD + left hemisphere STN-DBS) consisted of Parkinson's rats that received one hour of DBS in the left hemisphere STN for three consecutive days (Gut & Winn, 2015). Group 5 (PD + bilateral STN-DBS) included Parkinson's rats that received one hour of bilateral DBS in both the left and right hemisphere STNs for three consecutive days (Gut & Winn, 2015). All methods and analyses were conducted in accordance with relevant guidelines and regulations, and the study protocol was approved by the Research Ethics Committee of Baqiyatallah University of Medical Sciences (Ethics code: IR.BMSU.REC.1398.232). The animal segment of this research was carried out in accordance with the ARRIVE guidelines and adhered to the U.K. Animals (Scientific Procedures) Act of 1986, along with its related guidelines, the EU Directive 2010/63/EU on animal experiments, and the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). This research was conducted in compliance with animal welfare regulations and the guidelines established by both international and local ethical committees. We made every effort to minimize the number of rats involved in this experiment and to alleviate their pain and discomfort throughout the study.

Induction of a PD model by Injection of 6-OHDA

Injection of 6-OHDA into the SNc was performed surgically using a stereotaxic device (Stoelting Company, Germany) and an infusion pump. First, the animals were anesthetized via intraperitoneal injection of xylazine (5 mg/kg body weight) and ketamine (50 mg/kg body weight). After confirming complete anesthesia by the absence of corneal and toe reflexes, the animal's head was secured in the stereotaxic apparatus. The hair on the head was shaved, the scalp was disinfected with diluted betadine, and a midline incision was made to expose the skull. Using the Paxinos and Watson rat brain atlas and referencing the Bregma and Lambda points, the coordinates of the SNc were identified (AP: -5.0 mm, ML: ±2.1 mm, and DV: - 7.7 mm). A hole was drilled in the skull with an automatic drill to accommodate the cannula. Then, using a Hamilton syringe and

microinjection pump, 8 µg of 6-OHDA diluted in 2 µL of 0.9% normal saline containing 0.2% ascorbic acid was injected at a rate of 0.2 µL/min through a 23-gauge needle into the right, left, or bilateral SNc, serving as a guide cannula. After the injection, the incision site was sutured (Haddadi et al., 2015). To validate the animal model, several steps were performed. First, the Apomorphine-Induced Rotational Behavior test was performed (Bustelli et al., 2024; Hudson et al., 1993).

Three weeks post-treatment, the animals were assessed for catalepsy and motor imbalance using the bar test and rotarod test, respectively. Based on the results of these tests, PD was successfully induced in all rats.

Implantation of Electrodes in the STN

Rats were implanted unilaterally in either the right or left or bilaterally in both sides. They left STN with insulated stainless steel electrodes (250 µm in diameter, 0.55 mm of surface exposed). The bregma coordinates of electrode placement according to the Paxinos and Watson rat brain atlas were AP: 3, ML: 2.6, and DV: 3, with the incisor bar at 3.2 mm from the plane. Electrodes were implanted within the STN and fixed to the skull with dental cement (Campos et al., 2020).

Deep Brain Stimulation (DBS)

Rats in groups 3 to 5 received DBS. A portable stimulator (St. Jude MTS, St. Jude Medical, Plano, TX, USA) was used for targeting. A guide cannula was made for the target area based on the Paxinos and Watson rat brain atlas. This cannula allowed for deep injections. To perform cannulation, the animals were first anesthetized by injecting a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg), and then the SNc in the right hemisphere and the STN area in both hemispheres were cannulated using a stereotaxic device. The coordinate of the cannulas relative to the bregma reference point for the STN were determined as AP: 3, ML: 2.6, and DV: 3, respectively, based on the Paxinus atlas. The animals were given a week to recover, and then 2.5 µl of 0.9% saline solution containing 8 µg of neurotoxin 6-hydroxydopamine and 0.2% ascorbic acid was injected into the SNc. The sham group also received the same amount and concentration of saline and ascorbic acid. Two weeks after injection of the neurotoxin 6-hydroxydopamine, all animals were evaluated for catalepsy and balance during movement, using the bar and rotarod behavioral tests, respectively, and the creation of an experimental model of Parkinson's disease in these animals was confirmed by behavioral tests. The electrodes consisted of two parallel strands of platinum wires with a Teflon coating, which were connected to a socket on one side. The deep stimulation device (made at the Institute of Neuroscience and Behavior, Baqiyatallah University

of Medical Sciences, Iran) was connected to the socket via a stimulation cable and was placed inside a transparent cylindrical chamber so that the animal could move freely and not be affected by environmental factors. Then, the device was programmed and started at a frequency of 130 Hz, for 100 microseconds, and a current intensity of 2 volts (Gut & Winn, 2015). Stimulation lasted one hour each day for three days. Afterward, animals were assessed for catalepsy and motor imbalance using the bar test and rotarod test. Blood samples were collected 24 hours after the last stimulation for serum analysis and immediately centrifuged to separate serum.

