Barbigerone Potentially Alleviates Rotenone-Activated Parkinson's Disease in a Rodent Model by Reducing Oxidative Stress and Neuroinflammatory Cytokines

Khalid M. Alharthy,* Hassan N. Althurwi, Faisal F. Albaqami, Ali Altharawi, Sami I. Alzarea, Fahad A. Al-Abbasi, Muhammad Shahid Nadeem, and Imran Kazmi*
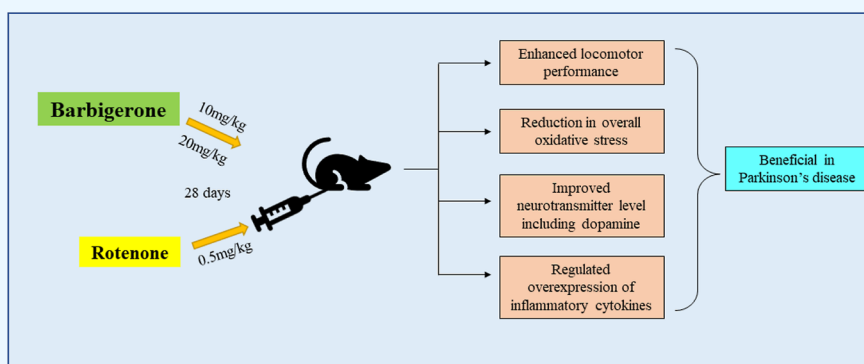
ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Background: Parkinson's disease (PD) is a common age-related and slowly progressive neurodegenerative disease that affects approximately 1% of the elderly population. In recent years, phytocomponents have aroused considerable interest in the research for PD treatment as they provide a plethora of active compounds including antioxidant and anti-inflammatory compounds. Herein, we aimed to investigate the anti-Parkinson's effect of barbigerone, a natural pyranoisoflavone possessing antioxidant activity in a rotenone-induced rat model of PD. Methods: To evaluate antioxidant activity, a 0.5 mg/kg dose of rotenone was injected subcutaneously into rats. Barbigerone (10 and 20 mg/kg) was administered to rats for 28 days 1 h prior to rotenone. All behavioral parameters were assessed before sacrificing the rats. On the 29th day, all of the rats were humanely killed and assessed for biochemical changes in antioxidant enzymes (superoxide dismutase, glutathione, malondialdehyde, and catalase), neurotransmitter levels (dopamine, 5-hydroxyindoleacetic acid, serotonin, dihydroxyphenylacetic acid, and homovanillic acid levels), and neuroinflammatory cytokines [interleukin (IL)-1β, tumor necrosis factor-α, nuclear factor kappa B, and IL-6]. Results: The data presented in this study has shown that barbigerone attenuated rotenone-induced motor deficits including the rotarod test, catalepsy, akinesia, and open-field test. Additionally, barbigerone has shown improvements in the biochemical and neuroinflammatory parameters in the rotenone-induced rat model of PD. Conclusion: The results demonstrated that barbigerone exhibits antioxidant and anti-inflammatory actions via reducing oxidative stress and inflammatory cytokines. Altogether, these findings suggest that barbigerone could potentially be utilized as a therapeutic agent against PD.

1. INTRODUCTION

Parkinson's disease (PD) is associated with long-term neuro-progressive cardinal symptoms including bradykinesia, tremors, postural imbalance, muscular stiffness, and secondary symptoms such as disturbance in gait, impaired gait, and difficulty in speech.[1,2] PD is diagnosed in older age groups, that is geriatric patients who are above 65 years. The prominent indication of PD is the damage to the dopaminergic nerve fiber and imbalance in the catecholamine (acetylcholine) levels in the nigrostriatal pathway.[3,4] The main risk factors associated with PD are genetic (genes—PARK1, PINK1, and parkin), environmental toxins (chemicals like n-hexane, carbon tetrachloride, pesticides, and heavy metals), age, and gender (men are more affected).[5,6] Mitochondrial dysfunction is the main pathological occurrence accompanying the cause of PD. The dopaminergic loss led to a rise in oxidative damage resulting in reactive oxygen species (ROS) generation. The

generated ROS inhibits the mitochondrial function and causes protein misfolding, leading to cell damage and apoptosis.[7,8] Rotenone, the most widely used rodent model, inhibits the NADH complex-I of the electron-transport chain, causing mitochondrial deterioration.[9,10] Chronic exposure to rotenone produces PD-like symptoms in humans. Rotenone administration induces biochemical and behavioral alterations akin to PD in rats.[11] Rotenone is associated with several mechanisms such as altered calcium signaling, mitochondrial dysfunction, oxidative damage, α-synuclein accumulation, and cell apoptosis. Rotenone as an inducing agent produces neuronal symptoms and motor deficit replicating human PD.[12,13]

There are currently many therapeutic options for the management of PD including the dopaminergic drug L-DOPA, monoamine oxidase B inhibitors, catecholamine-o-methyltransferase inhibitors, dopamine agonists, and anticholinergic agents. L-DOPA is the most efficacious and standard drug to relieve PD symptoms.[14,15] However, due to its side effects and peripheral degradation, L-DOPA cannot penetrate the blood–brain barrier and must be combined with carbidopa. Currently, these medications can only produce symptomatic relief in PD patients, thus the need for a more safe and effective option rises.[16,17]

Phytoconstituents are known for their medicinal value and possess a plethora of bioactivities ranging from antioxidant, anti-inflammatory, antimicrobial, antifungal, antiviral, antiplasmodial, anticarcinogenic, and several others. Flavonoids, for instance, exhibit biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilatory actions. However, of particular interest is the antioxidant activity of flavonoids because of their inherent ability to scavenge free radicals and reduce their formation. The anti-neuroinflammatory action of flavonoids has also been demonstrated and is probably related to their potential to modulate the inflammatory responses associated with neurodegenerative diseases. In fact, in vitro and in vivo studies have highlighted the anti-neuroinflammatory action of pure flavonoids (e.g., quercetin, genistein, hesperetin, and epigallocatechin-3-gallate) and enriched extracts.[18] Barbigerone (2′,4′,5′-trimethoxy-6″,6″-dimethyl-6″H-pyrano-[2″,3″:7,8]isoflavone) is a naturally occurring pyranoisoflavone obtained from the seeds of Tephrosia barbigeria and belongs to the family *Leguminosae*.[18] Barbigerone reportedly exhibits various pharmacological properties, such as antioxidant, antimutagenic, anti-plasmodial, and lipoxygenase-inhibiting activities. Recently, barbigerone has also shown antiproliferative activity against several cancer cell lines through inhibition of the mitogen-activated protein kinase pathway.[19] Another study showed the antidiabetic effect of barbigerone by reducing glucose and inflammatory cytokines in streptozotocin-induced diabetic rats.[20] While barbigerone's mechanism of action is unclear, its strong antioxidant properties may make it useful in treating diseases.[20] The present work investigated the anti-Parkinson's effect of barbigerone, particularly focusing on its role in reducing the oxidative stress and neuroinflammatory cytokines.

