

Unlocking the therapeutic potential of *Mucuna gigantea* seed extract on a rotenone-induced rat model of Parkinson's disease

Sharon F. Olago¹ , Charles G. Githinji^{1,2} , Jeswinder Sian-Hülsmann¹ , Boniface M. Chege^{1,3,*} ,
Elsie Date Odour¹ 

¹Department of Medical Physiology, Faculty of Health Sciences, University of Nairobi, GPO 30197-00100, Kenya

²Center for Bioequivalence Studies and Pharmaceutical Research, University of Nairobi, P.O Box 30197-00100, Nairobi, Kenya

³School of Health Sciences, Dedan Kimathi University of Technology, Private Bag-10143, Dedan Kimathi, Kenya

*Corresponding author

Boniface M. Chege
Department of Medical Physiology,
Faculty of Health Sciences,
University of Nairobi, GPO 30197-
00100, Kenya
Email: bmchege87@gmail.com

Academic editor

Md Jamal Uddin, PhD
ABEx Bio-Research Center
Bangladesh

Article info

Received: 26 March 2026

Accepted: 18 May 2026

Published: 04 June 2026

Keywords

Levodopa, *Mucuna gigantea*,
Neurodegenerative, Parkinson's
disease, Rotenone

ABSTRACT

Parkinson's disease (PD) is the second most prevalent neurodegenerative movement disorder, predominantly affecting older people. Despite increasing this global burden, the current therapeutics and medication strategies for the disease are not sufficient, and the treatment cost is unaffordable in many low-income countries. Thus, cost-effective strategies are crucial for the people of low-income territories. This study aimed to investigate the therapeutic potential of a freeze-dried aqueous extract of *Mucuna gigantea* seeds in a rotenone-induced rat model of PD. Thirty-six male Sprague Dawley rats were randomly allocated into six groups: normal, negative, positive, and extract-treated groups (low and high) doses of *M. gigantea* extract. Parkinsonism was induced using rotenone, and treatment was applied orally from day 22. In this study, motor performance, body weight, food intake, oxidative stress markers, striatal dopamine levels, faecal branched-chain amino acid levels, and histopathological changes in the substantia nigra. Treatment with the freeze-dried aqueous extract of *M. gigantea* significantly improved locomotor activity, coordination, gait, dopamine levels, antioxidant activity, and histological integrity while reducing oxidative stress and severity of motor deficits compared to the control group. The findings suggest that *M. gigantea* seed extract showed neuroprotective and anti-Parkinsonian properties, and it could be used as a potential affordable alternative to chemical therapy for PD.

INTRODUCTION

Parkinson's disease (PD) is the second most prevalent neurodegenerative movement disorder [1]. The disease is characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta [1,2]. The formation of Lewy bodies—mainly consisting of misfolded α -synuclein aggregates—is also observed in various regions of the nervous system [3]. In addition, characteristic motor symptoms include resting tremor, bradykinesia, postural instability, impaired gait, and muscle rigidity [3]. Non-motor symptoms also manifest, such as depression, hyposmia, REM sleep disturbances, and constipation, which can be experienced in the prodromal phase of the disorder [4].

The interplay between age, genetics, and environmental aspects is implicated in the pathogenesis of PD [4]. Although genetics plays a definitive role in some cases, about 90 % are idiopathic [5]. Mechanisms that have been postulated to contribute to the development of the disorder include mitochondrial dysfunction, α -synuclein misfolding and aggregation, neuroinflammation, protein degradation system malfunction, oxidative stress, apoptosis, toxic accumulation of iron, and gut dysbiosis [2,6]. There are currently no therapies that can reverse, halt, or effectively slow down the dopaminergic neuron destruction [7]. The current gold standard for management of PD is symptomatic therapy



Copyright: © by the authors. This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution 4.0 \(CC BY 4.0\)](https://creativecommons.org/licenses/by/4.0/) International license.

with L-DOPA, whose chronic administration is associated with dyskinesia development, and which is not widely available and affordable in low-income countries [8,9].

Plants of the *Mucuna* genus have been shown to contain natural levodopa [10–12]. In addition to containing levodopa, the seed extracts of *Mucuna pruriens* have been shown to have various neuroprotective properties, such as antioxidant, antiapoptotic, and anti-neuroinflammatory properties, and reduced risk for dyskinesias compared to the standard L-DOPA drug [13]. *Mucuna* extract is used as a complementary treatment for PD [14]. However, despite the growing interest in herbal therapies for neurodegenerative disorders, there remains limited scientific evidence regarding the neuropharmacological properties of many *Mucuna* species, particularly *Mucuna gigantea*, in the management of PD. Most of the studies have largely been focused on *Mucuna pruriens*, leaving extensive gaps in knowledge regarding the therapeutic potential, efficacy, and safety profile of other plant species within the genus. Besides, there are inadequate experimental findings evaluating the efficiency of freeze-dried aqueous seed extracts of *Mucuna gigantea* for validating animal models of PD, especially rotenone-induced models that mimic dopaminergic neurodegeneration and oxidative stress observed in PD. Furthermore, the potential neuroprotective mechanisms of *M. gigantea*, including its antioxidant and anti-inflammatory activities, remain largely uncharacterized. Therefore, it is crucial to address these fundamental gaps using plant-based therapeutics. In this current study, we unlocked the therapeutic potential of *M. gigantea* seed extract on a rotenone-induced rat model of PD. This therapeutic strategy is affordable and could be an alternative to chemical medicine used for PD healing.

MATERIALS AND METHODS

Experimental materials

In this study, a total of thirty-six (36) adult male Sprague-Dawley rats weighing from 250–290 g were considered for the experiments. All rats were adapted for one week so that they could acclimatize to the new environment prior to starting the main experiments. The rats were divided randomly into 6 groups, with 6 animals per cage. They were fed standard chow pellets, and tap water for drinking was provided ad libitum. The wood shavings in the cages were changed three times a week. A temperature of 21°C–23°C and a 12/12-hour light/dark cycle were maintained in the animal house.

Ethical considerations

The experimental protocol was approved by the Biosafety, Animal Use, and Ethics Committee of the Faculty of Veterinary Medicine, University of Nairobi (permit number FVM BAUEC/2021/318). All experiments were conducted in accordance with the ARRIVE guidelines. All methods were performed in accordance with the relevant guidelines and regulations. All surgery was performed under ketamine injection (80 mg/kg, i.p.) anesthesia, and all efforts were made to minimize suffering.