Validation of 6-OHDA PD model: Apomorphine-Induced Rotational Behavior

Apomorphine (Apomorphine hydrochloride hemihydrate: Sigma-Aldrich 6-OHDA) is a dopamine agonist that induces rotation toward the side opposite to the 6-OHDA-injured area in rats. The rotational behavior induced by apomorphine was assessed in all groups 21 days post-surgery. The animals were placed in a cylindrical chamber measuring 28 cm in diameter and 38 cm in height for 10 minutes to acclimate to the surroundings. Following that, apomorphine at a dosage of 0.5 mg/kg, dissolved in saline with 0.1% ascorbic acid, was administered intraperitoneally to the rats. One minute after the injection, the rats were again placed in the chamber, and their rotations toward both the injured area and the opposite area were recorded over one hour, with the total number of pure rotations calculated as the difference in the counts for the two directions (Bustelli et al., 2024; Hudson et al., 1993). Counting the number of rotations over 100 times in the opposite direction of the injury within 30 minutes was considered as a criterion for the amount of dopamine depletion in the nigrostriatal system and the occurrence of the PD model, and the disease model was confirmed.

Rotarod Test

Motor coordination in rats was assessed using the Rotarod test. The Rotarod device (Ugo Basile, Comerio, VA, Italy) features a rotating rod with a diameter of 4 cm and a rough surface. The rod is positioned 20 cm above the base and is divided into five equal sections by grooves. The latency to fall was recorded as the time taken for the animal to fall from the rotarod rotating at 18 rpm. Before the actual experiment, each animal underwent training to climb onto and move along the Rotarod. Balance and motor coordination were evaluated based on the latency to fall. For this purpose, all experimental rats underwent a four-day training regimen, wherein they were placed on the rotating rod four times daily for five minutes per session, interspersed with five-minute rest intervals. The initial training day featured a rotational velocity of 11 revolutions per minute (rpm),

progressively increasing to 14 rpm by the final training day. On the fifth day, formal testing was performed at 15 rpm, with the primary outcome measure being the latency, or duration, that the animals remained on the rotating rod at each stage. Data are presented as the mean duration of three trials per animal, with a 15-minute interval between each trial (Haddadi et al., 2015).

Bar Test

Catalepsy was assessed via the standardized bar test procedure. In this assay, both forelimbs of the animal were positioned on a metal rod measuring 0.9 cm in diameter and elevated 9 cm above the surface. The duration during which the animal maintained this fixed posture without voluntary movement was recorded. The test was terminated upon the subject's withdrawal of either forelimb from the rod or upon observable head movement to either side, indicative of exploratory behavior (Sharifi et al., 2013).

Measurement of Serum Levels of Inflammatory Markers TNF-α and IL-6

The levels of TNF-α and IL-6 in serum were measured using ELISA kits purchased from R&D Systems, Inc. (USA). All procedures were conducted according to the manufacturer's instructions. The concentrations of TNF-α and IL-6 are reported in pg/mL.

Measurement of Serum Levels of Oxidative Stress Markers

The photometric method was employed to measure oxidative stress markers, including superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and glutathione (GSH).

Determination of Serum SOD Activity

SOD activity was assessed using the method described by Kakkar et al. (1984) (Kakkar et al., 1984). Briefly, serum was homogenized with cold PBS at a concentration of 10 mM (pH 8.0) and centrifuged at 10,000 × g for 10 minutes at 4°C. The supernatants were collected, and 0.4 mM xanthine, 0.24 mM nitroblue tetrazolium (NBT), and 0.049 mL xanthine oxidase were added, followed by incubation for 20 minutes at 37°C. The reaction was stopped by adding 69 mM sodium dodecyl sulfate. Absorbance was measured at 560 nm. SOD enzyme activity is expressed in U/mL, where one unit is defined as the amount of enzyme causing 50% inhibition of NBT reduction.

Determination of Serum Catalase Activity

CAT activity was evaluated using a modified Aebi method (Aebi, 1984). Briefly, serum was homogenized in cold RIPA buffer containing 0.1 M PBS (pH 7.4), 5 mM EDTA, 0.01% digitonin, and 0.25% sodium chelate, then centrifuged at 10,000 × g for 30 minutes at 4°C. The supernatant

was incubated with phosphate buffer (50 mM, pH 7.0) containing 5 mM EDTA and 10 mM H2O2 for 10 minutes at 37°C. H2O2 decomposition was directly measured by monitoring the decrease in absorbance at 240 nm over 2 minutes. CAT activity is expressed in U/ml, where one unit of CAT corresponds to the micromoles of hydrogen peroxide degraded per minute.

Measurement of MDA Concentration

To measure the level of MDA, a key indicator of oxidative stress, the method described by Fernández et al. (1997) was employed (Fernández et al., 1997). This method is based on the colorimetric spectrophotometric measurement of the reaction between thiobarbituric acid (TBA) and MDA. Briefly, to measure serum MDA, 2 ml of 1.15% KCl was added to 500 µl of serum to prepare a 10% (w/v) homogenate, which was then mixed for 2 minutes at 4°C using a vortex mixer. It is important to note that this step was performed on ice. Next, 3 ml of 1% phosphoric acid and 1 ml of 0.6% TBA were added to 500 µl of the homogenate, and the resulting mixture was heated in a boiling water bath for 45 minutes. After cooling, 4 ml of n-butanol was added to the mixture and vortexed for one minute. The sample was then centrifuged at 3000 × g for 20 minutes. The upper organic phase was transferred to new tubes, and the absorbance was measured at 532 nm for each sample. To calculate the MDA concentration, a standard curve was plotted using MDA concentrations ranging from 0 to 100 nmol/ml. Finally, the MDA concentration was reported in µmol/L.