2. MATERIALS AND METHODS

2.1. Drugs and Reagents. Rotenone (Sigma-Aldrich), barbigerone, reagents, and kits (Modern Lab India) acquired from authenticate sources were of analytical quality. Nuclear factor kappa B (NF-κB), interleukin (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) were analyzed by the rat enzyme-linked immunosorbent assay kit (MyBioSource, USA).

3. ETHICAL STATEMENT

The study was examined and permitted by the Institutional Animal Ethics Committee, Trans-Genica Services, India (IAEC/TRS/PT/021/005). The animals were segregated and acclimatized for at least a week in quarantine. All of the rats were housed in propylene cages with enough supply of water and diet. The rats were maintained at a standard temperature (23 ± 5 °C) and relative humidity (55−60%) under a 12 h dark and 12 h light cycle. This research conducted as per the ARRIVE guidelines.

3.1. Acute Toxicity. The study was carried out by following The Organization for Economic Cooperation and Development guideline no. 425 (up and down procedure). All animals were examined for up to 14 days for any clinical signs, including variations in the skin, fur, mucous membrane, eyes, response to stimuli, and body weight. As per the previously published study, we chose the dose of barbigerone.[20]

3.2. Experimental Protocol. This experimental study was conducted on 24 adult Wistar rats (8 weeks of age and weighing about 180 ± 20 g). The rats were grouped into four cages, each group consisting of six rats.

Group I animals served as normal and treated with saline (5 mL/kg).

Group II served as rotenone control and treated with rotenone (0.5 mg/kg/day s.c. for 28 days).[21,22]

Group III and IV rats were treated with barbigerone (10 and 20 mg/kg/day),[23] respectively, for 28 days 1 h prior to rotenone (0.5 mg/kg s.c.).

On the 29th day, behavioral tests were conducted, and the animals were sacrificed under high anesthesia immediately. The brains were excised, collected, and stored in formalin solution for further neurochemical estimation (Figure 1).
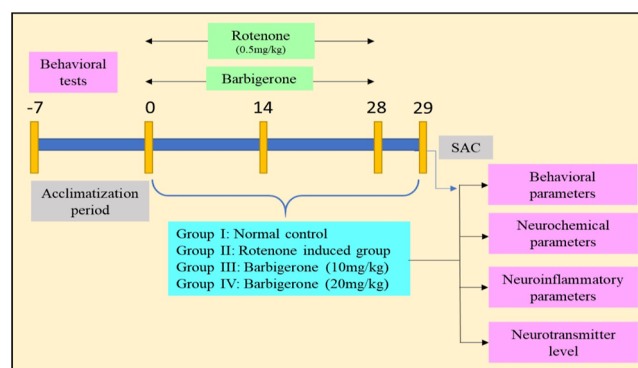

Figure 1. Brief presentation of the experimental plan.

3.3. Behavioral Parameters. *3.3.1. Catalepsy Test.* The catalepsy test was done by placing one of the rat's paws on a horizontal plank (9 cm above) with the other forepaw on a 3 cm high stage. The time at which any movement occurred was recorded. The test was based on a scoring system given by performing three stages which are as follows:

Stage 1: If the rat showed normal movement when placed on a flat table, the score was 0, and if on gentle touch, the rat moved or otherwise remained still, the score given was 0.5.

Stage 2: The hind paw was kept on a 3 cm high box and if there was no movement observed within 10 s, the score received was 0.5.

Stage 3: Only one of the hind paws was kept on a 9 cm wooden plank, while the other was left without any support. A
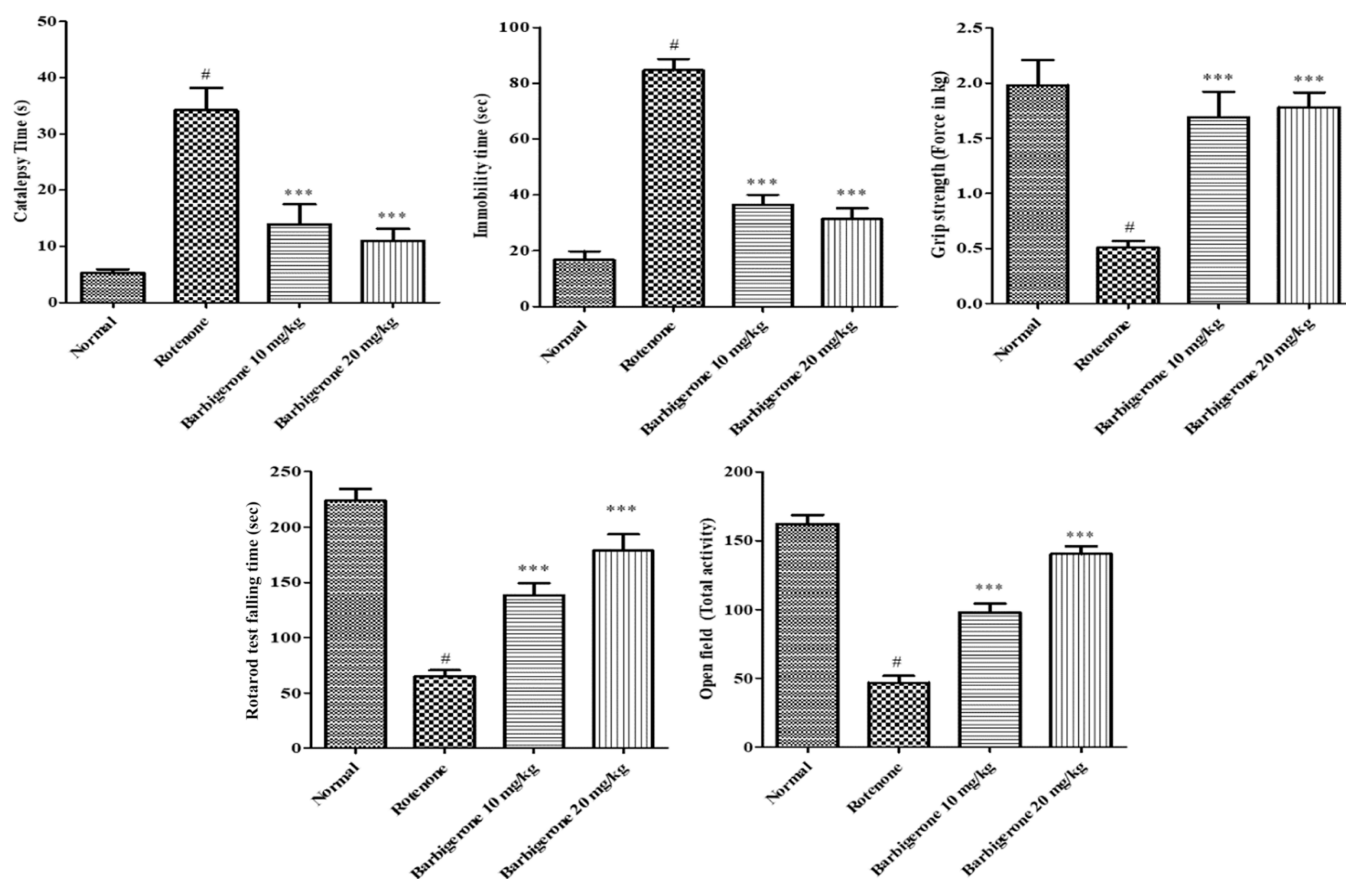
Figure 2. Effect of barbigerone on behavioral parameters. All values are presented as mean ± SEM. Correlation among the groups was done using Tukey's test by one-way ANOVA. p values < 0.001 were expressed as ***. # Significant as corelated to the control group.

