Research Article

Neuroprotective effects of *Madhuca longifolia* leaf extract and voglibose in a rotenone-induced rat model of Parkinson's disease

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ABSTRACT

Degeneration of dopaminergic neurons in the substantia nigra pars compacta, followed by dopamine depletion in the striatum, causes Parkinson's disease (PD), a progressive neurodegenerative disease that results in both motor and non-motor impairments. The purpose of this study was to examine the neuroprotective effects of Voglibose and *Madhuca longifolia* leaf extract in an animal model of Parkinson's disease. The rats were induced with Parkinsonism using rotenone (1.5 mg/kg, s.c) for 28 days and assessed behaviorally through the Open Field Test, Rotarod, and grip strength tests. Rats were treated with *M. longifolia* leaf extract (200 and 500 mg/kg, p.o.) and Voglibose (10 mg/kg, p.o.) individually and combined. Following the treatment schedule, biochemical estimations were conducted using brain homogenates to assess markers of oxidative stress, such include catalase, reduced glutathione, and lipid peroxidation (LPO). Histopathology was then undertaken on the striatum and substantia nigra and involved observing the brains of the rats for neuronal integrity. Overall, *M. longifolia* and Voglibose treatment improved behavioral activity, reduced oxidative stress (through reducing LPO – increasing antioxidant enzymes), and preserved neuronal architecture. For these reasons, it suggests that *M. longifolia* and Voglibose have neuroprotective roles and represent alternative therapeutic prospects for researching the management of PD.

Keywords: *Madhuca longifolia*, neuroprotective effect, Parkinson's disease, rotenone, voglibose

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder that causes bradykinesia, rigidity, resting tremors, and postural instability, among other motor deficits, by gradually destroying dopaminergic neurons in the substantia nigra pars compacta. These symptoms significantly affect daily functioning and quality of life.[1] Globally, in 2019, PD impacted more than 8.5 million people, with projections suggesting more than 17 million cases by 2024. While men are generally at higher risk, regional variations exist due to genetics, lifestyle, environmental exposures, and healthcare access.[2] Although the precise etiology of PD still unknown, number of factors, such as genetic predisposition, mitochondrial dysfunction, oxidative stress, environmental toxins, and neuroinflammation, have been implicated in its pathogenesis.[3]

The hallmark pathological features of PD are the development of intracellular Lewy bodies made mostly of aggregated α-synuclein and the selective degeneration of dopaminergic neurons in the substantia nigra.[4] Oxidative stress is major factor in neurodegeneration. It arises when reactive oxygen species (ROS) generation exceeds the capacity of endogenous antioxidant systems, causing DNA damage, protein oxidation, lipid peroxidation (LPO), and impaired cellular function.[5] Mitochondrial dysfunction, particularly inhibition of the electron transport chain's complex I, is a significant contributor to ROS production.[6] Dysregulated dopamine metabolism further

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exacerbates oxidative stress, as excess cytosolic dopamine undergoes enzymatic or auto-oxidation to form dopamine quinones that covalently modify key proteins, such as parkin, α-synuclein, DJ-1, and UCH-L1, impairing proteostasis and promoting neuronal death.[7] Vesicular monoamine transporter 2 sequesters dopamine into synaptic vesicles, reducing cytosolic ROS formation and protecting neurons from oxidative injury.[8]

Neuroinflammation is another important factor in PD progression. Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-6, and interferon gamma are released by activated microglia, resulting in a vicious cycle of oxidative stress and neuronal damage.[9] Dopaminergic degradation is accelerated by persistent neuroinflammation in the substantia nigra and striatum.[10] Apoptotic cell death, triggered by oxidative stress, mitochondrial dysfunction, and adenosine triphosphate (ATP) depletion, contributes further to progressive neuronal loss, with caspase-mediated pathways and the balance of pro- and anti-apoptotic genes determining the susceptibility of dopaminergic neurons.[11]

Neurotoxin-based models of PD are widely employed to study its pathogenesis and evaluate potential neuroprotective strategies. Dopaminergic neurodegeneration is caused by toxins such 6-hydroxydopamine, MPTP, rotenone, and paraquat through glial activation, mitochondrial inhibition, oxidative stress, and proteasomal dysfunction.[12] For example, after crossing the blood-brain barrier, MPTP is converted to MPP+, which is taken up by dopamine transporters, causing selective SNpc degeneration and motor deficits.[13] Rotenone, a lipophilic insecticide, inhibits mitochondrial complex I, increases ROS production, reduces dopamine and glutathione (GSH) levels, and leads to progressive dopaminergic neuron loss.[14] Paraquat generates intracellular ROS through redox cycling and disrupts antioxidant defense mechanisms, further contributing to dopaminergic cell death.[15] These models have been instrumental in understanding PD mechanisms and testing potential therapeutic agents.