Determination of Reduced GSH

Serum GSH was measured using the method described by Moron et al. (Moron et al., 1979). This method is based on the reaction of free sulfhydryl groups with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) reagent in an alkaline medium. Briefly, serum samples from different homogeneous groups were prepared as 10% (w/v) solutions in phosphate-buffered saline (PBS) at pH 7.4. These samples were then mixed with 10% trichloroacetic acid (TCA) in a 1:1 ratio and centrifuged at 2,500 × g for 10 minutes. The supernatant was subsequently mixed with 2 mL of PBS (pH 8.0), and after adding 0.5 mL of DTNB reagent, a yellow 5-thio-2-nitrobenzoic acid (TNB) complex was formed. Absorbance was measured at 412 nm. To calculate the GSH content, a standard curve was generated using GSH concentrations ranging from 0 to 150 µmol/mL, and the GSH concentration was reported in µmol/mL.

Statistical Analysis

If the data were normally distributed, parametric tests (One-Way ANOVA) were used to compare the means, and Fisher's or Tukey's tests were applied to compare percentages and scores. However, if the distribution was not normal, non-parametric Kruskal-Wallis H tests were used to compare the means, and the Mann-Whitney U test was employed for percentages and scores. A p-value of less than 0.05 was considered statistically significant. All analyses were performed using SPSS software, version 21.

Results

The Effects of 6-OHDA Injection and STN-DBS on the Motor Coordination

Motor coordination was assessed using the rotarod test. As shown in Figure 1, 6-OHDA induced significant motor impairment in the PD group compared to the normal group ($p < 0.001$) (Figure 1). DBS of the STN in the right hemisphere, left hemisphere, and bilaterally (both hemispheres) significantly improved motor coordination ($p < 0.001$). Bilateral stimulation had a greater effect on motor coordination compared to stimulation of either the right or left hemisphere alone ($p < 0.01$).

The Effects of 6-OHDA Injection and STN-DBS on Catalepsy

Catalepsy was assessed using the bar test. The 6-OHDA treatment induced significant catalepsy in the PD group compared to the control group ($p < 0.001$). DBS treatment significantly alleviated the severity of catalepsy across all groups ($p < 0.001$). Bilateral stimulation produced a greater improvement in catalepsy than stimulation of either the right or left hemisphere alone ($p < 0.01$) (Figure 2).

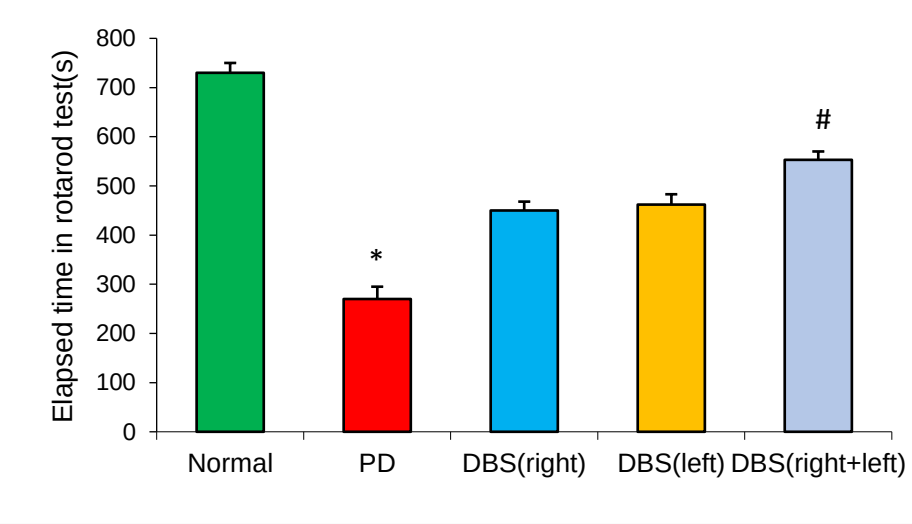


Figure 1: The effects of 6-OHDA and STN-DBS on the right, left, and bilateral hemispheres in the rotarod test, measured by elapsed time. Data are presented as the mean ± standard deviation (SD).

* P < 0.001 compared to all groups, and # P < 0.01 compared to the DBS (right) and DBS (left) groups.