score of 1 was allocated for a complete measure of rigidity in 10 s. The rat with complete catalepsy (stiffness) was assigned a score of 3.5. The position of the placement of rats was in a half rearing position with both the forefront paws on the wooden level. The time from when the rats upheld the position on the wooden bar with two hindlimbs was recorded. The sessions were video-recorded for evaluating the time and comparing the performance of each rat. The cut-off time for each rat was fixed to 3 min.[24,25]

3.3.2. Akinesia Test. This test determines the difficulty in movement which is commonly observed in PD. The rats were acclimatized for 5−10 min on a flat surface before conduction of the test. Then, the rats were placed on a wooden platform (40 cm D× 40 cm H ×30 cm W) for 180 s, and the time at which all paws were moved from the position was noted. The test was repeated six times a day, and the mean was computed.[26,27]

3.3.3. Grip Strength Test. This test was developed to assess muscular strength in the rodents. A rat was held by the tail and slowed to hold a trapeze with its front paws. Then, the rat was grabbed until it released the grip. The maximum force with which it grabbed until the release was observed in kilogram-force. Before performing the test, all rats were weighed to note the effect of body weight on grip strength. During a 30 min interval, readings were taken, and the mean of the obtained readings was used as a representative grip force for the animal.[28,29]

3.3.4. Rotarod Test. The rotarod test was done to examine the motor functionality, balance, and coordination. All of the rats were trained by placing them on a suspended rod which

was rotated at 5−20 revolutions per min (25−30 rpm) for 60 s one by one. The test was repeated in the treated rats after administration of the drug. The animals were allowed to move on the rod, and the time at which the animal fell was noted. The max time for one animal on the rod should be 180 s[30]

3.3.5. Open-Field Test. To assess locomotor performance, the test system consisted of a wooden floor, rectangular, open field apparatus which was $100 \times 100 \times 40$ cm equally divided into 25 squares (20×20 cm^2). Initially, the rats were placed at the center for at least 10 min. Then the rats were evaluated for parameters, that is, distance travelled by each rat (mins), time spent at the initial position (center), number of entries in the center square, number of squares crossed, and rearing number for 30 min. The overall activity for the test was calculated.[31]

3.4. Neurochemical Analysis. *3.4.1. Homogenization of Brain Tissues.* On the 29th day, immediately after a behavioral assessment, the animals were separated for the estimation of neurochemical and neurotransmitter levels and neuroinflammatory markers. The collected brains were homogenized using a phosphate buffer. The supernatant was obtained by centrifuging the homogenate at 15 000−25 000 rpm for 15 min.

3.4.2. Malondialdehyde Determination. The test involved the addition of trichloroacetic acid and thiobarbituric acid-reactive substances (TBARS) solution in the separated supernatant, allowing it to incubate in boiling water for 90 min, and cooled in ice-cold water. The mixture was then centrifugated at 1500g for at least 15 min and measured spectrophotometrically at 532 nm. The quantity of malondial-
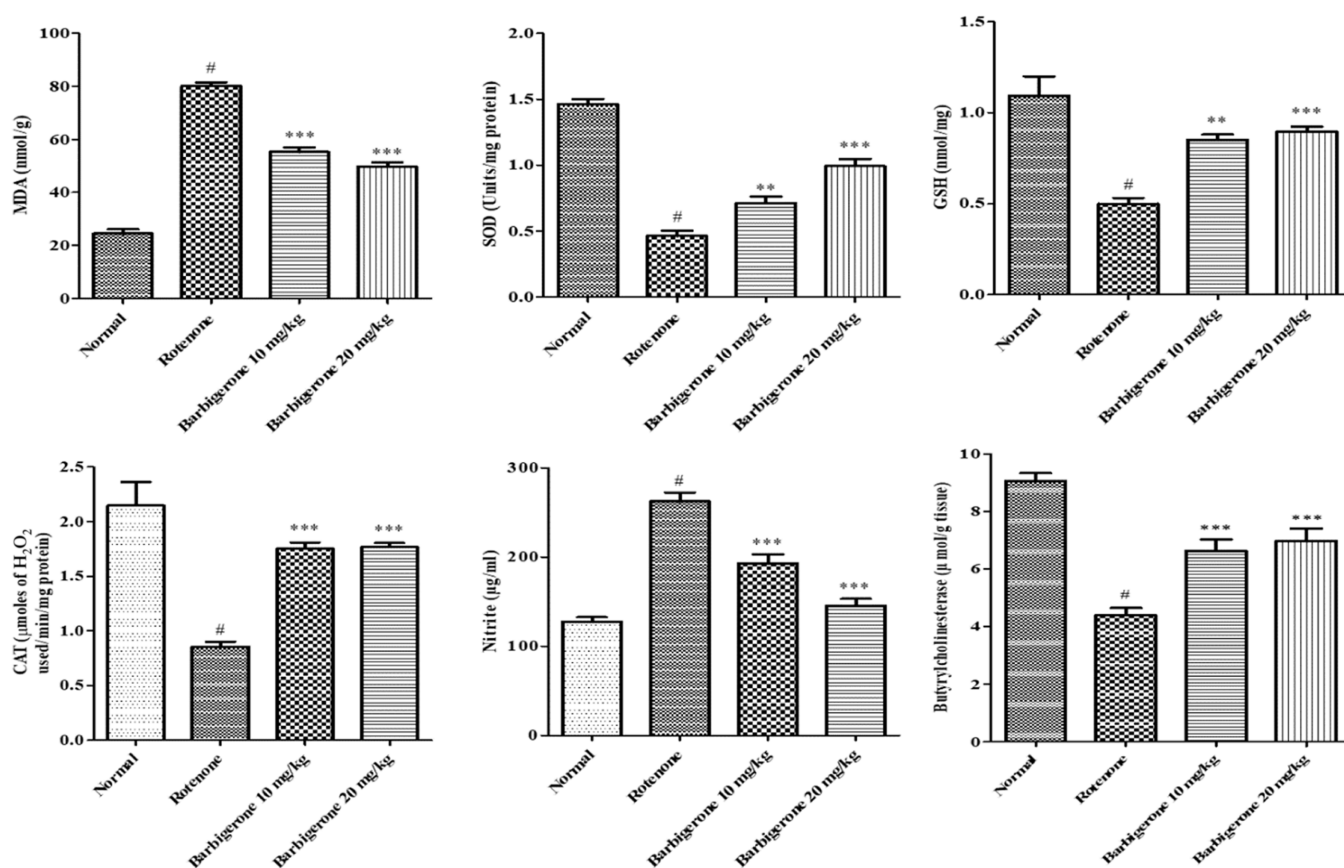
Figure 3. Effect of barbigerone on antioxidant enzyme activities. All values are presented as mean $\pm$ SEM. Correlation among the groups was done using Tukey's test by one-way ANOVA. p values < 0.01 and 0.001 were expressed as ** and ***, respectively. # Significant as correlated to the control group ($p < 0.001$).

