ORIGINAL ARTICLE

Fasudil Improves Motor Functions Against Rotenone-Induced Neurotoxicity in Rats

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Abstract

Background: Parkinson's disease (PD), the second most frequent neurodegenerative disease, involves the progressive loss of dopaminergic neurons in the substantia Nigra, resulting in motor dysfunction. Recently, Rho-associated protein kinase (ROCK) has been associated with Parkinson's pathology. Fasudil (FSD), a ROCK inhibitor, offers neuroprotection; however, its precise mechanism remains to be explored in the rotenone (ROT)-exposed animal model. **Objectives:** This study aimed to evaluate the therapeutic effectiveness of FSD in mitigating ROT -induced neurotoxicity by attenuating oxidative stress (OS) in a Parkinsonian rat model. **Materials and Methods:** Wistar rats were exposed to ROT (2.5 mg/kg; i.p.,) for 28 days to induce Parkinson's-like symptoms. Subsequently, FSD (10 mg/kg; p.o.) was administered daily for 28 days in the treatment group. The behavioral and biochemical assessments were conducted to evaluate motor functions, OS, dopamine (DA) levels, and mitochondrial functions. **Results:** Administration of ROT caused increased motor dysfunction and OS, along with a reduction in dopaminergic content. FSD treatment reversed the motor and biochemical impairments caused by ROT by restoring DA levels, enhancing enzymatic antioxidant activity, and reducing lipid peroxidation, thereby protecting dopaminergic neurons. **Conclusion:** The present study demonstrated that FSD has a neuroprotective effect in ROT -induced neurotoxicity, mediated by inhibiting the ROCK pathway, which results in the restoration of antioxidant and DA levels.

Key words: Dopamine, fasudil, oxidative stress, Parkinson's disease, rho-associated protein kinase, rotenone

INTRODUCTION

Parkinson's disease (PD) is the fastest-growing neurodegenerative disease, with a global burden projected to exceed 12 million cases by 2040, is characterized by akinesia, bradykinesia, postural instability, and muscular rigidity, primarily due to striatal dopamine (DA) depletion caused by dopaminergic neuronal loss.[1,2] The loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) is a key pathological feature of PD, resulting in up to an 80% decline in striatal DA levels.[3] Various etiological factors in PD, including oxidative stress (OS), mitochondrial dysfunction, neuroinflammation, cellular apoptosis, neurodegeneration, protein accumulation, and the generation of reactive oxygen species (ROS), collectively contribute to the progression of PD.[4] Multiple factors seem to work together to cause neurodegeneration rather than one. Moreover, long-term exposure to PD-related pharmacotherapeutic approaches, particularly allopathic medications, has been reported to cause severe side effects, often leading to complications in disease management.[5] Despite extensive research, the actual specific underlying cause of PD pathogenesis remains ambiguous. Consequently, the development of an alternative therapy for PD pharmacotherapy is necessary.

Emerging documented data suggest that increased OS significantly contributes to dopaminergic neuronal death by increased reactive nitrogen species generation, decreased cellular antioxidant defenses, and consequent mitochondrial damage, lipid peroxidation, protein oxidation, and which ultimately leads to neuronal apoptosis.[6] Lotharius and O'Malley suggested that defective mitochondrial complex-I could be the site for oxidative damage in DA

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Received: 08-08-2025
Revised: 20-09-2025
Accepted: 28-09-2025

metabolism.[7] and the generation of ROS during its complex cycles.[8,9] Moreover, reduced glutathione (GSH), superoxide dismutase (SOD), and increased metabolism of DA and malondialdehyde (MDA) could participate in neuronal death.[10]

Another study suggested that depleted GSH levels and ROS-mediated damage to the proteins, lipids, and DNA were found in the postmortem studies in PD-affected brains, indicating that the substantia nigra is at the level of OS.[11] Although various alternative options are available to mitigate the pathogenesis of PD, they have failed to do so. Rotenone (ROT) is a neurotoxin that inhibits mitochondrial complex-I activity, resulting in substantial mitochondrial damage in PD patients, and can replicate many PD-related pathological features, including oxidative damage, microglial activation, mitochondrial dysfunction, α-synuclein aggregation, iron accumulation, neurochemicals' depletion, substantial dopaminergic neuronal degeneration, and defective nigrostriatal pathway.[12] Moreover, long-term exposure to ROT has been shown to degenerate dopaminergic neurons in various experimental models.[13,14] Based on these studies, ROT is an invaluable tool for exploring novel neuroprotective agents in PD-like symptoms.