Despite advances in understanding its pathophysiology, PD remains incurable. The main goal of current therapy is to control symptoms rather than stop the disease's progression. Levodopa, a dopamine precursor, is the best treatment for stiffness and bradykinesia, frequently used in conjunction with carbidopa to stop peripheral metabolism.[16] Catechol-O-methyltransferase inhibitors and dopamine agonists, including pramipexole and ropinirole, like entacapone, enhance dopaminergic signaling and prolong levodopa's efficacy [40-42]. However, these therapies do not address the underpinning neurodegenerative mechanisms, emphasizing the necessity for novel neuroprotective interventions.[17]

Medicinal plants have long been recognized as bioactive compounds source that may have potential therapeutic benefits. *Madhuca longifolia*, traditionally used in southern India for various ailments including headaches, snakebites, and gastrointestinal disorders, contains phytochemicals such as myricetin, quercetin, β-carotene, oleanolic acid, and β-sitosterol, which have anti-inflammatory and antioxidant properties relevant to neuroprotection.[18] These compounds control inflammatory mediators such nuclear factor-kappa B, TNF-α, IL-6, IL-1β, and caspase-3, scavenge free radicals, and reduce oxidative stress, thereby helping preserve neuronal integrity.[19] Similarly, voglibose, an α-glucosidase inhibitor with established antidiabetic effects, has shown potential in neuroprotection by reducing oxidative stress and suppressing pro-inflammatory cytokines, suggesting its dual role in metabolic and neuronal health.[20] Combined evaluation of *M. longifolia* and voglibose in PD models may provide insight into novel strategies for slowing neurodegeneration and improving functional outcomes in Parkinson's The disease.[21]

Materials and Methods

Materials

Rotenone, Levodopa-Carbidopa, Voglibose, ethanol, hydrogen peroxide (H_2O_2), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), phosphate buffer, nitrobluetetrazolium, ethylenediaminetetraacetic acid, hydroxylamine hydrochloride, trichloroacetic acid (TCA), thiobarbituric acid (TBA), α-naphthol solution, behaviour acid (H_2SO_4), Benedict's reagent, Wagner's reagent, Mayer's reagent, ferric chloride, iodine solution, and magnesium turnings were procured from standard commercial suppliers. All reagents used were of analytical grade.

Instruments

Behavioral studies were conducted using a rota-rod, grip strength meter, and open field apparatus. Additional equipment included an electronic balance, hot-air oven, ultraviolet-visible spectrophotometer, centrifuge, enzyme-linked immunosorbent assay (ELISA) kit, and Maze-Master software.

Experimental animals

Healthy adult Wistar rats (200–250 g, 3–4 months old, both sexes) were obtained from the National Institute of Bioscience (Pune, India). Animals ($n = 30$) were housed under controlled conditions (22 ± 2°C, 50–60% humidity, 12 h light/dark cycle) with ad libitum access to a standard pellet diet and water. Experimental protocols followed CPCSEA guidelines (Reg. No. 2117/PO/RcBiBt/S/20/CPCSEA), and all efforts were made to minimize animal suffering.

Preparation of extract and phytochemical screening

Fresh leaves of *M. longifolia* were shade dried (10–15 days), powdered, and extracted with ethanol using Soxhlet extraction (60 g of leaf powder in 300 mL ethanol, 7 h). The solvent was evaporated at room temperature to yield 4 g of concentrated extract (6.67% yield), which was stored at 4°C. Preliminary phytochemical screening was performed using standard qualitative tests to identify flavonoids, alkaloids, phenols, saponins, proteins, carbohydrates, steroids, and terpenoids.

Induction of PD

In this study, PD was modeled in rodents using subcutaneous rotenone (1.5 mg/kg), a widely accepted approach that replicates

the neurodegenerative features of idiopathic PD. Rotenone, a natural pesticide and mitochondrial complex I inhibitor, impairs ATP production and increases ROS, leading to oxidative stress, inflammation, and dopaminergic neuron degeneration in the substantia nigra pars compacta. It also promotes α-synuclein aggregation, contributing to progressive motor deficits. This model reliably mirrors the biochemical, behavioral, and histological characteristics of PD, making it suitable for evaluating pharmacological interventions.