dehyde (MDA) produced was expressed as nM of MDA/g of wet tissue.[32,33]

3.4.3. Reduced Glutathione Assay. For the determination of glutathione (GSH) levels in the brain, 1 ml of trichloroacetic acid was used to precipitate an equal amount of brain homogenate. To the supernatant, phosphate buffer solution (PBS) and 5-5′-dithio-bis(2-nitrobenzoic acid) (DTNB) reagent were added. The measurement of absorbance was done at 412 nm by a UV spectrophotometer. The concentration of GSH was obtained by plotting a standard curve. The results were presented in nmol GSH/mg.[34,35]

3.4.4. Superoxide Dismutase Activity. The collected supernatant was mixed with xanthine and xanthine oxidase and then incubated in potassium phosphate buffer for 30 min. To this mixture, nitro blue tetrazolium was added and mixed to form a blue-colored formazan product, which was measured spectrophotometrically at 550 nm. One nitrite unit of superoxide dismutase (SOD) activity was calculated by the quantity of protein that inhibited 50% nitroblue tetrazolium (NBT) reduction.[36]

3.4.5. Catalase Activity. The assay mixture included the supernatant of the brain homogenate and PBS (50 nM). To this mixture, hydrogen peroxide (H_2O_2) was added, and the absorbance was estimated spectrophotometrically at 240 nm every 15 s. The activity was presented in micromoles of H_2O_2 used per min per mg of protein.[37]

3.4.6. Nitrite Content Assay. The level of nitrite formed was due to the oxidative stress generated in the brain. An equal amount of brain homogenate and Griess reagent (mixture of

N-1-naphthyl ethylenediamine dihydrochloride and sulfanila-mide in phosphoric acid) were incubated for 10−15 min and measured at 546 nm using a UV spectrophotometer.[38,39]

3.4.7. Estimation of Butyrylcholinesterase Activity. In colorimetric assay, hydrolysis of butyrylthiocholine to butyrate and thiocholine in the presence of cholinesterase was assessed. The thiocholine formed was then reacted with DTNB to obtain a colored product, which was then measured at 405 nm. The increase in absorbance depends on the cholinesterase activity in the homogenate.[40]

3.4.8. Neurotransmitter Levels. Following the standard procedure, enzyme-linked immunosorbent assay (ELISA, MyBioSource, USA) kits was used to estimate the level of neurotransmitters including serotonin (5-HT, MBS725497), dopamine (DA, MBS725908), and their corresponding metabolites, homovanillic acid (HVA, MBS051114), 3,4-dihydroxyphenylacetic acid (DOPAC, MBS7269503), and 5-hydroxyindoleacetic acid (5-HIAA, MBS024867).

3.4.9. Neuroinflammatory Markers. Cytokines such as NF-κB (MBS453975), IL-1β (MBS825017), IL-6 (MBS269892), and TNF-α (MBS825075) were determined by respective ELISA kits. The proteins separated from the homogenate were pipetted in an antibody-coated ELISA plate. The level of cytokines was measured by following the standard assay protocol.

3.4.10. Statistical Analysis. The results of the following methods were calculated as mean $\pm$ SEM. The data were interpreted by using one-way ANOVA following Tukey's comparison test using Graph pad prism software. The ANOVA
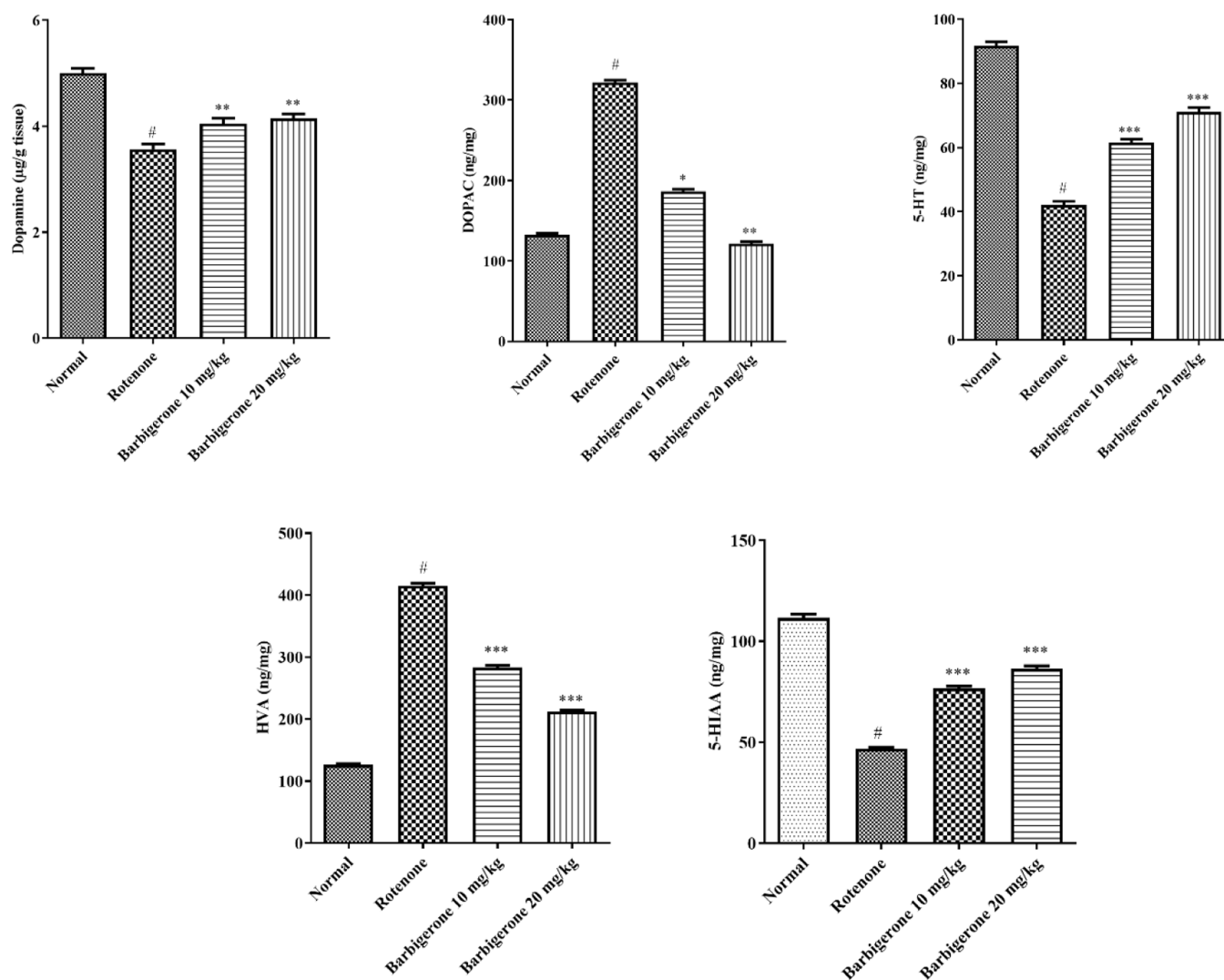
Figure 4. Effect of barbigerone on neurotransmitter levels. All values are presented as mean ± SEM. Correlation among the groups was done using Tukey's test by one-way ANOVA. p values < 0.05, 0.01, and 0.001 were expressed as *, **, and ***, respectively. # Significant as correlated to the control group ($p < 0.001$).