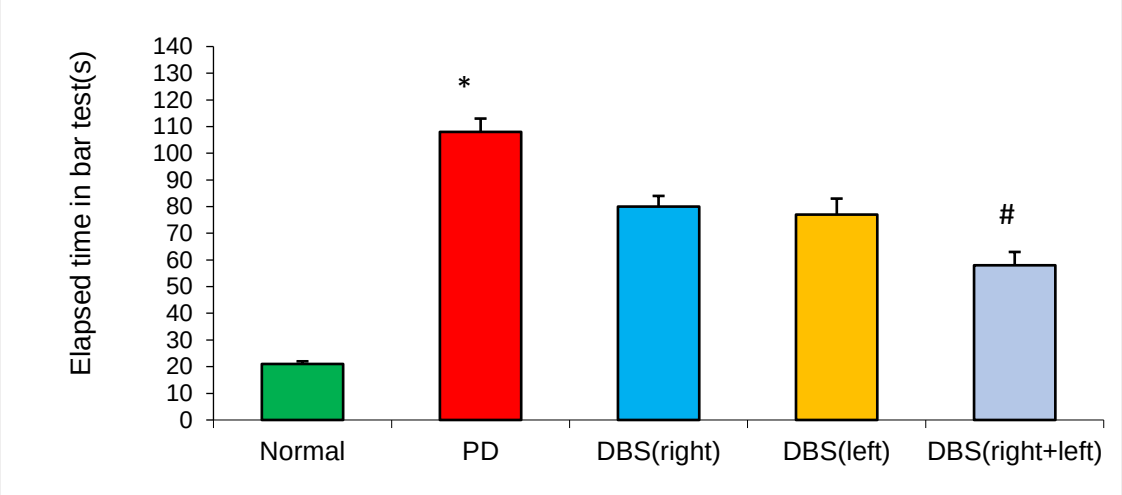


Figure 2: The effects of 6-OHDA and STN-DBS on catalepsy in the right, left, and bilateral hemispheres. Data are presented as mean ± standard deviation (SD).

* P < 0.001 compared to all groups, and # P < 0.01 compared to the DBS (right) and DBS (left) groups.

The Effects of 6-OHDA Injection and STN-DBS on MDA Levels

The results of serum MDA measurements showed that cannulation of the STN in the brains of the PD group significantly increased MDA levels compared to the normal group ($p < 0.001$). In

contrast, the groups receiving 6-OHDA with DBS of the right, left, and bilateral hemispheres exhibited significantly decreased MDA levels compared to the PD group ($p < 0.001$) (Figure 3).

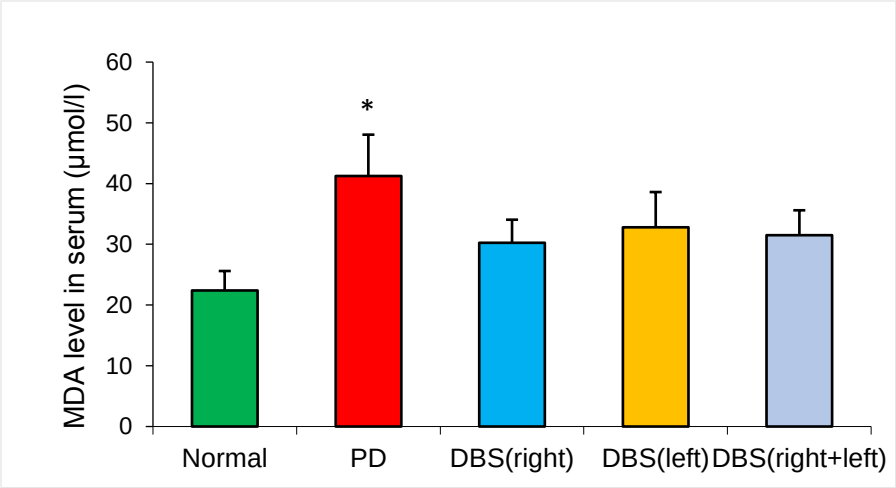


Figure 3: The effects of 6-OHDA and STN-DBS on MDA levels in serum from the right, left, and bilateral hemispheres. Data are presented as mean ± SD.

* $P < 0.001$ compared with all other groups.

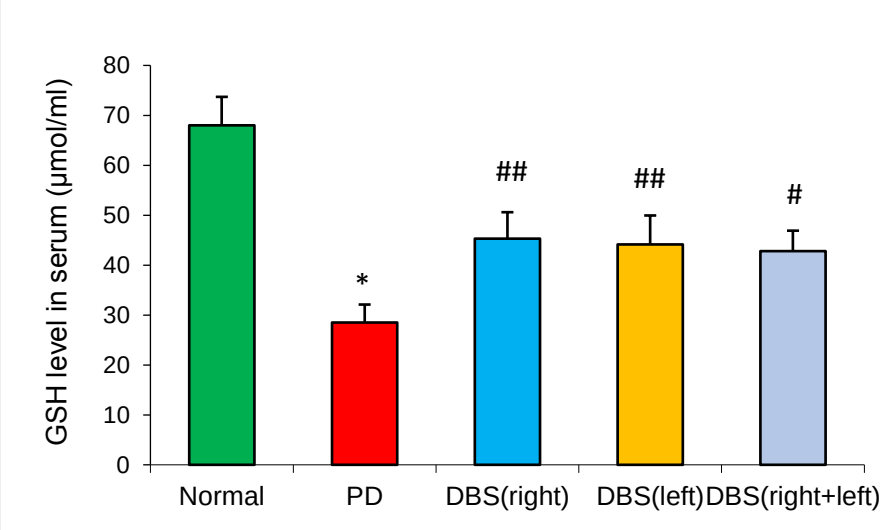


Figure 4: The effects of 6-OHDA and STN-DBS on GSH levels in the serum of the right, left, and bilateral hemispheres. Data are presented as mean ± SD.