Experimental design

Rats were randomly assigned into six groups (n=6 per group) as follows: normal control (rotenone vehicle only - DMSO diluted in sunflower oil (2% DMSO)); negative control (Rotinone 2 mg/kg/day i.p.); positive control (levodopa/carbidopa 100/10 mg/kg/day p.o.); low dose *M. gigantea* extract (100 mg/kg/day p.o.); medium dose *M. gigantea* extract (200 mg/kg/day p.o.); high dose *M. gigantea* extract (400 mg/kg/day p.o.). The high,

medium, and low dose *M. gigantea* groups also received carbidopa in similar ratios (10mg/kg, 5mg/kg, and 2.5mg/kg respectively) (Figure 1). The rotenone dose was selected based on previous studies [15,16]. The extract doses were determined based on previous studies done with related species such as *Mucuna pruriens* [13].

Behavioural tests were conducted at 3 time points: baseline to ensure that there were no significant preexisting differences between the groups, pretreatment to verify that the normal group was performing significantly better than the other groups, and therefore as a marker of induction, and finally posttreatment to compare the performance of the different groups after *Mucuna gigantea* extract administration. Baseline behavioural tests were carried out first. Then, the rats in which PD was to be induced (groups 2 to 6) received rotenone at a dose of 2 mg/kg/day i.p., from day 1 of the experiment to day 21. Behavioural tests were carried out at this time point to determine if there were significant differences. From days 22 to 35, the treatment groups (4,5, and 6) and positive control group (2) began receiving their respective doses of extract and Sinemet (L-DOPA/carbidopa). Rotenone was also continually administered to groups 2 to 6 until day 35. Final behavioral motor tests were carried out, and fresh faeces were collected before the rats were sacrificed by ketamine injection (80 mg/kg, i.p.), which was used as the anaesthetic, followed by cervical dislocation, and their brains were harvested for further analysis.

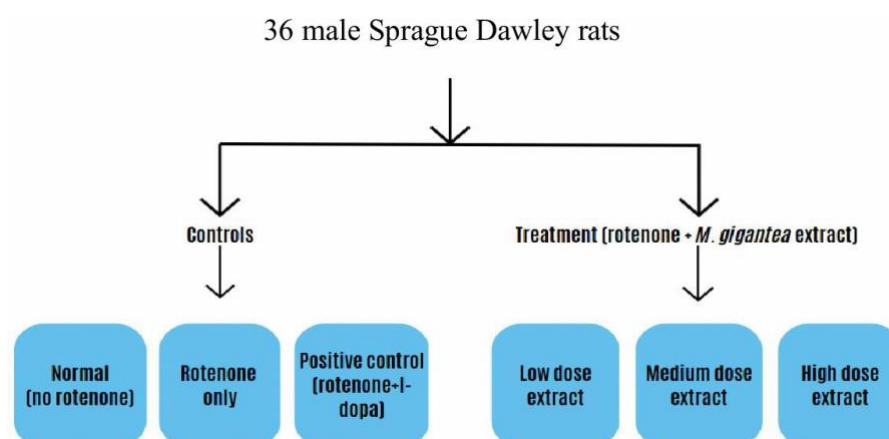


Figure 1. A flow chart represents an experimental overview of the whole study.

Preparation of rotenone

Rotenone was dissolved in DMSO and diluted with sunflower oil such that the sunflower oil-DMSO mixture consisted of 2% DMSO [17]. The solution was prepared fresh each day.

Preparation of plant extract

Mucuna gigantea seeds were obtained from western Kenya. Their identity was verified at the herbarium in the Department of Botany, School of Biological Sciences, University of Nairobi (Voucher No: SOUON2023/001). The seeds were air-dried for three days in the shade. They were then ground into a powder and sieved to obtain a fine powder. The seed powder was mixed with water, shaken thoroughly every five minutes for thirty minutes, and then allowed to settle. The mixture was then decanted and filtered using filter paper, and the filtrate was lyophilized.

Assessment of motor function

Open field test

The open field test was used to measure the general locomotor activity of the rats. The open field box measured 72 x 72 x 36 cm with transparent walls. The floor of the box was drawn into an 18 x 18 cm square pattern using a blue marker pen. A video camera was mounted, capturing the entire box. Each rat was placed in the box for five minutes, and its behaviour was observed. The rat was then removed, and the box was cleaned with 5 % alcohol before the next rat was placed in it. The parameters assessed for each rat included the number of line crossings and the time inactive in the open field.

Footprint test

This test was used to assess the gait of the rats. The rats were trained to walk across a white sheet of paper without pausing in a space 100cm long and 12cm wide, enclosed by 40cm high boards. To acquire the footprint patterns, the forepaws and hind paws of each rat were dipped in red and blue ink, respectively, before allowing them to walk across the sheet of paper. A fresh sheet of paper was used for each rat. The stride length (distance between consecutive steps on the same side of the body, using the clear hind paw prints) was measured in centimetres. The stride lengths between four consecutive steps were averaged.

Beam walking test

This test was used to assess motor coordination and balance. A flat beam 1 metre long and 4 cm wide was fixed to two poles fixed 50 cm above the ground. Boxes with wood shavings were placed below to cushion the rats in case they fell off the beam. The respective home cage of the rats was placed at the endpoint to attract them to the finish point. There were two-day training periods to familiarise the rats with the task. On test days, the time each rat took to cross the beam, as well as the number of times the paws slipped off the beam, was recorded. A cut-off time of 60 seconds was applied.

Bar test

The bar test was used to assess catalepsy. Catalepsy is characterised by muscle rigidity and fixed posture, showing difficulty in the control of voluntary movement. The forepaws of each rat were placed on a bar fixed 9 cm above the ground. The time taken for the rat to initiate correction of its posture and remove both forepaws from the bar was measured. A cut-off time of 180 seconds was set.

Oxidative stress and dopamine analysis

Homogenate preparation

Rat brain striata and midbrains were excised, rinsed, and weighed. Each sample was homogenised in ice-cold phosphate-buffered saline (0.1 M, pH 7.4), and the homogenate was centrifuged at 10,000 $\times g$ for 25 min to obtain the supernatant (maintained at 4° Celsius), which was used for biochemical tests such as malondialdehyde (MDA), catalase, and dopamine assays.

Malondialdehyde levels

Midbrain malondialdehyde levels were determined using the Ohkawa method [18]. 2 ml of 10% TCA was added to 1 ml of homogenate, and the mixture was centrifuged for five minutes at 10000 $\times g$. 0.5ml of 1% TBA was added to 2 ml of the supernatant, and the mixture was heated in a water bath at 95° Celsius for 60 minutes. The mixture was then cooled under tap water, and the absorbance at 532 nm was determined. The reagents were used as a blank. Concentrations were calculated using the molar extinction coefficient of $0.156 \times 10^6 \text{ L mol}^{-1} \text{ cm}^{-1}$.