test was used for evaluating the variations among the two groups by setting the significance criteria at $p < 0.05$.

4. RESULTS

4.1. Acute Toxicity Study. In the acute toxicity study, no morbidity or clinical appearance of symptoms were observed for 14 days. Thus, based on acute oral toxicity and the previous studies, we chose 10 and 20 mg/kg barbigerone for performing the main study.

4.2. Effect of Barbigerone on Behavioral Parameters. Rotenone administration in rats significantly increased ($p < 0.001$) the catalepsy time, prolonged the immobility time in akinesia, decreased the falling time in the rotarod test, lowered the overall activity during the open-field test, and decreased the force applied in the grip strength test as compared to the normal group. Barbigerone-treated rats dependently ameliorated ($p < 0.001$) the behavioral impairments when compared with rotenone-induced rats (Figure 2).

4.2.1. Neurochemical Analysis. To assess the antioxidant activity of barbigerone, assays for SOD, CAT, GSH, MDA, nitrite, and butyrylcholinesterase were measured using brain homogenate in the control and the treated rats. The MDA and

nitrite content ($p < 0.001$) was markedly enhanced, while activities of SOD, GSH, CAT, and butyrylcholinesterase were decreased ($p < 0.001$) in rotenone-induced rats. On barbigerone administration, the MDA and nitrite levels were remarkably diminished ($p < 0.001$) and attenuated other antioxidant enzymes in the rotenone-induced group ($p < 0.001$), indicating the antioxidant role of barbigerone at the doses of 10 and 20 mg/kg via lowering down the overall oxidative stress SOD and GSH ($p < 0.01$; $p < 0.001$), CAT, and butyrylcholinesterase ($p < 0.001$) (Figure 3).

4.3. Effect of Barbigerone on Neurotransmitter Levels. Rotenone administration decreased dopamine, 5-HIAA, and 5-HT levels while increasing the DOPAC and HVA levels ($p < 0.001$) when correlated with the controls. After treating with barbigerone, it significantly increased dopamine ($p < 0.01$), 5-HIAA, and 5-HT ($p < 0.001$) levels as correlated to the rotenone-induced group. When treated with 10 mg/kg barbigerone, it lowered both DOPAC ($p < 0.05$) and HVA ($p < 0.001$) levels, while 20 mg/kg barbigerone also reduced DOPAC ($p < 0.01$) and HVA ($p < 0.001$) levels (Figure 4).

4.4. Effect of Barbigerone on Neuroinflammatory Markers. A noticeable rise ($p < 0.001$) in IL-6, IL-1β, TNF-α,
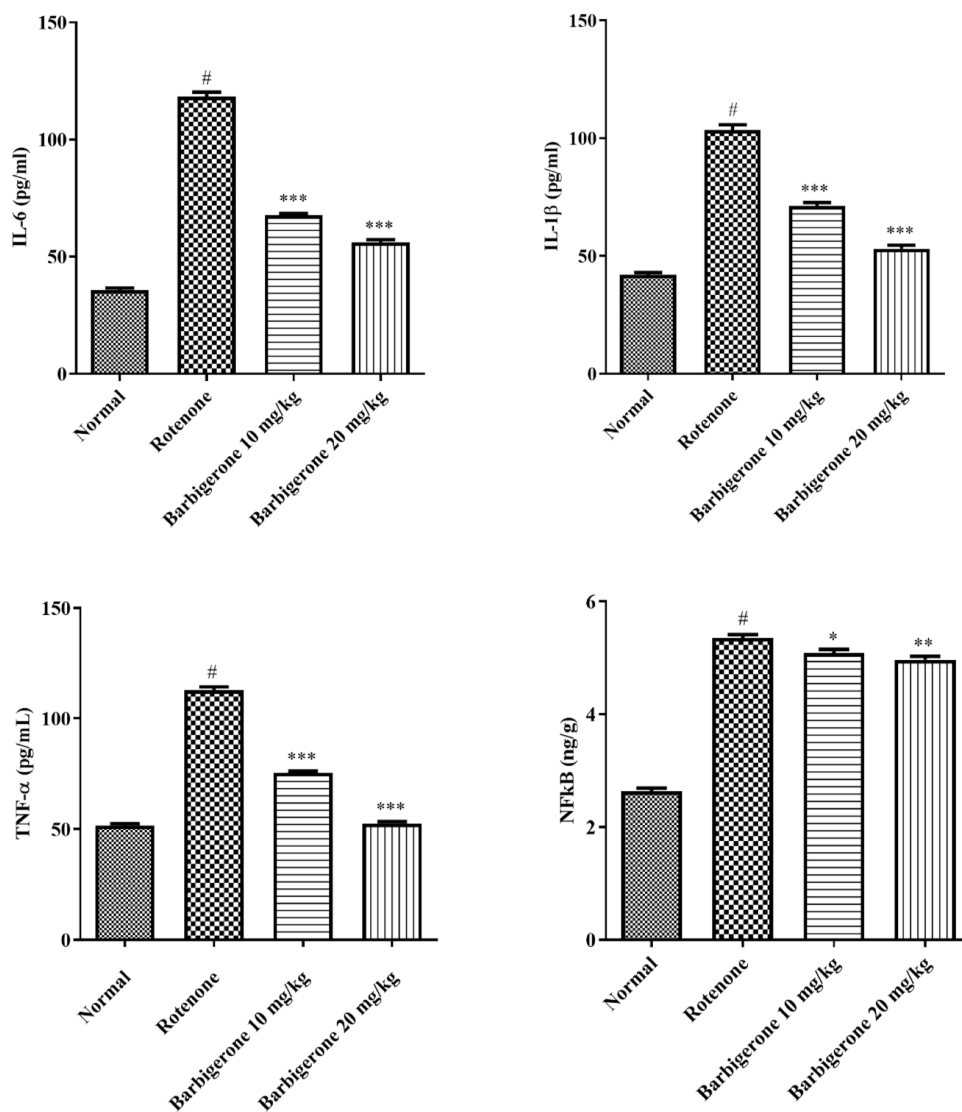
Figure 5. Effect of barbigerone on neuroinflammatory parameters. All values are presented as mean ± SEM. Correlation among the groups was done using Tukey's test by one-way ANOVA. p values < 0.05, 0.01, and 0.001 were expressed as *, **, and ***, respectively. # Significant as correlated to the control group ($p < 0.001$).