* *P* < 0.001 compared to the Normal group; #*P* < 0.05 and ##*P* < 0.01 compared to the PD group.

The Effects of 6-OHDA Injection and STN-DBS on GSH Levels

The results showed that serum GSH levels in the PD group were significantly reduced compared to the normal group ($p < 0.001$). In contrast, the groups receiving 6-OHDA combined with DBS

of the right hemisphere ($p < 0.01$), left hemisphere ($p < 0.01$), and bilateral stimulation (both hemispheres) exhibited significantly increased GSH levels compared to the PD group (Figure 4).

The Effects of 6-OHDA Injection and STN-DBS on SOD

As shown in Figure 5, serum SOD activity in the PD group was significantly decreased compared to the normal group ($p < 0.001$). However, in groups receiving 6-OHDA combined with DBS of the STN in the left hemisphere ($p < 0.001$) and bilaterally ($p < 0.05$) (both right and left hemispheres), SOD activity was significantly increased compared to the PD group (Figure 5).

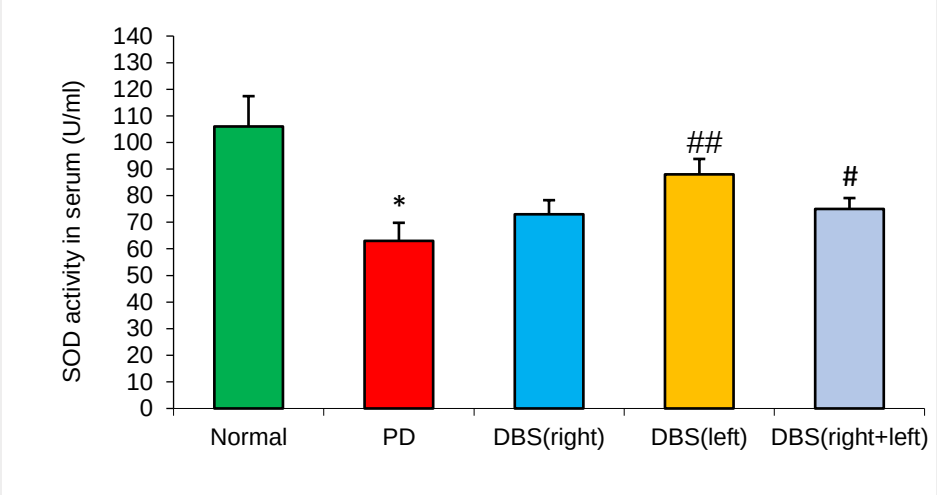


Figure 5: The effects of 6-OHDA and STN-DBS on SOD activity in serum from the right, left, and bilateral hemispheres. Data are presented as mean ± SD.

* $P < 0.001$ compared to the normal group; # $P < 0.05$, ## $P < 0.001$ compared to the PD group.

The Effects of 6-OHDA Injection and STN-DBS on CAT

The results of measuring CAT activity in serum showed that cannulation in the PD group significantly increased CAT activity compared to the normal group ($p < 0.001$). Additionally, in the groups receiving 6-OHDA combined with DBS of the left, right, or bilateral (both left and right) STN, CAT activity significantly decreased compared to the PD group (Figure 6).

The Effects of 6-OHDA Injection and STN-DBS on TNF-α

Cannulation in the PD group resulted in a significant increase in TNF-α concentration compared to the control group ($p < 0.001$). In contrast, groups receiving 6-OHDA combined with DBS of

the right hemisphere, left hemisphere, or bilateral STN showed a significant decrease in serum TNF-α levels compared to the PD group ($p < 0.001$) (Figure 7).

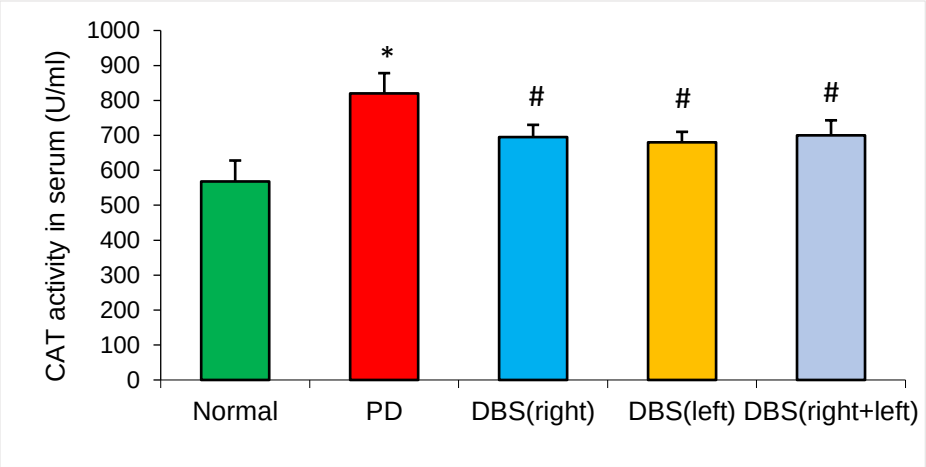


Figure 6: The effects of 6-OHDA and STN-DBS on CAT activity in the serum of the right, left, and bilateral hemispheres. Data are presented as mean ± standard deviation (SD).