Experimental design

Thirty-five rats were randomly divided into seven groups ($n = 5$ each): Group I (normal saline control), Group II (rotenone, negative control), Group III (Levodopa-Carbidopa + rotenone, positive control), Group IV (low-dose *M. longifolia* extract, 200 mg/kg + rotenone), Group V (high-dose *M. longifolia* extract, 500 mg/kg + rotenone), Group VI (Voglibose 10 mg/kg + rotenone), and Group VII (combination therapy with Voglibose 10 mg/kg + *M. longifolia* extract 500 mg/kg + rotenone). Treatments were administered orally for 28 days. Behavioral assessments were performed on days 7, 14, 21, and 28, followed by biochemical, neuroinflammatory, and histological evaluations [Table 1].

Behavioral assessments

Motor coordination, grip strength, and locomotor activity were evaluated using the rotarod, grip strength, and open field tests, respectively. For the rotarod test, pre-trained rats were placed on a 7 cm-diameter rod rotating at 25 rpm, and fall latency was recorded with a 180 s cutoff. Forelimb grip strength was measured using a digital grip force meter by allowing rats to grasp the grid and gently pulling to record force in Kgf. Locomotor activity was assessed in an 80 × 80 × 40 cm arena divided into 16 squares, where rats were placed at the center for 5 min, and behaviors such as paw movement latency, rearing, grooming, and total distance traveled were recorded through overhead video.

Biochemical analyses

At the end of the treatment period, rats were sacrificed, and brains were collected, homogenized in phosphate buffer (pH 7.4), and centrifuged at 10,000 rpm for 15 min at 4°C [90]. Biochemical assessments included reduced GSH, catalase (CAT), and LPO. GSH was measured by adding 0.5 mL supernatant to 0.25 M phosphate buffer (pH 7.4) and 0.04% DTNB solution, with absorbance read at 412 nm and expressed as nmol GSH/g tissue. CAT activity was determined by incubating supernatant with 10 mM H_2O_2 in phosphate buffer (pH 7) and measuring the decomposition rate at 240 nm, expressed as unit's/g tissue. LPO was assessed by incubating 0.5 mL homogenate with 15% TCA, 15% TBA, and 5 N HCl at 90°C for 20 min, centrifuging at 2,000 rpm for 15 min, and reading absorbance at 512 nm, expressed as nmol/mg tissue. All reagents were freshly prepared according to standard protocols.

Neuroinflammatory markers

IL-6 and TNF-α levels were estimated using commercially available ELISA kits (Krishegen Biosystem, India) following the manufacturer's protocol. All standards and samples were analyzed in duplicate, and a fresh standard curve was generated for each assay to ensure accuracy and reproducibility. Briefly, 100 µL of standards and test samples were added to the ELISA plate, followed by incubation with biotinylated detection antibody and Streptavidin-HRP conjugate under specified conditions. After successive wash steps, 100 µL of TMB substrate solution was added, and the plates were incubated in the dark. The reaction was terminated by adding stop solution, and absorbance was recorded at 450 nm using a microplate reader within 10–15 min.

Histopathological examination

Following sacrifice, brains were excised, and the midbrain region was isolated for histopathological analysis. Transverse sections (7–9 µm) were fixed in formalin, embedded in paraffin, and subjected to hematoxylin and eosin staining. Briefly, sections were dewaxed in xylene, rehydrated through graded alcohol series, stained with hematoxylin, differentiated in 90% alcohol, counterstained with 1% eosin, dehydrated, and cleared in xylene. The stained sections were examined under a light microscope for histopathological alterations.

Statistical analysis

Data are expressed as mean ± scanning electron microscope (SEM). Statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by appropriate *post hoc* tests. Differences were considered statistically significant at $P < 0.05$.