and NF-κB was observed in the rotenone-induced group as compared to controls. Barbigerone administration markedly reduced the IL-6, IL-1β, and TNF-α ($p < 0.001$) levels when correlated with the rotenone-induced group. A higher dose (20 mg/kg) of barbigerone lowered the NF-κB ($p < 0.01$) levels than the lower dose (10 mg/kg, $p < 0.05$) as correlated to the rotenone group (Figure 5).

5. DISCUSSION

PD is a slow progression to neuronal death which is caused due to imbalance of dopamine and catecholamine in the nigrostriatal pathway. The prominent motor and non-motor symptoms have multisystem effects, thus downregulating the functions of the nervous system.[41,42] The pathogenesis of PD is closely associated to the oxidative stress-generated ROS which lead to neuroinflammation of the brain.[43] The current treatment options have serious adverse effects and do not therapeutically resolve the condition. The current study focuses on the effect of barbigerone in rotenone-activated PD.

A strong lipophilic mitochondrial complex inhibitor, rotenone is a widely utilized pesticide. Rotenone can easily penetrate the blood–brain barrier and mimic neurological, behavioral, and neuropathological alterations of PD.[44] Earlier investigation revealed that rotenone induction caused dopaminergic damage in the substantia nigra leading to memory deficits in rats.[45] In accordance with previous investigations, the present study has shown a downfall in the behavioral pattern, antioxidant status, and neuroinflammatory markers when 0.5mg/kg rotenone was administered in rats for 28 days.[46] The dopaminergic loss alters the behavior, causing impaired motor and non-motor performance. However, treatment with barbigerone improved all behavioral parameters, thus alleviating locomotor activity.

Mitochondria is the main powerhouse for ROS generation and its dysfunction increases ROS production, causing oxidative damage to the tissues.[47] The present study showed that rotenone is directly responsible in oxidative injury which hinders the mechanistic function of anti-oxidant enzymes, as reported in earlier studies.[48,49] The study displayed that barbigerone corrected all antioxidant enzymes including SOD, GSH, MDA, catalase, nitrite, and butyrylcholinesterase which were altered on rotenone administration.

The neuronal damage/loss in the motor coordination is caused by dysfunction in the neurotransmitter levels, mainly a decrease in the dopaminergic neurons. In the current work, a sharp decline in the dopamine levels in the rotenone-induced group was observed which is comparable to what was reported in previous findings.[50] The barbigerone-treated rats improved the altered neurotransmitter levels, that is, an increase in dopamine, 5-HT, and 5-HIAA and a decrease in the levels of DOPAC and HVA. The treatment-related change in the neurotransmitter level may lead to enhancement of memory and cognition.

Another factor contributing to PD is neuroinflammation. Neuronal inflammation is caused due to overexpression of the inflammatory cytokines, which activates the degenerative pathway leading to neuronal damage.[51] As mentioned in previous studies, rotenone showed upregulation in the cytokine levels as rotenone administration leads to neuronal inflammation.[52,53] The results indicated that barbigerone at both doses (10 and 20mg/kg) attenuated rotenone-induced alterations by decreasing the cytokine levels, including IL-6, IL-1ß, TNF-α, and NFkB, which indicate its anti-inflammatory action. These findings also suggest that barbigerone may also contribute to neuroprotective effects on rotenone-activated PD in a rodent paradigm by reducing oxidative stress and restoring neurotransmitter levels as well as neuroinflammatory cytokines as it is a naturally occurring isoflavone with strong antioxidant activity. Limitations of this study are the short duration of study and the small number of animals used. Future studies, along with more mechanistic cellular and antioxidant factors, should be needed to better understand and confirm the mechanism of barbigerone. Furthermore, research on the effect of higher doses of barbigerone in PD could be considered an option in preclinical and clinical research.

6. CONCLUSIONS

The present work clearly indicated that barbigerone improved the motor symptoms in the rat-induced Parkinson's model. Moreover, barbigerone showed neuroprotective activity via decreasing overall oxidative stress and inflammatory cytokines. Further, 20 mg/kg dose of barbigerone was found to be more effective in improving neurochemical and behavioral findings.-fabbasi@kau.edu.sa/fahad.alabbasi3@gmail.com.

■ AUTHOR INFORMATION

Corresponding Authors

Khalid M. Alharthy − *Department of Pharmacology, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia*; Email: k.alharthy@psau.edu.sa

Imran Kazmi − *Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia*; orcid.org/0000-0003-1881-5219; Phone: +966-543970731; Email: ikazmi@kau.edu.sa

Authors

Hassan N. Althurwi − *Department of Pharmacology, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia*

Faisal F. Albaqami − *Department of Pharmacology, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia*

Ali Altharawi − *Department of Pharmaceutical Chemistry, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia*; orcid.org/0000-0002-6491-6611

Sami I. Alzarea − *Department of Pharmacology, College of Pharmacy, Jouf University, Sakaka 72341, Saudi Arabia*

Fahad A. Al-Abbasi − *Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia*

Muhammad Shahid Nadeem − *Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia*

Complete contact information is available at:
https://pubs.acs.org/10.1021/acsomega.2c05837

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Raza, C.; Anjum, R.; Shakeel, A. Parkinson's disease: Mechanisms, translational models and management strategies. *Life Sci.* **2019**, *226*, 77−90.