* $P < 0.001$ compared to the Normal group; # $P < 0.01$ compared to the PD group.

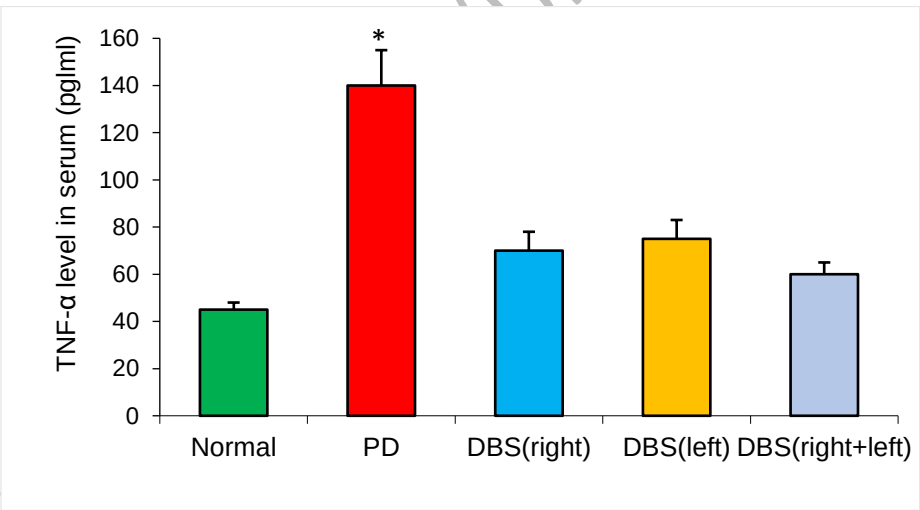


Figure 7: The effects of 6-OHDA and STN-DBS on TNF-α levels in serum from the right, left, and bilateral hemispheres. Data are presented as mean ± standard deviation (mean ± SD).

* $P < 0.001$ compared to all other groups.

The Effects of 6-OHDA Injection and STN-DBS on IL-6 Levels

Cannulation in the PD group resulted in a significant increase in IL-6 concentration compared to the control group ($p < 0.001$). In contrast, groups receiving 6-OHDA combined with DBS of the

right hemisphere, left hemisphere, or bilateral STN showed a significant decrease in serum IL-6 levels compared to the PD group ($p < 0.001$) (Figure 8).

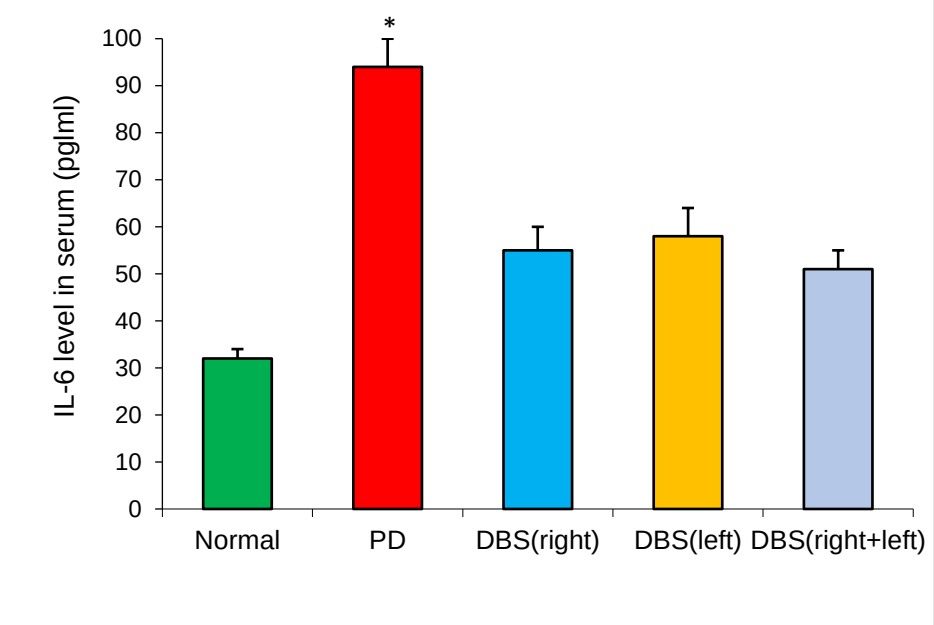


Figure 8: The effects of 6-OHDA and STN-DBS on IL-6 levels in serum from the right, left, and bilateral hemispheres. Data are presented as mean ± standard deviation (SD).

* $p < 0.001$ compared to all other groups.