Results

Behavioral assessment

Rota-rod test

Rotenone-treated rats exhibited a progressive decline in motor coordination, with a significant reduction in rotarod fall-off time

Table 1: Experimental design

Groups	Description	Treatment
I	Control group	Received normal saline (5 mL/kg) for 28 days
II	Negative control group	Induced with Rotenone (1.5 mg/kg S.C) for 28 days
III	Positive control group	Received standard drug Levodopa-Carbidopa (10 mg/kg oral) + Rotenone (1.5 mg/kg) for 28 days
IV	Low dose extract	Received *Madhuca longifolia* extract (200 mg/kg oral) + Rotenone (1.5 mg/kg) for 28 days
V	High-dose extract	Received *Madhuca longifolia* extract (500 mg/kg oral) + Rotenone (1.5 mg/kg) for 28 days
VI	Voglibose group	Received Voglibose (10 mg/kg oral) + Rotenone (1.5 mg/kg) for 28 days
VII	Combination group	Received Voglibose (10 mg/kg) and *Madhuca longifolia* (500 mg/kg) + Rotenone (1.5 mg/kg) for 28 days

observed by day 28 compared to control rats (Negative Control: 77 ± 2.6 s on day 7 to 28 ± 2.8 s on day 28 vs. Control: 105 ± 2.3 s to 104 ± 2.4 s). Treatment with *M. longifolia* extract (200 and 500 mg/kg), voglibose (10 mg/kg), their combination (500 mg/kg), and levodopa/carbidopa (10 mg/kg), administered 1 h before rotenone, significantly attenuated this decline. Fall-off times in treated groups were higher compared to the negative control across all time points (e.g., 500 mg/kg *M. longifolia*: 103 ± 2.2 s to 78 ± 2.5 s; combination: 104 ± 2.1 s to 90 ± 2.4 s), comparable to the standard treatment (102 ± 2.1 s to 88 ± 2.6 s) [Figure 1].

Grip strength test

Rotenone infusion significantly reduced forelimb grip strength in rats compared to the vehicle-treated control group, reflecting neuromuscular impairment (Day 7: 0.25 ± 0.1 kg; Day 14: 0.15 ± 0.23 kg; Day 21: 0.14 ± 0.5 kg; Day 28: 0.10 ± 0.3 kg vs. control: Day 7: 0.68 ± 0.2 kg; Day 14: 0.7 ± 0.3 kg; Day 21: 0.8 ± 0.1 kg; Day 28: 0.7 ± 0.1 kg) (***$P < 0.001$). Treatment with *M. longifolia* extract (200 mg/kg and 500 mg/kg), voglibose, and their combination significantly improved grip strength in a time-dependent manner across all observation days (*$P < 0.05$ to ***$P < 0.001$ vs. negative control). The effect of *M. longifolia* was dose-dependent, with the 500 mg/kg dose exhibiting greater efficacy than the 200 mg/kg dose. Notably, the combination of *M. longifolia* (500 mg/kg) with voglibose produced the most substantial improvement in gripping ability by Day 28 (0.39 ± 0.1 kg), suggesting an enhanced neuroprotective effect. These results indicate that both *M. longifolia* and voglibose, individually and in combination, effectively mitigate rotenone-induced neuromuscular deficits in rats [Table 2].

Table 2 presents the effects of *Madhuca longifolia* extract, voglibose, and their combination on forelimb grip strength in rotenone-treated rats. Grip strength (kg) was recorded on Days 7, 14, 21, and 28. Data are expressed as mean ± SEM ($n = 5$) and analyzed using one-way ANOVA followed by Tukey's post hoc test. Rotenone administration significantly reduced grip strength compared to the control group (***$P < 0.001$). Treatment with M. longifolia extracts (200 mg/kg and 500 mg/kg), voglibose, and their combination significantly restored grip strength (*$P < 0.05$ to ***$P < 0.001$ vs. negative control), demonstrating their protective effect against rotenone-induced neuromuscular deficits.

Open field test

Rats infused with rotenone exhibited a significant decline in locomotor activity, as reflected by reduced distance travelled, rearing frequency, and line crossings in the open field test compared to the vehicle-treated control group (Day 7: 6.16 ± 0.3 m vs. 11.65 ± 0.4 m; Day 14: 4.75 ± 0.2 m vs. 14.4 ± 0.3 m; Day 21: 3.39 ± 0.2 m vs. 14.1 ± 0.3 m; Day 28: 1.94 ± 0.1 m vs. 14.53 ± 0.4 m) (***$P < 0.001$), indicating impaired motor function and heightened anxiety-like behavior.