(2) Xia, R.; Mao, Z. H. Progression of motor symptoms in Parkinson's disease. *Neurosci. Bull.* **2012**, *28*, 39−48.

(3) Moustafa, A. A.; Chakravarthy, S.; Phillips, J. R.; et al. Motor symptoms in Parkinson's disease: A unified framework. *Neurosci. Biobehav. Rev.* **2016**, *68*, 727−740.

(4) Lotharius, J.; Brundin, P. Pathogenesis of parkinson's disease: dopamine, vesicles and α-synuclein. *Nat. Rev. Neurosci.* **2002**, *3*, 932−942.

(5) Wirdefeldt, K.; Adami, H. O.; Cole, P.; Trichopoulos, D.; Mandel, J. Epidemiology and etiology of Parkinson's disease: a review of the evidence. *Eur. J. Epidemiol.* **2011**, *26*, 1.

(6) Goldman, S. M. Environmental Toxins and Parkinson's Disease. *Annu. Rev. Pharmacol. Toxicol.* **2014**, *54*, 141−164.

(7) Blesa, J.; Trigo-Damas, I.; Quiroga-Varela, A.; Jackson-Lewis, V. R. Oxidative stress and Parkinson's disease. *Front. Neuroanat.* **2015**, *9*, 91.

(8) Zhou, C.; Huang, Y.; Przedborski, S. Oxidative Stress in Parkinson's Disease. *Ann. N. Y. Acad. Sci.* **2008**, *1147*, 93−104.

(9) Wrangel, C.; Schwabe, K.; John, N.; Krauss, J. K.; Alam, M. The rotenone-induced rat model of Parkinson's disease: Behavioral and electrophysiological findings. *Behav. Brain Res.* **2015**, *279*, 52−61.

(10) Cannon, J. R.; Tapias, V.; Na, H. M.; Honick, A. S.; Drolet, R. E.; Greenamyre, J. T. A highly reproducible rotenone model of Parkinson's disease. *Neurobiol. Dis.* **2009**, *34*, 279−290.

(11) Sherer, T. B.; Kim, J. H.; Betarbet, R.; Greenamyre, J. T. Subcutaneous Rotenone Exposure Causes Highly Selective Dopaminergic Degeneration and α-Synuclein Aggregation. *Exp. Neurol.* **2003**, *179*, 9−16.

(12) Sonia Angeline, M.; Chaterjee, P.; Anand, K.; Ambasta, R. K.; Kumar, P. Rotenone-induced parkinsonism elicits behavioral impairments and differential expression of parkin, heat shock proteins and caspases in the rat. *Neuroscience* **2012**, *220*, 291−301.

(13) Alam, M.; Schmidt, W. J. Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. *Behav. Brain Res.* **2002**, *136*, 317−324.

(14) Connolly, B. S.; Lang, A. E. Pharmacological Treatment of Parkinson Disease. *JAMA* **2014**, *311*, 1670−1683.

(15) Rezak, M. Current Pharmacotherapeutic Treatment Options in Parkinson's Disease. *Disease-a-Month* **2007**, *53*, 214−222.

(16) Oertel, W.; Schulz, J. B. Current and experimental treatments of Parkinson disease: A guide for neuroscientists. *J. Neurochem.* **2016**, *139*, 325−337.

(17) Lee, D. J.; Dallapiazza, R. F.; De Vloo, P.; Lozano, A. M. Current surgical treatments for Parkinson's disease and potential therapeutic targets. *Neural Regener. Res.* **2018**, *13*, 1342−1345.

(18) Wei, Z.; Yang, Y.; Xie, C.; et al. Synthesis and biological evaluation of pyranoisoflavone derivatives as anti-inflammatory agents. *Fitoterapia* **2014**, *97*, 172−183.

(19) Li, Z. G.; Zhao, Y. L.; Wu, X.; et al. Barbigerone, a natural isoflavone, induces apoptosis in murine lung-cancer cells via the mitochondrial apoptotic pathway. *Cell. Physiol. Biochem.* **2009**, *24*, 95−104.

(20) Al-Abbasi, F. A. Potential antidiabetic activity of barbigerone on glucose and inflammatory cytokine levels in streptozotocin activated diabetic rats. *J. King Saud Univ., Sci.* **2022**, *34*, 102249.

(21) Teerapattarakan, N.; Benya-aphikul, H.; Tansawat, R.; Wanakhachornkrai, O.; Tantisira, M. H.; Rodsiri, R. Neuroprotective effect of a standardized extract of Centella asiatica ECa233 in rotenone-induced parkinsonism rats. *Phytomedicine* **2018**, *44*, 65−73.

(22) Sharma, S.; Raj, K.; Singh, S. Neuroprotective Effect of Quercetin in Combination with Piperine Against Rotenone- and Iron Supplement-Induced Parkinson's Disease in Experimental Rats. *Neurotoxic. Res.* **2020**, *37*, 198−209.

(23) Touqeer, S.; Saeed, M. A.; Ajaib, M. A review on the phytochemistry and pharmacology of genus Tephrosia. *Phytopharmacology* **2013**, *4* (3), 598−637.

(24) Dekundy, A.; Pietraszek, M.; Schaefer, D.; Cenci, M. A.; Danysz, W. Effects of group I metabotropic glutamate receptors blockade in experimental models of Parkinson's disease. *Brain Res. Bull.* **2006**, *69*, 318−326.

(25) Sarkaki, A.; Badavi, M.; Hoseiny, N.; Gharibnaseri, M. K.; Rahim, F. Postmenopausal effects of intrastriatal estrogen on catalepsy and pallidal electroencephalogram in an animal model of Parkinson's disease. *Neuroscience* **2008**, *154*, 940−945.

(26) Dhanalakshmi, C.; Janakiraman, U.; Manivasagam, T.; et al. Vanillin Attenuated Behavioural Impairments, Neurochemical Deficts, Oxidative Stress and Apoptosis Against Rotenone Induced Rat Model of Parkinson's Disease. *Neurochem. Res.* **2016**, *41*, 1899−1910.

(27) Bhattamisra, S. K.; Shak, A. T.; Xi, L. W.; et al. Nose to brain delivery of rotigotine loaded chitosan nanoparticles in human SH-SY5Y neuroblastoma cells and animal model of Parkinson's disease. *Int. J. Pharm.* **2020**, *579*, 119148.

(28) Maurissen, M.; Marable, B. R.; Andrus, A. K.; Stebbins, K. E. Factors affecting grip strength testing. *Neurotoxicol. Teratol.* **2003**, *25*, 543−53.