Discussion

This study investigated the effects of Subthalamic Nucleus Deep Brain Stimulation (STN-DBS) on behavioral abnormalities and serum levels of oxidative stress markers and inflammatory factors in a PD rat model. DBS improved motor coordination and reduced catalepsy in the PD model. Serum levels of MDA and CAT activity were significantly higher in the PD group compared to the healthy control group, whereas GSH levels and SOD activity were decreased. In groups receiving 6-OHDA combined with STN-DBS applied to the right hemisphere, left hemisphere, or both hemispheres, MDA levels significantly decreased compared to the PD group, while GSH levels increased. Furthermore, in groups treated with 6-OHDA and STN-DBS on the left hemisphere or bilaterally, CAT activity decreased, and SOD activity increased significantly compared to the PD group.

A lower level of antioxidants, a higher concentration of substrates for oxidation, and the presence of unidentified plasma pro-oxidants in PD patients may contribute to increased lipid peroxidation and, consequently, elevated MDA levels (Sharma et al., 2008). Consistent with this finding,

Naduthota et al. reported significantly higher MDA levels in PD patients (Naduthota et al., 2017). Similarly, Wei et al., in their systematic review, found increased MDA levels in PD patients (Wei et al., 2018). They also demonstrated that CAT and GSH concentrations were reduced in PD patients (Wei et al., 2018); however, in the present study, CAT activity was increased in PD model rats. In the study by de Farias et al., PD was characterized by increased MDA and SOD activity, along with decreased CAT activity (de Farias et al., 2016). Although their findings regarding MDA were consistent with the present study, their results for CAT and SOD activity contrasted with ours. Additionally, Abraham et al. reported significantly lower SOD and CAT activities in PD patients (Abraham et al., 2005). Similarly, Yuan et al. found reduced SOD levels in PD patients, consistent with our findings (Yuan et al., 2000).

The results of the present study showed that the concentrations of TNF-α and IL-6 in the serum of the PD group increased significantly compared to the normal group. However, in the groups receiving 6-OHDA combined with STN-DBS in the right, left, and bilateral hemispheres, the concentrations of TNF-α and IL-6 decreased significantly compared to the PD group.

TNF-α plays a crucial role in the progressive degeneration of dopaminergic neurons mediated by neuroinflammation in PD (Montgomery & Bowers, 2012). Additionally, the signaling pathway induced by TNF-α may significantly contribute to apoptotic cell death in PD (Nagatsu & Sawada, 2005). Studies have shown that TNF-α levels are significantly elevated in the nigrostriatal region of the brain and the lumbar cerebrospinal fluid of PD patients (Nagatsu & Sawada, 2005). Clinical research has further demonstrated that TNF-α is involved in the pathogenesis of PD (Dufek et al., 2009; Reale et al., 2009; Varani et al., 2010). Consistent with these findings, Kouchaki et al. reported increased TNF-α concentrations in the serum of PD patients (Kouchaki et al., 2018). Similarly, a study by Çomoğlu et al. revealed that TNF-α levels are significantly elevated in the tears of PD patients (Çomoğlu et al., 2013). In line with this, Mogi et al. found that IL-6 levels were elevated five- to twelve-fold in the striatal dopaminergic regions of patients with PD (Mogi et al., 1994). Moreover, a systematic review by Qin et al. demonstrated that IL-6 concentrations were significantly increased in PD patients across 13 studies (Qin et al., 2016).

Mechanisms underlying the activation of inflammatory cytokines in the brains of patients with PD include active absorption and transport, disruption of the blood-brain barrier by cytokines, and increased permeability of the blood-brain barrier to iron mediated by cortisol. Additionally, there is enhanced activation of autoimmune antibodies, including the spontaneous activation of antibodies targeting sympathetic and dopaminergic neurons in both the blood and cerebrospinal

fluid (CSF), as well as increased lymphocyte activation in the bloodstream (Chiba et al., 1995; Fiszer et al., 1994). Other contributing factors include stimulation of toxin production by glial cells, induction of peripheral antigen production by other cells, and activation of reflex pathways that promote peripheral inflammation. Furthermore, stimulation of the hypothalamic-pituitary-gonadal axis and inhibition of mitochondrial oxidoreductase activity result in decreased cellular energy and increased oxidative stress (Dobbs et al., 1999). Studies of brains from individuals with PD have demonstrated activation of microglial cells in the striatal region, leading to elevated expression of pro-apoptotic cytokines such as TNF-α, IL-6, and IL-1β, as well as activation of dopaminergic neurons and their receptors (Mogi et al., 1994).

It was reported that bilateral DBS of the STN and GPi significantly improved motor function in patients who did not respond to medical therapy (Obeso et al., 2001). Similarly, Varma et al. (2003) demonstrated that bilateral STN-DBS improved motor mobility in patients who had discontinued Apomorphine treatment (Varma et al., 2003). In a study by Krack et al. involving 49 patients with advanced PD, bilateral DBS targeting the STN significantly improved motor function and daily activities for five years without the need for L-DOPA. Additionally, dyskinesia showed marked improvement when patients resumed medication (Krack et al., 2003).