Treatment with *M. longifolia* extract (200 mg/kg and 500 mg/kg), voglibose, and their combination significantly enhanced locomotor performance and exploratory behavior over the 28-day study period (*$P < 0.05$ to ***$P < 0.001$ vs. negative control). The effect of *M. longifolia* was both dose- and time-dependent, with the 500 mg/kg dose producing superior improvements in distance travelled compared to the 200 mg/kg dose. Notably, the combination of *M. longifolia* (500 mg/kg) and voglibose demonstrated the most pronounced enhancement in open field activity (Day 28: 7.3 ± 0.3 m), indicating an additive protective effect against rotenone-induced motor deficits and neurotoxicity [Figure 2].

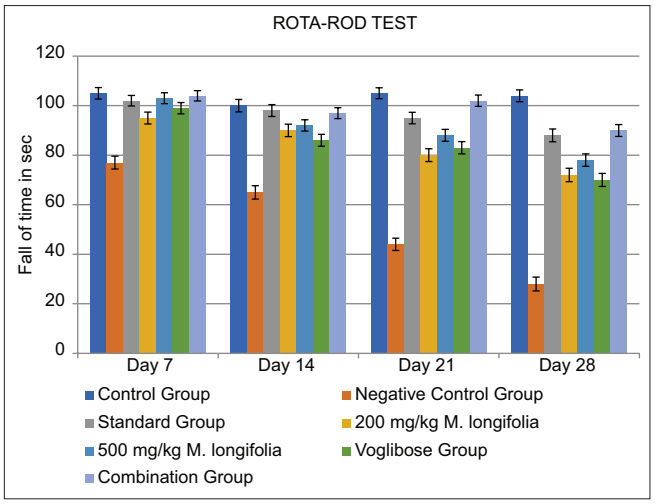

Figure 1: Effect of *Madhuca longifolia* extract, voglibose, and their combination on motor coordination in rotenone-treated rats. Rotarod fall-off time (in seconds) was measured on days 7, 14, 21, and 28. Data are expressed as mean ± SEM ($n = 5$) and analyzed using one-way ANOVA followed by Tukey's *post hoc* test. Rotenone treatment significantly reduced fall-off time compared to the control group (***$P < 0.001$). Administration of standard drug, *M. longifolia* extract (200 mg/kg and 500 mg/kg), voglibose, and their combination significantly increased fall-off time compared to the negative control group (**$P < 0.01$, *$P < 0.05$), indicating protective effects on motor coordination. SEM: Scanning electron microscope, ANOVA: Analysis of variance

Table 2: Effect of *Madhuca longifolia* and Voglibose treatment on grip strength activity in rotenone-treated rats

Sr. No.	Groups	Grip strength in kg			
		Day 7	Day 14	Day 21	Day 28
1	Control group	0.68±0.2	0.7±0.3	0.8±0.1	0.7±0.1
2	Negative group	0.25±0.1***	0.15±0.23	0.14±0.5	0.10±0.3
3	Standard group	0.56±0.3***	0.48±0.21***	0.42±0.5***	0.39±0.4***
4	200 mg/kg extract	0.50±0.21**	0.44±0.2**	0.36±0.3**	0.32±0.3**
5	500 mg/kg extract	0.54±0.36**	0.42±0.3**	0.34±0.1**	0.35±0.1**
6	Voglibose group	0.46±0.1*	0.34±0.1*	0.31±0.12*	0.31±0.2*
7	Combination group	0.52±0.1***	0.46±0.4***	0.41±0.3***	0.39±0.1 ***

Biochemical parameters

In the present study, rotenone-treated rats showed a significant rise in malondialdehyde (MDA) levels (2.55 ± 0.4 nmol/g protein), indicating enhanced LPO, along with a decrease in