(29) Bandookwala, M.; Sahu, A. K.; Thakkar, D.; Sharma, M.; Khairnar, A.; Sengupta, P. Edaravone-caffeine combination for the effective management of rotenone induced Parkinson's disease in rats: An evidence based affirmative from a comparative analysis of behavior and biomarker expression. *Neurosci. Lett.* **2019**, *711*, 134438.

(30) Zhang, Z. N.; Zhang, J. S.; Xiang, J.; et al. Subcutaneous rotenone rat model of Parkinson's disease: Dose exploration study. *Brain Res.* **2017**, *1655*, 104−113.

(31) Yu, L.; Wang, X.; Chen, H.; Yan, Z.; Wang, M.; Li, Y. Neurochemical and behavior deficits in rats with iron and rotenone co-treatment: Role of redox imbalance and neuroprotection by Biochanin A. *Front Neurosci.* **2017**, *11*, 657.

(32) Sedaghat, R.; Roghani, M.; Khalili, M. Neuroprotective Effect of thymoquinone, the nigella sativa bioactive compound, in 6-hydroxydopamine-induced hemi-Parkinsonian rat model. *Iran. J. Pharm. Res.* **2014**, *13*, 227−34.

(33) Roghani, M.; Niknam, A.; Jalali-Nadoushan, M. R.; Kiasalari, Z.; Khalili, M.; Baluchnejadmojarad, T. Oral pelargonidin exerts dose-dependent neuroprotection in 6-hydroxydopamine rat model of hemi-parkinsonism. *Brain Res. Bull.* **2010**, *82*, 279−283.

(34) Bais, S.; Gill, N. S.; Kumar, N. Neuroprotective Effect of Juniperus communis on Chlorpromazine Induced Parkinson Disease in Animal Model. *Chin. J. Biol.* **2015**, *2015*, 1.

(35) Khadrawy, Y. A.; Salem, A. M.; El-Shamy, K. A.; Ahmed, E. K.; Fadl, N. N.; Hosny, E. N. Neuroprotective and Therapeutic Effect of Caffeine on the Rat Model of Parkinson's Disease Induced by Rotenone. *J. Diet. Suppl.* **2017**, *14*, 553−572.

(36) S, S.; Chaudhary, S.; R.S, R. Hydroalcoholic extract of *Stevia rebaudiana bert. leaves* and stevioside ameliorates lipopolysaccharide induced acute liver injury in rats. *Biomed. Pharmacother.* **2017**, *95*, 1040−1050.

(37) Bhangale, J. O.; Acharya, S. R. Anti-Parkinson Activity of Petroleum Ether Extract of Ficus religiosa (L.) Leaves. *Adv. Pharmacol. Sci.* **2016**, *2016*, 1.

(38) Sandhua, K. S.; Rana, A. C. Evaluation of anti Parkinson's activity of Nigella sativa (kalonji) seeds in a chlorpromazine-induced experimental animal model. *Mortality* **2013**, *22* (5), 23.

(39) Kumar, A.; Sharma, N.; Gupta, A.; Kalonia, H.; Mishra, J. Neuroprotective potential of atorvastatin and simvastatin (HMG-CoA reductase inhibitors) against 6-hydroxydopamine (6-OHDA) induced Parkinson-like symptoms. *Brain Res.* **2012**, *1471*, 13−22.

(40) Abdel-Salam, O. M. E.; Youness, E. R.; Ahmed, N. A.; et al. *Bougainvillea spectabilis flowers* extract protects against the rotenone-induced toxicity. *Asian Pac. J. Trop. Med.* **2017**, *10*, 478−490.

(41) Giza, E.; Gotzamani-Psarrakou, A.; Bostantjopoulou, S. Imaging beyond the striatonigral dopaminergic system in Parkinson's disease. *Hell J Nucl Med* **2012**, *15*, 224−32.

(42) Ferreira, M.; Massano, J. An updated review of Parkinson's disease genetics and clinicopathological correlations. *Acta Neurol. Scand.* **2017**, *135*, 273−284.

(43) Hwang, O. Role of Oxidative Stress in Parkinson's Disease. *Exp. Neurobiol.* **2013**, *22*, 11−17.

(44) Betarbet, R.; Sherer, T. B.; MacKenzie, G.; Garcia-Osuna, M.; Panov, A. V.; Greenamyre, J. T. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.* **2000**, *3*, 1301−1306.

(45) Wang, T.; Li, C.; Han, B.; et al. Neuroprotective effects of Danshensu on rotenone-induced Parkinson's disease models in vitro and in vivo. *BMC Complementary Med. Ther.* **2020**, *20*, 20.

(46) Rajasankar, S. Demethoxycurcumin ameliorates rotenone-induced toxicity in rats. *Front. Biosci.* **2019**, *11*, 1−11.

(47) Henchcliffe, C.; Beal, M. F. Mitochondrial biology and oxidative stress in Parkinson disease pathogenesis. *Nat. Clin. Pract. Neurol.* **2008**, *4*, 600−609.

(48) Avcı, B.; Günaydın, C.; Güvenç, T.; Yavuz, C. K.; Kuruca, N.; Bilge, S. S. Idebenone Ameliorates Rotenone-Induced Parkinson's Disease in Rats Through Decreasing Lipid Peroxidation. *Neurochem. Res.* **2021**, *46*, 513−522.

(49) Alikatte, K.; Palle, S.; Rajendra Kumar, J.; Pathakala, N. Fisetin Improved Rotenone-Induced Behavioral Deficits, Oxidative Changes, and Mitochondrial Dysfunctions in Rat Model of Parkinson's Disease. *J. Diet. Suppl.* **2021**, *18*, 57−71.

(50) Monti, B.; Gatta, V.; Piretti, F.; Raffaelli, S. S.; Virgili, M.; Contestabile, A. Valproic Acid is Neuroprotective in the Rotenone Rat Model of Parkinson's Disease: Involvement of α-Synuclein. *Neurotoxic. Res.* **2010**, *17*, 130−141.

(51) More, S. V.; Kumar, H.; Kim, I. S.; Song, S. Y.; Choi, D. K. Cellular and Molecular Mediators of Neuroinflammation in the Pathogenesis of Parkinson's Disease. *Mediators Inflammation* **2013**, *2013*, 1.

(52) Li, C.; Chen, X.; Zhang, N.; Song, Y.; Mu, Y. Gastrodin inhibits neuroinflammation in rotenone-induced Parkinson's disease model rats. *Neural Regener. Res.* **2012**, *7*, 325−331.

(53) Michel, H. E.; Tadros, M. G.; Esmat, A.; Khalifa, A. E.; Abdel-Tawab, A. M. Tetramethylpyrazine Ameliorates Rotenone-Induced Parkinson's Disease in Rats: Involvement of Its Anti-Inflammatory and Anti-Apoptotic Actions. *Mol. Neurobiol.* **2017**, *54*, 4866−4878.