The exact mechanism of action of DBS has not been fully elucidated. Regulation of neural activity, including the modulation of abnormal excitatory and inhibitory patterns within the basal ganglia, appears to disrupt pathological electrical activity in neurons, leading to significant improvements in both motor and non-motor cognitive symptoms (Krack et al., 2003). One primary mechanism by which DBS reduces motor symptoms is through overstimulation or depolarization of neurons. Evidence suggests that high-frequency DBS may decrease neuronal activity via overstimulation or depolarization, potentially involving the inactivation of sodium channels (Beurrier et al., 2001; Do & Bean, 2003). Notably, immune cells such as macrophages (Black & Waxman, 2013), lymphocytes (Fraser et al., 2004; Lo et al., 2012), and microglia (Pappalardo et al., 2016) also express voltage-dependent sodium channels. Recent studies indicate that the inflammatory response, particularly involving monocytes and macrophages, may be modulated through these voltage-dependent sodium channels (Zhou et al., 2013).

This effect is likely associated with the inhibition of sodium channels. As demonstrated in an animal model of multiple sclerosis, phenytoin administration significantly decreased the activation of macrophages and microglia by inhibiting sodium channels (Craner et al., 2005). DBS likely inhibits sodium channels as well (Beurrier et al., 2001), which may explain how DBS interferes

with neuronal communication. High-frequency DBS in the anterior thalamic nucleus further reduces the expression of inflammatory cytokines, including TNF-α and IL-6 (Chen et al., 2017). Additionally, high-frequency DBS decreases the levels of apoptosis-related proteins in the hippocampus, submandibular gland, and anterior thalamic nucleus, thereby reducing neuronal loss (Fischer et al., 2017). Recent studies have shown that DBS prevents apoptosis through the intracellular receptor PPAR-γ; DBS increases the number of PPAR-γ receptors, which in turn inhibits neuronal apoptosis (Liu et al., 2017).

The STN receives direct excitatory input from layer V cortical neurons [52-54], referred to as the cortico-subthalamic hyperdirect pathway (HDP) because it bypasses the striatum, as well as indirect cortical input through the basal ganglia loops (Albin et al., 1989; DeLong, 1990). It has been suggested that the activation of the HDP is crucial for the effectiveness of STN-DBS (Anderson et al., 2018; Chen et al., 2020; Gradinaru et al., 2009; Kuriakose et al., 2010; Li et al., 2012; Miocinovic et al., 2018; Sanders & Jaeger, 2016; Walker et al., 2012), indicating the HDP's role in the pathology of PD. The primary physiological function of the HDP appears to be the inhibition of actions (Chen et al., 2020; Gradinaru et al., 2009; Gurney et al., 2001; Nambu, 2005). The HDP primarily relays inhibitory signals to the STN. Extensive cortical fibers project to the STN (Canteras et al., 1988; Haynes & Haber, 2013). Correspondingly, medium-latency evoked cortical responses resulting from STN-DBS demonstrate a broad cortical distribution that varies based on the STN subsection that is stimulated (Miocinovic et al., 2018). These medium-latency evoked cortical responses may even act as a potential biomarker for the therapeutic effects of STN-DBS. Moreover, the connectivity between the STN and cortical regions is associated with the effectiveness of STN-DBS (Akram et al., 2017; Maurice et al., 1998). Furthermore, STN-DBS induces hyperdirect high beta interruption and facilitates cortical high gamma entrainment in PD.

Conclusion

The results of this study demonstrated that the levels of MDA, TNF-α, IL-6, and the CAT enzyme in the serum of the PD group increased significantly, while GSH and SOD levels decreased. Additionally, in the groups receiving 6-OHDA combined with STN-DBS in the right, left, and bilateral hemispheres, the concentrations of MDA, TNF-α, and IL-6 decreased significantly, whereas GSH levels increased. Furthermore, in the groups treated with 6-OHDA and STN-DBS in the left and bilateral hemispheres, CAT levels decreased, and SOD levels increased significantly. However, due to variations in stimulation regions, disease models, stimulation intensities (ranging from 30 to 100 μA), and stimulation durations, accurate comparisons are

challenging. Nonetheless, research indicates that DBS exerts neuroprotective and anti-inflammatory effects. Therefore, further studies are necessary to elucidate the precise mechanisms by which DBS confers neuroprotection and anti-inflammatory benefits.

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Author Contributions

RM performed the biochemical analyses and wrote the initial draft of the paper. **AM** and **MAT** served as supervisors in the design, management, and statistical analysis and finalized the manuscript.

Ethical consideration

All methods and analyses were conducted in accordance with relevant guidelines and regulations, and the study protocol was approved by the Research Ethics Committee of Baqiyatallah University of Medical Sciences (Ethics code: IR.BMSU.REC.1398.232). The animal segment of this research was carried out in accordance with the ARRIVE guidelines and adhered to the U.K. Animals (Scientific Procedures) Act of 1986, along with its related guidelines, the EU Directive 2010/63/EU on animal experiments, and the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). This research was conducted in compliance with animal welfare regulations and the guidelines established by both international and local ethical committees. We made every effort to minimize the number of rats involved in this experiment and to alleviate their pain and discomfort throughout the study.

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Declaration of competing interest

The authors declared no conflict of interest.

Data availability

Data will be made available on request.

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