reduced GSH (3.79 ± 0.1 µmol/mg protein) and CAT activity (18.03 ± 0.5 µmol H_2O_2/min/mg protein) compared with the control group (5.04 ± 0.2 µmol/mg protein, 0.62 ± 0.1 nmol/g protein, and 29.0 ± 0.2 µmol H_2O_2/min/mg protein, respectively). Pretreatment with *M. longifolia* extract (200 and 500 mg/kg) or voglibose (10 mg/kg), administered 1 h before rotenone exposure, significantly mitigated these oxidative alterations. Both doses of *M. longifolia* extract reduced MDA (1.55 ± 0.12 and 1.10 ± 0.21 nmol/g protein) and improved GSH (4.90 ± 0.2 and 4.91 ± 0.2 µmol/mg protein) and CAT (22.46 ± 0.2 and 23.91 ± 0.4 µmol H_2O_2/min/mg protein) activities. Voglibose alone also exhibited antioxidant activity, restoring GSH (4.60 ± 0.3 µmol/mg protein) and CAT (23.6 ± 1.0 µmol H_2O_2/min/mg protein) with reduced MDA (1.6 ± 0.3 nmol/g protein). The combination of *M. longifolia* (500 mg/kg) with voglibose produced the most prominent neuroprotective effect, maintaining near-normal GSH (4.93 ± 0.1 µmol/mg protein) and CAT (26.02 ± 0.2 µmol H_2O_2/min/mg protein) levels and lowering MDA (1.25 ± 0.3 nmol/g protein), comparable to the standard group (4.94 ± 0.3 µmol/mg protein, 0.95 ± 0.1 nmol/g protein, and 26.30 ± 0.3 µmol H_2O_2/min/mg protein, respectively). These findings confirm that *M. longifolia*, particularly in combination with voglibose, effectively prevents rotenone-induced oxidative stress by enhancing antioxidant defense and reducing LPO in rat brain tissue [Table 3].

Effect of M. longifolia extract, voglibose, and their combination on oxidative stress parameters (GSH, LPO, and CAT) in rotenone-treated rats. Values are expressed as mean ± SEM ($n = 5$). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.**$P < 0.001$, *$P < 0.05$, **$P < 0.002$ versus Negative group; ***$P < 0.001$ versus control group.

Inflammatory mediators

In the present study, rotenone-treated rats exhibited a significant increase in pro-inflammatory cytokines, with IL-6 (140 ± 0.2 pg/mL) and TNF-α (145 ± 0.3 pg/mL) levels markedly elevated compared to the control group (57 ± 0.5 pg/mL and 42 ± 0.5 pg/mL, respectively), indicating pronounced neuroinflammation. Pretreatment with *M. longifolia* extract at 200 mg/kg and 500 mg/kg, voglibose, or their combination administered 1 h before rotenone exposure significantly attenuated these increases. The 200 mg/kg extract reduced IL-6 and TNF-α to 109 ± 0.5 pg/mL and 106 ± 0.3 pg/mL, respectively, while the 500 mg/kg extract further decreased them to 104 ± 0.2 pg/mL and 90 ± 0.2 pg/mL. Voglibose alone lowered IL-6 to 114 ± 0.5 pg/mL and TNF-α to 96 ± 0.5 pg/mL, whereas the combination of *M. longifolia* (500 mg/kg) with voglibose produced the most pronounced anti-inflammatory effect, reducing IL-6 and TNF-α levels to 102 ± 0.2 pg/mL and 80 ± 0.2 pg/mL, respectively, comparable to the standard treatment group (92 ± 0.5 pg/mL and 72 ± 0.5 pg/mL). These results demonstrate that *M. longifolia*, particularly in combination with voglibose, effectively mitigates rotenone-induced neuroinflammation in rat brain tissue [Table 4].

Data are presented as mean ± SEM ($n = 5$). IL-6 levels were significantly elevated in the negative (rotenone-treated) group compared to the control group (***$P < 0.001$). Standard treatment and all other treatment groups (*M. longifolia* 200 and 500 mg/kg, voglibose, and the combination) significantly reduced IL-6 levels compared to