Catalase activity

Midbrain catalase activity was determined using the Aebi kinetic method [19]. 1.9 ml of 50 mM phosphate buffer (pH 7) was added to 0.1ml of brain homogenate. Then, 1 mL of 30 mM H_2O_2 was added to the mixture. The initial and final absorbance in one minute were determined at 240 nm. The reagents were used as a blank. Concentrations were calculated using the molar extinction coefficient of $43.6 \text{ L mol}^{-1} \text{ cm}^{-1}$.

Striatal dopamine

Brain homogenate (0.1 ml) was added to 0.1 ml of 5 mM ferric chloride and 0.1 ml of 5 mM potassium ferricyanide and topped to 2.5 ml with phosphate buffer (pH 4.0). The mixture was left for 35 min at room temperature before the absorbance was read at 735 nm. The reagents were used as the blank. The concentrations of dopamine were calculated using the Molar extinction coefficient $3.2 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$.

Gut metabolites

Raman spectroscopy of faeces was done at the end of the study. The faecal levels of branched-chain amino acids l-leucine, l-valine, and l-isoleucine in the different experimental groups were compared by analysing the respective Raman spectra.

Histological analysis

Rat brain tissues were fixed in 10% neutral buffered formalin for two days to harden them for sectioning. The samples were washed with ice-cold saline solution and dehydrated with increasing concentrations of alcohol. They were then cleared in xylene and embedded in paraffin. Sections of the specimen were made using a microtome. The sections were allowed to dry overnight and were prepared for hematoxylin and eosin staining. After staining, they were examined under a light microscope at $\times 40$ magnification for histopathological changes, and photographs were taken.

Statistical analysis

One-way ANOVA was used to analyse the data, and the data were expressed as mean $\pm$ SEM. Tukey's post hoc test was then used to identify the specific groups that significantly differed from each other. The significance level was set at $p < 0.05$. GraphPad Prism 9.5.1 was used for analysis.

RESULTS

Effect of *M. gigantea* seed extract on the number of line crossings

There were significant differences in the mean number of line crossings among the six experimental groups in the posttreatment phase: [109.8 $\pm$ 5.344 (normal control) vs 1.667 $\pm$ 0.7601 (negative control) vs 7.833 $\pm$ 0.6009 (low dose) vs 28.33 $\pm$ 1.926 (medium dose) vs 57.00 $\pm$ 7.230 (high dose) vs 70.67 $\pm$ 6.902 (positive control): $p < 0.0001$] (See Table 2). Post hoc analysis using Tukey's multiple-comparison test revealed that there were significant differences between the normal control group and all other treatment groups ($p < 0.0001$) (Table 1).

Impact of *M. gigantea* seed extract on inactivity time

There were significant differences in the mean inactive time among the six experimental groups in the posttreatment phase: [16.27 $\pm$ 3.373 seconds (normal control) vs 250.7 $\pm$ 16.35 seconds (negative control) vs 152.4 $\pm$ 20.44 seconds (low dose) vs 90.05 $\pm$ 6.054 seconds (medium dose) vs 71.97 $\pm$ 6.622 seconds (high dose) vs 72.43 $\pm$ 4.427 seconds (positive control): $p < 0.0001$] (Table 2). Post hoc analysis using Tukey's multiple-comparison test revealed that there were significant differences between the normal control group and all other treatment groups ($p < 0.0001$) (Table 1).

Effect of *M. gigantea* seed extract on the narrow beam

There were significant differences in the mean amount of time taken on the beam among the six experimental groups in the posttreatment phase: [5.025 $\pm$ 0.3135 seconds (normal control) vs 35.92 $\pm$ 0.3679 seconds (negative control) vs 28.33 $\pm$ 0.3612 seconds (low dose) vs 24.96 $\pm$ 0.2541 seconds (medium dose) vs 21.63 $\pm$ 0.3463 seconds (high dose) vs 20.44 $\pm$ 0.2881 seconds (positive control): $p < 0.0001$]. Post hoc analysis using Tukey's multiple-comparison test revealed that there were significant differences between the normal control group and all other treatment groups ($p < 0.0001$) (Table 1).

Role of *M. gigantea* seed extract on the number of foot slips

There were significant differences in the mean number of foot slips among the six experimental groups in the posttreatment phase: [0.6667 $\pm$ 0.3333 (normal control) vs 12.17 $\pm$ 0.4014 (negative control) vs 9.333 $\pm$ 0.3333 (low dose) vs 8.000 $\pm$ 0.3651 (medium dose) vs 6.333 $\pm$ 0.3333 (high dose) vs 5.833 $\pm$ 0.3073 (positive control): $p < 0.0001$] (See Table 2). Post hoc analysis using Tukey's multiple-comparison test revealed that there were significant differences between the normal control group and all other treatment groups ($p < 0.0001$) (Table 1).

Impact of *M. gigantea* seed extract on catalepsy

There were significant differences in the mean descent latency among the six experimental groups in the posttreatment phase: [3.085 $\pm$ 0.2336 seconds (normal control) vs 32.85 $\pm$ 0.46706 seconds (negative control) vs 26.41 $\pm$ 0.2288 seconds (low dose) vs 22.35 $\pm$ 0.514 seconds (medium dose) vs 16.99 $\pm$ 0.2009 seconds (high dose) vs 15.87 $\pm$ 0.1939 seconds (positive control): $p < 0.0001$]. Post hoc analysis using Tukey's multiple-

comparison test revealed that there were significant differences between the normal control group and all other treatment groups ($p < 0.0001$) (Table 1).

Table 1. Showing the effects of *Mucuna gigantea* extract on behavioural and motor function.