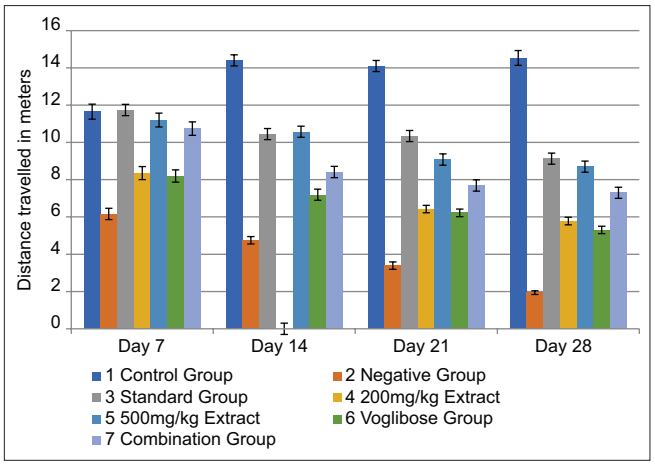

Figure 2: Effect of *Madhuca longifolia* extract, voglibose, and their combination on locomotor activity (open field test) in rotenone-treated rats. Distance travelled (in meters) was recorded on days 7, 14, 21, and 28. Data are presented as mean ± SEM ($n = 5$) and analyzed using one-way ANOVA followed by Tukey's *post hoc* test. Rotenone-treated rats showed a significant reduction in locomotor activity compared to the control group (***$P < 0.001$), indicating impaired movement due to neurotoxicity. Treatment with standard drugs, *M. longifolia* extracts (200 mg/kg and 500 mg/kg), voglibose, and their combination significantly improved locomotor performance (*$P < 0.05$ to ***$P < 0.001$ vs. negative control), suggesting protective effects against rotenone-induced motor dysfunction. SEM: Scanning electron microscope, ANOVA: Analysis of variance

Table 3: Effect of *Madhuca longifolia* and Voglibose treatment on oxidative marker in rotenone-treated rats

Sr. No.	Groups	GSH (µmol/mg protein)	Lipid peroxidation (nmol/g protein)	Catalase (µmol H_2O_2/min/mgprotein)
1	Control group	5.04±0.2	0.62±0.1	29.0±0.2
2	Negative group	3.79±0.1**	2.55±0.4***	18.03±0.5***
3	Standard group	4.94±0.3**	0.95±0.1***	26.30±0.3***
4	200 mg/kg extract	4.90±0.2*	1.55±0.12**	22.46±0.2**
5	500 mg/kg extract	4.91±0.2**	1.1±0.21***	23.91±0.4***
6	Voglibose group	4.60±0.3*	1.6±0.3**	23.6±01**
7	Combination group	4.93±0.1**	1.25±0.3***	26.02±0.2***

GSH: Glutathione

Table 4: Inflammatory mediators

Sr. No.	Groups	IL-6	TNF-α
1	Control group	57±0.5	42±0.5
2	Negative group	140±0.2***	145±0.3***
3	Standard group	92±0.5**	72±0.5***
4	200 mg/kg extract	109±0.5*	106±0.3*
5	500 mg/kg extract	104±0.2**	90±0.2**
6	Voglibose group	114±0.5	96±0.5**
7	Combination group	102±0.2**	80±0.2***

IL-6: Interleukin-6, TNF-α: Tumor necrosis factor-alpha

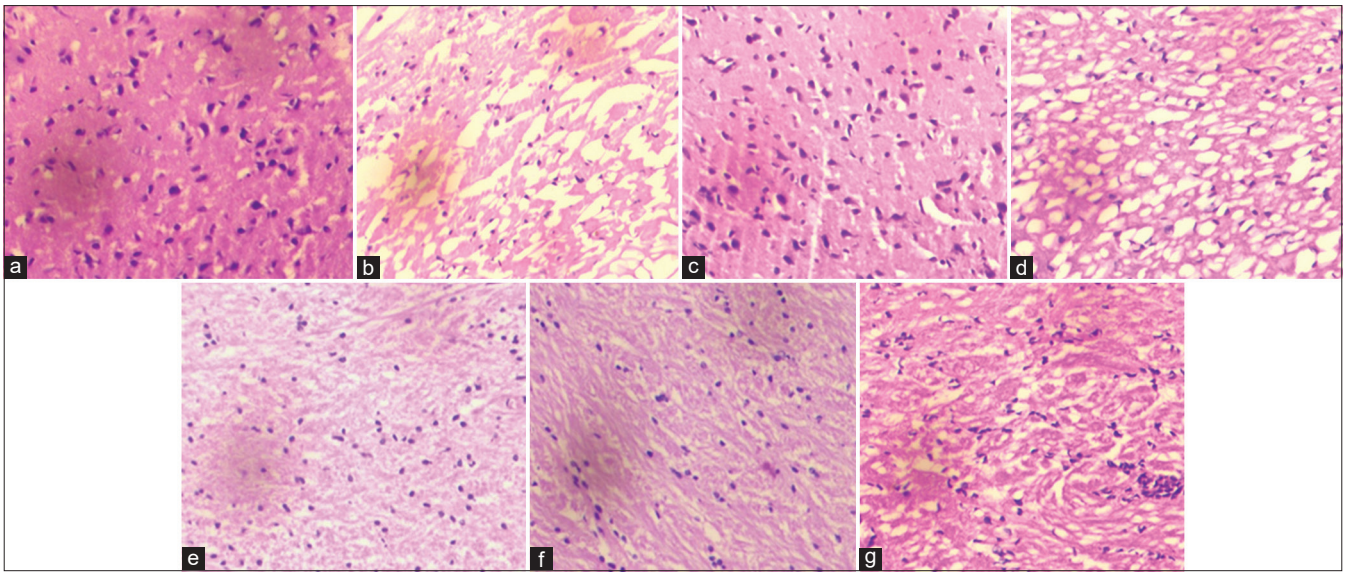

Figure 3: Histopathological study of midbrain of Control (a), rotenone-treated negative group (b), standard drug-treated group (c), 200 mg/kg *Madhuca longifolia* extract-treated group (d), 500 mg/kg *M. longifolia* extract-treated group (e), voglibose-treated group (f), and combination of *M. longifolia* (500 mg/kg) with voglibose-treated group (g) was carried out. Observations were done under a light microscope at ×40 magnification

the negative group (*$P < 0.05$, **$P < 0.01$), demonstrating anti-inflammatory effects. TNF-α levels were also markedly increased in the negative group compared to control (***$P < 0.001$). Standard treatment significantly decreased TNF-α levels (***$P < 0.001$), while treatment with *M. longifolia* extract at 200 mg/kg (*$P < 0.05$) and 500 mg/kg (**$P < 0.01$), voglibose (**$P < 0.01$), and the combination (***$P < 0.001$) significantly reduced TNF-α levels compared to the negative group. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test.

Histopathological evaluation

Histopathological analysis of the midbrain revealed no neuronal damage in the vehicle-treated control group. In contrast, rotenone-treated rats exhibited marked neuronal cell damage, indicating neurodegeneration. Co-treatment with standard drugs notably reduced neuronal damage compared to the rotenone group. *M. longifolia* extract demonstrated a dose-dependent neuroprotective effect, with 500 mg/kg showing greater preservation of neuronal architecture than 200 mg/kg. Voglibose treatment also ameliorated neuronal damage relative to the rotenone-treated group. The combination of *M. longifolia* (500 mg/kg) with voglibose provided the most pronounced neuroprotection, showing minimal neuronal damage and preservation of normal neuronal morphology [Figure 3].

Discussion

In a rotenone-induced rat model of Parkinson's disease, the present study aimed to evaluate the neuroprotective potential of both Voglibose and *M. longifolia* extract, individually and in combination. As observed by reduced performance on the rotarod, grip strength meter, and open field tests, rotenone treatment significantly affected motor coordination, muscle strength, and exploratory behavior. Due to dopaminergic neuronal loss, these behavioral abnormalities mirror the classic motor signs of PD.[22] Notably, these impairments were significantly reduced by therapy with *M. longifolia* (200 and 500 mg/kg), voglibose, and particularly their combination, suggesting a restoration of neuromuscular function and motor activity.[23] These results were further corroborated by biochemical analyses. Elevated MDA levels and decreased GSH and CAT activity were indicative of rotenone-induced oxidative stress.[24] Particularly with the greater dose of extract and the combination treatment, treatment groups showed a considerable improvement in GSH and CAT levels and a significant decrease in MDA, indicating strong antioxidant activity. IL-6 and TNF-α, two neuroinflammatory indicators, were likewise extremely higher in the group of rotenone but much lower after therapy; the combination group's levels were similar to those of the standard group.[25] The rotenone group's midbrain showed significant neuronal damage, according to histopathological analysis, but the treated groups, particularly the combination group – showed low neuronal loss and preserved neuronal integrity.[26] Together, these findings demonstrate that voglibose and *M. longifolia* provide neuroprotection through their anti-inflammatory and antioxidant processes, and that their combination provides increased effectiveness in reducing Parkinsonian symptoms in this animal model.[27]

Conclusion

This study demonstrates that *M. longifolia* extract and Voglibose, individually and in combination, exert notable neuroprotective effects in a rotenone-induced rat model of PD. The combination therapy produced the greatest benefits, improving motor function, enhancing antioxidant defenses, reducing neuroinflammation and oxidative stress, and preserving neuronal integrity. These findings suggest a synergistic interaction that holds potential as a therapeutic approach for PD, though to elucidate the underlying molecular mechanisms and long-term effectiveness, more research is required.

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