Dependent variable	Group	Group	Mean $\pm$ SEM	p-Value
Open Field Line crossings	Normal 109.8 $\pm$ 5.344	Negative control	1.667 $\pm$ 0.7601	< 0.0001****
		Low dose	7.833 $\pm$ 0.6009	< 0.0001****
		Medium dose	28.33 $\pm$ 1.926	< 0.0001****
		High dose	57.00 $\pm$ 7.230	< 0.0001****
		Positive control	70.67 $\pm$ 6.902	< 0.0001****
	Negative 1.667 $\pm$ 0.7601	High dose	57.00 $\pm$ 7.230	< 0.0001****
		Medium dose	28.33 $\pm$ 1.926	0.00460**
		Positive control	70.67 $\pm$ 6.902	< 0.0001****
Open Field Inactivity (seconds)	Normal 16.27 $\pm$ 3.373	Negative control	250.7 $\pm$ 16.35	< 0.0001****
		Low dose	152.4 $\pm$ 20.44	< 0.0001****
		Medium dose	90.05 $\pm$ 6.054	0.0011**
		High dose	71.97 $\pm$ 6.622	0.0205*
		Positive control	72.43 $\pm$ 4.427	0.0191*
	Negative 250.7 $\pm$ 16.35	High dose	57.00 $\pm$ 7.230	< 0.0001****
		Medium dose	28.33 $\pm$ 1.926	0.0046**
		Low dose	152.4 $\pm$ 20.44	< 0.0001****
		Positive control	70.67 $\pm$ 6.902	< 0.0001****
Time on Narrow Beam (seconds)	Normal 5.025 $\pm$ 0.3135	Negative control	35.92 $\pm$ 0.3679	< 0.0001****
		Low dose	28.33 $\pm$ 0.3612	< 0.0001****
		Medium dose	24.96 $\pm$ 0.2541	0.0011**
		High dose	21.63 $\pm$ 0.3463	0.0205*
		Positive control	20.44 $\pm$ 0.2881	0.0191*
	Negative 35.92 $\pm$ 0.3679	High dose	21.63 $\pm$ 0.3463	< 0.0001****
		Medium dose	24.96 $\pm$ 0.2541	0.0046**
		Low dose	28.33 $\pm$ 0.3612	< 0.0001****
		Positive control	20.44 $\pm$ 0.2881	< 0.0001****
Number of foot slips in narrow beam	Normal 0.6667 $\pm$ 0.3333	Negative control	12.17 $\pm$ 0.4014	< 0.0001****
		Low dose	9.333 $\pm$ 0.3333	< 0.0001****
		Medium dose	8.000 $\pm$ 0.3651	0.0011**
		High dose	6.333 $\pm$ 0.3333	< 0.0001****
		Positive control	5.833 $\pm$ 0.3073	< 0.0001****
	Negative 12.17 $\pm$ 0.4014	High dose	6.333 $\pm$ 0.3333	< 0.0001****
		Medium dose	8.000 $\pm$ 0.3651	< 0.0001****
		Low dose	9.333 $\pm$ 0.3333	< 0.0001****
		Positive control	5.833 $\pm$ 0.3073	< 0.0001****
Effect of catalepsy (Seconds)	Normal 3.085 $\pm$ 0.2336	Negative control	32.85 $\pm$ 0.46706	< 0.0001****
		Low dose	26.41 $\pm$ 0.2288	< 0.0001****
		Medium dose	22.35 $\pm$ 0.514	< 0.0001****
		High dose	16.99 $\pm$ 0.2009	< 0.0001****
		Positive control	15.87 $\pm$ 0.1939	< 0.0001****
	Negative 32.85 $\pm$ 0.46706	High dose	16.99 $\pm$ 0.2009	< 0.0001****
		Medium dose	22.35 $\pm$ 0.514	< 0.0001****
		Low dose	26.41 $\pm$ 0.2288	< 0.0001****
		Positive control	15.87 $\pm$ 0.1939	< 0.0001****
Effect of stride length (cm)	Normal 14.88 $\pm$ 0.1922	Negative control	10.00 $\pm$ 0.4405	< 0.0001****
		Low dose	11.27 $\pm$ 0.1563	< 0.0001****
		Medium dose	11.87 $\pm$ 0.0333	< 0.0001****
		High dose	13.15 $\pm$ 0.2320	< 0.0001****
		Positive control	13.33 $\pm$ 0.1820	< 0.0001****
	Negative 10.00 $\pm$ 0.4405	High dose	13.15 $\pm$ 0.2320	< 0.0001****
		Medium dose	11.87 $\pm$ 0.0333	< 0.0001****
		Low dose	11.27 $\pm$ 0.1563	< 0.0001****
		Positive control	13.33 $\pm$ 0.1820	< 0.0001****

The behavioural and motor function parameters, including open field line crossings, open field inactivity duration, time taken on the narrow beam test, number of foot slips on the narrow beam, catalepsy score, and stride length across the different experimental groups. Data are presented as mean $\pm$ SEM.

Effect of *M. gigantea* seed extract on stride length

There were significant differences in the mean stride length (hind paw) among the six experimental groups in the posttreatment phase: [14.88 ± 0.1922 centimetres (normal control) vs 10.00 ± 0.4405 centimetres (negative control) vs 11.27 ± 0.1563 centimetres (low dose) vs 11.87 ± 0.0333 centimetres (medium dose) vs 13.15 ± 0.2320 centimetres (high dose) vs 13.33 ± 0.1820 centimetres (positive control): $p < 0.0001$] Table 2. Post hoc analysis using Tukey's multiple comparison test revealed that there were significant differences between the normal control group and high dose group ($p = 0.0002$), normal control group and medium dose group ($p < 0.0001$), normal control group and low dose group ($p < 0.0001$), normal control group and positive control group ($p = 0.0010$), normal control group and negative control group ($p < 0.0001$) (Table 1).

M. gigantea seed extract regulates catalase activity

There were significant differences in the mean catalase activity in the midbrain among the six experimental groups at the end of the five week study: [55.79 ± 2.469 U/g (normal control) vs 13.88 ± 0.1430 U/g (negative control) vs 25.11 ± 0.1705 U/g (low dose) vs 35.96 ± 0.1519 U/g (medium dose) vs 46.19 ± 0.3968 U/g (high dose) vs 13.71 ± 0.1828 U/g (positive control): $p < 0.0001$]. Post hoc analysis using Tukey's multiple-comparison test revealed that there were significant differences between the normal control group and all other treatment groups ($p < 0.0001$) (Table 2).

M. gigantea seed extract regulates malondialdehyde content

There were significant differences in the mean malondialdehyde levels in the midbrain among the six experimental groups at the end of the five week study: [10.12 ± 0.4133 nmol/g (normal control) vs 41.02 ± 0.4474 nmol/g (negative control) vs 32.48 ± 0.3605 nmol/g (low dose) vs 26.80 ± 0.1694 nmol/g (medium dose) vs 17.77 ± 0.2693 nmol/g (high dose) vs 40.33 ± 0.1710 nmol/g (positive control): $p < 0.0001$]. Post hoc analysis using Tukey's multiple-comparison test revealed that there were significant differences between the normal control group and all other treatment groups ($p < 0.0001$) (Table 2).

Impact of *M. gigantea* seed extract on striatal dopamine level

There were significant differences in the striatal dopamine levels among the six experimental groups at the end of the five week study: [77.79 ± 2.700 nmol/g (normal control) vs 22.04 ± 0.3153 nmol/g (negative control) vs 36.69 ± 0.3388 nmol/g (low dose) vs 52.56 ± 0.2915 nmol/g (medium dose) vs 65.13 ± 0.6060 nmol/g (high dose) vs 67.66 ± 0.5501 nmol/g (positive control): $p < 0.001$]. Post hoc analysis using Tukey's multiple-comparison test revealed that there were significant differences between the normal control group and all other treatment groups ($p < 0.0001$) (Table 2).

Effect of *M. gigantea* seed extract on L-valine

There were significant differences in the mean l-valine Raman intensities among the six experimental groups at the end of the five-week study: [49.22 ± 2.338 a.u. (normal control) vs 23.66 ± 2.117 a.u. (negative control) vs 26.07 ± 3.572 a.u. (low dose) vs 27.46 ± 3.060 a.u. (medium dose) vs 31.05 ± 4.856 a.u. (high dose) vs 31.64 ± 2.226 a.u. (positive

control): $p=0.0001$]. Post hoc analysis using Tukey's multiple comparison test revealed that there were significant differences between the normal control group and high dose group ($p=0.0056$), normal control group and medium dose group ($p=0.0008$), normal control group and low dose group ($p=0.0004$), normal control group and positive control group ($p=0.0076$), and normal control group and negative control group ($p<0.0001$) (Table 2 and Figure 2).

Table 2. Showing the effects of *Mucuna gigantea* extract on catalase activity, MDA levels, striatal dopamine levels, and Raman spectral intensities of selected amino acids, including L-valine, leucine, and L-isoleucine, across the different experimental groups.

Dependent variable	Group	Group	Mean $\pm$ SEM	p-Value
Effect on catalase activity (U/g)	Normal 55.79 $\pm$ 2.469	Negative control	13.88 $\pm$ 0.1430	< 0.0001****
		Low dose	25.11 $\pm$ 0.1705	< 0.0001****
		Medium dose	35.96 $\pm$ 0.1519	< 0.0001****
		High dose	46.19 $\pm$ 0.3968	< 0.0001****
		Positive control	13.71 $\pm$ 0.1828	< 0.0001****
	Negative 13.88 $\pm$ 0.1430	High dose	46.19 $\pm$ 0.3968	< 0.0001****
Effect on malondialdehyde levels (nmol/g)	Normal 10.12 $\pm$ 0.4133	Negative control	41.02 $\pm$ 0.4474	< 0.0001****
		Low dose	32.48 $\pm$ 0.3605	< 0.0001****
		Medium dose	26.80 $\pm$ 0.1694	< 0.0001****
		High dose	17.77 $\pm$ 0.2693	< 0.0001****
		Positive control	40.33 $\pm$ 0.1710	< 0.0001****
	Negative 1.02 $\pm$ 0.4474	High dose	17.77 $\pm$ 0.2693	< 0.0001****
Effect on striatal dopamine levels (nmol/g)	Normal 77.79 $\pm$ 2.700	Negative control	22.04 $\pm$ 0.3153	< 0.0001****
		Low dose	36.69 $\pm$ 0.3388	< 0.0001****
		Medium dose	52.56 $\pm$ 0.2915	< 0.0001****
		High dose	65.13 $\pm$ 0.6060	< 0.0001****
		Positive control	67.66 $\pm$ 0.5501	< 0.0001****
	Negative 22.04 $\pm$ 0.3153	High dose	65.13 $\pm$ 0.6060	0.001***
Effect on L-valine Raman intensities (a.u.)	Normal 49.22 $\pm$ 2.338	Negative control	23.66 $\pm$ 2.117	0.001***
		Low dose	26.07 $\pm$ 3.572	0.001***
		Medium dose	27.46 $\pm$ 3.060	0.001***
		High dose	31.05 $\pm$ 4.856	0.001***
		Positive control	31.64 $\pm$ 2.226	0.001***
Effect on L-leucine Raman intensities (a.u.)	Normal 653.4 $\pm$ 15.3	Negative control	392.8 $\pm$ 62.8	0.003.**
		Low dose	384.1 $\pm$ 47.31	0.0021**
		Medium dose	393.2 $\pm$ 31.01	0.003**
		High dose	375.9 $\pm$ 53.10	0.0015**
		Positive control	324.2 $\pm$ 28.31	0.0002***
Effect on L-isoleucine Raman intensities (a.u.)	Normal 705.3 $\pm$ 18.08	Negative control	350.1 $\pm$ 38.65	0.001**
		Low dose	415.4 $\pm$ 51.54	0.001**
		Medium dose	417.6 $\pm$ 31.81	0.001**
		High dose	402.6 $\pm$ 54.46	0.001
		Positive control	348.4 $\pm$ 29.32	0.001

Data are presented as mean $\pm$ SEM.

Role of *M. gigantea* seed extract on L-leucine

There were significant differences in the mean l-leucine Raman intensities among the six experimental groups at the end of the five-week study: [653.4 $\pm$ 15.3 a.u. (normal control) vs 392.8 $\pm$ 62.8 a.u. (negative control) vs 384.1 $\pm$ 47.31 a.u. (low dose) vs 393.2 $\pm$ 31.01 a.u. (medium dose) vs 375.9 $\pm$ 53.10 a.u. (high dose) vs 324.2 $\pm$ 28.31 a.u. (positive

control): $p=0.0002$]. Post hoc analysis using Tukey's multiple-comparison test revealed that there were significant differences between the normal control group and high-dose group ($p=0.0015$), normal control group and medium dose group ($p=0.0030$), normal control group and low dose group (Table 2 and Figure 2).

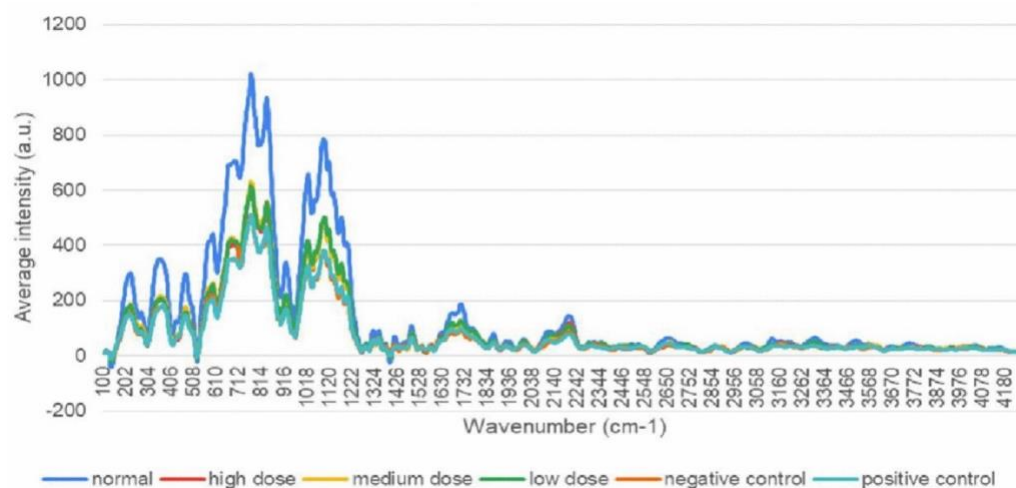


Figure 2. The graph shows the average Raman spectra of faecal samples obtained from the different experimental groups, illustrating variations in spectral intensity and peak distribution across the groups.

Impact of *M. gigantea* seed extract on L-isoleucine

There were significant differences in the mean l-isoleucine Raman intensities among the six experimental groups at the end of the five-week study: $[705.3 \pm 18.08$ a.u. (normal control) vs 350.1 ± 38.65 a.u. (negative control) vs 415.4 ± 51.54 a.u. (low dose) vs 417.6 ± 31.81 a.u. (medium dose) vs 402.6 ± 54.46 a.u. (high dose) vs 348.4 ± 29.32 a.u. (positive control): $p<0.0001$]. Post hoc analysis using Tukey's multiple comparison test revealed that there were significant differences between the normal control group and high dose group ($p<0.0002$), normal control group and medium dose group ($p<0.0004$), normal control group and low dose group ($p<0.0003$), normal control group and positive control group ($p<0.0001$), and normal control group and negative control group ($p<0.0001$) (Table 2 and Figure 2).

Effect of *M. gigantea* seed extract on histology

Normal neuronal architecture is displayed in the normal group. There is evidence of increased vacuolation in the remaining groups. The number of normal neuronal cells also appears to decrease in a dose-dependent manner from the high dose group, with the lowest numbers present in the positive control and negative control (normal-34, high dose-23, medium-19, low dose-11, positive-3, negative-2). The positive and negative controls show more evidence of pyknotic nuclei (darkly stained, shrunken) and reactive gliosis than the other groups (Figure 3).

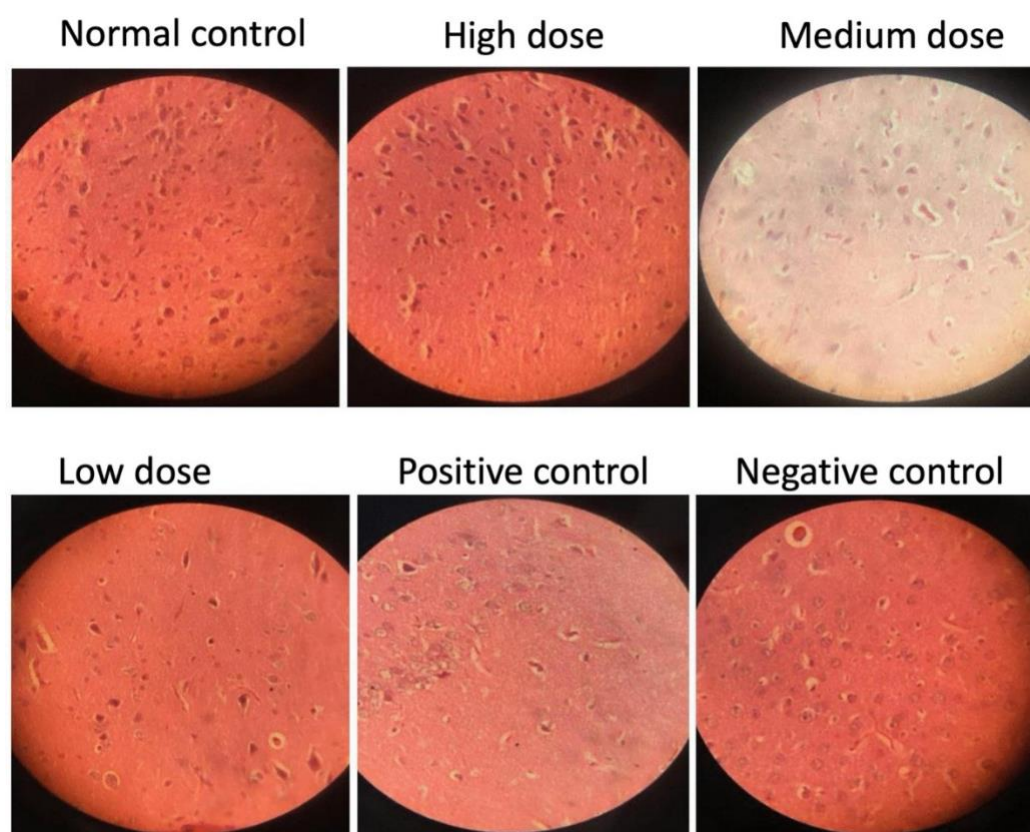


Figure 3. Representative images of hematoxylin and eosin (H&E)-stained sections of the nigral region of the midbrain from the different experimental groups, showing the histological architecture and neuronal morphology. Images were captured at $\times 40$ magnification.

DISCUSSION

PD is a progressive neurodegenerative disorder characterized by motor dysfunction, oxidative stress, dopaminergic neuronal degeneration, and neuroinflammation, with prevalence increasing alongside the ageing population [20]. Although levodopa/carbidopa remains the cornerstone of PD management, it does not halt disease progression and is associated with complications such as dyskinesia, wearing-off effects, limited accessibility, and high cost in low-income settings [7–9]. In this current study, the freeze-dried *M. gigantea* water extracts showed significant neuroprotective and anti-Parkinsonian effects, as it revealed by a rotenone-induced rat model.

The *M. gigantea* plant extract considerably enhanced motor performance by regulating of catalepsy, enhancing locomotor activity, along with stride length. These findings are consistent with previous reports demonstrating that rotenone administration induces motor impairments through degeneration of nigrostriatal dopaminergic neurons and depletion of striatal dopamine [15,22,23]. The dose-dependent improvement observed following treatment with *Mucuna gigantea* suggests restoration of dopaminergic neurotransmission. Almost similar neuroprotective effects have been documented by other studies in *M. pruriens*; this related species triggers an anti-Parkinsonian response [13]. However, the response may vary among different plant species and the labels of extract doses used in experiments. The levodopa/carbidopa-treated group supports the therapeutic potential of *M. gigantea*.

The elevated level of striatal dopamine observed in the treatment groups indicates the enhancement of motor symptoms. Although the levodopa-containing extract was comparably low, histological outputs indicate the inhibition of dopaminergic neurons,

suggesting that the extract leads to neuroprotective effects beyond the dopamine replacement. However, rotenone induces PD-like pathology initially through inhibition of mitochondrial complex I, which induces oxidative stress, neuroinflammation, and dopaminergic neuronal death [15,21]. In the current study, the decline in reactive gliosis, lower consequences of pyknotic nuclei, and enhancement of neuronal density in the substantia nigra of the treated animals indicate an attenuation tendency of neuroinflammation and neuronal degeneration. These findings corroborate earlier studies that lead to neuroprotection and contribute to inhibition of dopaminergic neurotransmission [13].

Oxidative stress is one of the key indicators of PD pathogenesis and rotenone-induced neurotoxicity. *M. gigantea* extracts involving treatment considerably decline MDA levels, while the catalase activity increased in the midbrain, suggesting the induction of antioxidant defense. These outputs are corroborated with the previous studies where increased lipid peroxidation and reduced antioxidant enzyme activity following rotenone administration [16,38]. However, the MDA level is considered a marker of lipid peroxidation, oxidative stress, and cellular injury, while catalase plays a vital role in detoxifying reactive oxygen species (ROS) [38,39]. In this current study, the antioxidant activity of *M. gigantea* might be involved in protecting nigral dopaminergic neurons, along with improvement of behavioural performance.

The extract also improved food intake and body weight in rotenone-treated rats. Weight loss is a common feature of PD and has been associated with disease severity and impaired energy balance [24]. Reduced food intake in PD may result from motor impairments that interfere with feeding behavior as well as dysfunction of dopaminergic reward pathways [25,26]. Degeneration of dopaminergic projections to the dorsal and ventral striatum has been implicated in impaired feeding motivation [27]. The higher striatal dopamine levels observed in the treatment groups may therefore have improved feeding behavior and contributed to the recovery of body weight. Similar observations have been reported in other rotenone-induced PD studies [22,23].

The findings related to gait and locomotor activity further support the anti-Parkinsonian effects of the extract. Rotenone administration resulted in impaired beam walking performance, increased foot slips, reduced stride length, and decreased locomotor activity, findings that are comparable to previous studies [23,34,36]. Gait disturbances in PD are characterized by bradykinesia, hypokinesia, and impaired postural control due to dysfunction within the basal ganglia circuitry [31,37]. The dose-dependent improvement in these parameters following treatment with *Mucuna gigantea* may therefore be attributed to enhanced dopaminergic neurotransmission. Similar improvements have been observed following dopamine-enhancing therapies in experimental models of PD [34,35].

Though the plant extracts exhibit significant neuroprotective effects, but it not significantly reverse the decrease in faecal branched-chain amino acid (BCAA) levels induced by rotenone. Reduced BCAA synthesis is linked with gut dysbiosis in PD patients and experimental PD models [40,41]. Since gut microbial BCAA production leads to regulating BCAA levels and CNS glutamate metabolism. However, the reduced BCAA levels impair neuronal protection against glutamate excitotoxicity [42]. The persistence of reduced faecal BCAA levels among the treatment groups suggests that *M. gigantea* effectively modulates oxidative stress and dopaminergic dysfunction. Furthermore, its effects on gut microbial metabolic activity may decline due to prolonged treatments.

Overall study findings suggest that *M. gigantea* extract regulates neuroprotective effects through multiple mechanisms, including the development of dopaminergic neurotransmission, the reduction of oxidative stress, and the reduction of neuroinflammation. These combined effects justify the improved behavioural, biochemical, and histopathological parameters in the rotenone-induced PD model. In further study, limitations should be addressed precisely. First, the study has been conducted using an animal model. Therefore, it may decline direct extrapolation of the outputs to human PD. Second, the specific bioactive compounds responsible for the observed neuroprotective effects have not been isolated and categorized. With these, alterations of molecular pathways related to neuroinflammation, apoptosis, and mitochondrial dysfunction need to be extensively studied.

CONCLUSIONS

This study provides *M. gigantea* extract involving the therapeutic potential of PD using a rat model. The *M. gigantea* plant-based compound significantly improved motor function, increased striatal dopamine levels, reduced oxidative stress, and minimized histopathological damage in a rotenone-induced rat model of PD. However, this study suggests that the neuroprotective effects are dose-dependent. It further suggests that *M. gigantea* would be a promising therapeutic source and acts as an anti-PD agent. These overall findings support the potential use of medicinal seed extracts as affordable, cost-effective, and complementary therapies for neurodegenerative disorders.

ACKNOWLEDGEMENTS

The authors want to express their gratitude for the valuable technical support provided by Ms. Abigeal Mutua and Mr. Horo Mwaura.

AUTHOR CONTRIBUTIONS

SFO: Writing – review & editing, Writing – original draft, Validation, Formal analysis, Conceptualization. CGG: Writing – review & editing, Project administration, Funding acquisition. JS-H: Validation, Formal analysis, Data curation. BMC: Writing – original draft, Validation, Methodology. EDO: Writing – review & editing.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

REFERENCES

- [1] Murthy M, Cheng YY, et al. Neurodegenerative movement disorders: An epigenetics perspective and promise for the future. *Neuropathol Appl Neurobiol.* 2021;47: 897–909.
- [2] Amro MS, Teoh SL, et al. The potential role of herbal products in the treatment of Parkinson's disease. *La Clinica Terapeutica.* 2018; 23–33.
- [3] Balestrino R, Schapira AHV. Parkinson disease. *Euro J Neurol.* 2020;27: 27–42.
- [4] Kouli A, Torsney KM, et al. Parkinson's Disease: Etiology, Neuropathology, and Pathogenesis. Exon Publications. 2018; 3–26.
- [5] Klein C, Westenberger A. Genetics of Parkinson's Disease. *Cold Spring Harbor Perspectives in Medicine.* 2012;2: a008888–a008888.
- [6] Zhu M, Liu X, et al. Gut Microbiota: A Novel Therapeutic Target for Parkinson's Disease. *Front Immunol.* 2022;13: 937555.

- [7] Troncoso-Escudero P, Parra A, et al. Outside in: Unraveling the role of neuroinflammation in the progression of parkinson's disease. *Front Neurol.* 2018;9: 860.
- [8] Merello M. The Dark Side of Globalization: Lack of Universal Levodopa Availability. *Move Disord Clin Pract.* 2019;6: 7–8.
- [9] Perez-Lloret S, Negre-Pages L, et al. L- DOPA -induced dyskinesias, motor fluctuations and health-related quality of life: the COPARK survey. *Euro J Neurol.* 2017;24: 1532–1538.
- [10] Raina AP, Misra RC. Chemical evaluation of *Mucuna* species for L- dopa content-an anti-parkinson's drug yielding medicinal plant from India. *IJTK.* 2018. 17 (1): 148-154.
- [11] Suryawanshi S, Kamble P, et al. Parkinsonism and Potential of *Mucuna* Beans. In: F. Heston T, Ray S, editors. *Bioethics in Medicine and Society.* IntechOpen; 2021.
- [12] Tse GG, Kim BB, et al. Case of levodopa toxicity from ingestion of *Mucuna gigantea*. *Hawaii J Med Public Health.* 2013;72: 157–160.
- [13] Rai SN, Chaturvedi VK, et al. *Mucuna pruriens* in Parkinson's and in some other diseases: recent advancement and future prospective. *3 Biotech.* 2020;10: 522.
- [14] Tse GG, Kim BB, et al. Case of levodopa toxicity from ingestion of *Mucuna gigantea*. *Hawaii J Med Public Health.* 2013;72: 157–160.
- [15] Johnson ME, Bobrovskaya L. An update on the rotenone models of Parkinson's disease: Their ability to reproduce the features of clinical disease and model gene–environment interactions. *Neurotoxicol.* 2015;46: 101–116.
- [16] Ogunraku OO, Ogunyemi BO, et al. Modulation of dopamine metabolizing enzymes and antioxidant status by *Capsicum annuum* Lin in rotenone-intoxicated rat brain. *Toxicology Rep.* 2019;6: 795–802.
- [17] Ablat N, Lv D, et al. Neuroprotective effects of a standardized flavonoid extract from safflower against a rotenone-induced rat model of parkinson's disease. *Molecules.* 2016;21: 1107.
- [18] Ohkawa H, Ohishi N, et al. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979; 95: 351–358.
- [19] Aebi H. Catalase in vitro. *Methods in Enzymology.* Elsevier; 1984. pp. 121–126.
- [20] Fothergill-Misbah N, Maroo H, et al. Could *Mucuna pruriens* be the answer to Parkinson's disease management in sub-Saharan Africa and other low-income countries worldwide? *Park Relat Disord.* 2020;73: 3–7.
- [21] Radad K, Al-Shraim M, et al. Rotenone: from modelling to implication in Parkinson's disease. *Folia Neuropathol.* 2019;57: 317–326.
- [22] Troshev D, Berezchnoy D, et al. The dynamics of nigrostriatal system damage and neurobehavioral changes in the rotenone rat model of Parkinson's disease. *Brain Res Bull.* 2021;173: 1–13.
- [23] Kumar S, Kumar S, et al. Trans-cinnamaldehyde mitigates rotenone-induced neurotoxicity via inhibiting oxidative stress in rats. *Pharmacol. Res. Mod. Chin. Med.* 2023;6: 100209.
- [24] Ma K, Xiong N, et al. Weight loss and malnutrition in patients with parkinson's disease: current knowledge and future prospects. *Front Aging Neurosci.* 2018; 19(10):1.
- [25] Fagerberg P, Klingelhoefer L, et al. Lower energy intake among advanced vs. early parkinson's disease patients and healthy controls in a clinical lunch setting: a cross-sectional study. *Nutrients.* 2020;12: 2109.
- [26] Baik J-H. Dopaminergic control of the feeding circuit. *Endocrinol Metab.* 2021;36: 229–239.
- [27] Alberico SL, Cassell MD, et al. The vulnerable ventral tegmental area in Parkinson's disease. *Basal Ganglia.* 2015;5: 51–55.
- [28] Sharma N, Khurana N, et al. Pharmacological evaluation of vanillic acid in rotenone-induced Parkinson's disease rat model. *Eur J Pharmacol.* 2021;903: 174112.
- [29] Sanberg PR, Ossenkopp K-P, et al. Motor activity and movement disorders: research issues and applications. totowa, NJ: Humana Press; 1996.
- [30] Taherianfard M, Riyahi M, et al. The cataleptic, asymmetric, analgesic, and brain biochemical effects of parkinson's disease can be affected by *Toxoplasma gondii* infection. *BioMed Res Intern.* 2020; 2020: 2546365.
- [31] Magrinelli F, Picelli A, et al. Pathophysiology of motor dysfunction in parkinson's disease as the rationale for drug treatment and rehabilitation. *Parkinson Dis.* 2016;2016: 1–18.
- [32] Hong M, Perlmutter JS, et al. Enhancement of rigidity in Parkinson's disease with activation. *Mov Disord.* 2007;22: 1164–1168.
- [33] Frequin HL, Schouten J, et al. Levodopa response in patients with early parkinson disease: further observations of the leap study. *Neurology.* 2023;100.
- [34] Sharma N, Jamwal S, et al. Beneficial effect of antidepressants against rotenone induced Parkinsonism like symptoms in rats. *Pathophysiol.* 2016;23: 123–134.
- [35] Wang JY, Yang JY, et al. Neuroprotective Effect of Pseudoginsenoside-F11 on a Rat Model of Parkinson's Disease Induced by 6-Hydroxydopamine. *Evid Based Complement Alternat Med.* 2013;2013: 1–9.
- [36] Madiha S, Tabassum S, et al. Assessment of gait dynamics in rotenone-induced rat model of Parkinson's disease by footprint method. *Pak J of Pharm Sci.* 2017;30: 943–948.
- [37] Bryant MS, Rintala DH, et al. Gait variability in Parkinson's disease: influence of walking speed and dopaminergic treatment. *Neurologic Res.* 2011;33: 959–964.

- [38] Azimullah S, Meeran MFN, et al. Tannic acid mitigates rotenone-induced dopaminergic neurodegeneration by inhibiting inflammation, oxidative stress, apoptosis, and glutamate toxicity in rats. *Int J Mol Sci.* 2023;24: 9876.
- [39] Nandi A, Yan L-J, et al. Role of catalase in oxidative stress- and age-associated degenerative diseases. *Oxid Med Cell Longev.* 2019;2019: 9613090.
- [40] Chu C, Yu L, et al. *Lactobacillus plantarum* CCFM405 against rotenone-induced Parkinson's Disease mice via regulating gut microbiota and branched-chain amino acids biosynthesis. *Nutrients.* 2023;15: 1737.
- [41] Zhang T, Mo H. Reinforcement learning for robot research: A comprehensive review and open issues. *Int J Adv Robot Syst.* 2021;18: 17298814211007305.
- [42] Zhang J, Chi R, et al. Preoperative administration of branched-chain amino acids reduces postoperative insulin resistance in rats by reducing liver gluconeogenesis. *Nutr Metab.* 2022;19: 78.
- [43] Badawi GA, Abd El Fattah MA, et al. Sitagliptin and liraglutide modulate l-dopa effect and attenuate dyskinetic movements in rotenone-lesioned rats. *Neurotox Res.* 2019;35: 635–653.