Rho-associated protein kinase (ROCK) has played an extensive role in PD pathophysiology.[15] It has been suggested that excessive ROCK expression in the central nervous system is responsible for generating OS, uncontrolled neuroinflammation, immune abnormalities, neuronal loss, and disrupted neurohumoral transmission, thereby promoting neurodevelopment disorders.[16] ROCK signaling may contribute to DA neuronal loss through the activation of microglial cells, the inhibition of cofilin, or the stimulation of endocytic trafficking.[17-19] Another study demonstrated that inhibition of the ROCK signaling pathway can lead to axonal regeneration, increased neurite outgrowth, the release of anti-inflammatory mediators, and cell survival.[20] It has been suggested that ROCK was acknowledged as a novel molecular target in neurological disorders, especially PD.[21-25] In addition, in cell culture and animal models, fasudil (FSD), a rho kinase inhibitor, has been shown to reduce the loss of dopaminergic cells.[24] Based on various clinical trials, several ROCK inhibitors have been reported to be effective in treating diseases, such as ophthalmological conditions,[26] cardiovascular diseases,[27] neurological disorders,[28] and cancers.[29] In addition, FSD also increases anti-inflammatory and antioxidant effects in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mouse model.[30] Furthermore, it has been observed that FSD also reduces the buildup of α-synuclein in a cell-based model.[31] Therefore, it can be assumed that FSD improves the PD-like symptoms in ROT-induced neurotoxicity in rats. Thus, the neuroprotective activity of FSD was evaluated against ROT -induced mitochondrial dysfunction, OS, and DA depletion in rats exhibiting PD-like symptoms.

MATERIALS AND METHODS

Experimental animals

Adult Wistar rats (male), weighing approximately 200 ± 30 g, were obtained from the Institute of Pharmaceutical Research, GLAU, Mathura, Uttar Pradesh, India. The animals were randomly divided into three groups, each containing six rats. During the experimental period, all the animals were housed in polyacrylic cages under standard conditions: a 12:12-h light/dark cycle, temperature maintained at 25 ± 2°C, and ambient humidity of 45–55%. The animals received a standard pellet diet and had free access to water throughout the study. All procedures adhered strictly to Committee for Control and Supervision of Experiments on Animals (CCSEA) guidelines and received approval from the Institutional Animal Ethics Committee (1260/PO/Re/S/09/CCSEA).

Chemicals and reagents

ROT, FSD, and all other analytical-grade chemicals and reagents were procured from Sigma (St. Louis, MO, USA) and Merck Pvt. Ltd. (New Delhi, India), respectively.

Experimental design

Figure 1 illustrates the 28-day experimental design, in which animals were assigned to three groups following a 1-week acclimatization period.

- Group I: Control
- Group II: ROT (2.5 mg/kg/day/b.w.; i.p.)
- Group III: ROT + FSD (10 mg/kg/day/b.w.; p.o.)

ROT was injected during the experimental protocol from D-1 to D-28 after preparation by dissolving in 100% DSMO (1 mL) and diluted in olive oil to get the final concentration of the solution of 0.5 mg/mL.[32] FSD was administered daily after ROT injection.[33] In the present experimental schedule, the control group received only a regular diet without any chemical treatment. All animals were subjected to behavioral and biochemical observations at the end of the experimental protocol. Biochemical parameters were measured from the dissected brain and were isolated from sacrificed animals by cervical dislocation.

Neurobehavioral tests

Narrow beam walk test

The narrow beam walk test assessed hind-limb impairment by evaluating the time taken to cross the beam, and the number of both hind-paw slips was noted. In this test, animals were allowed to cross a suspended beam, 100 cm long and 4 cm wide, for 120 s during a training session. The objective for the animals was to reach a dark target box, 25 × 20 × 18 cm, placed at the other end of the beam. After training, the

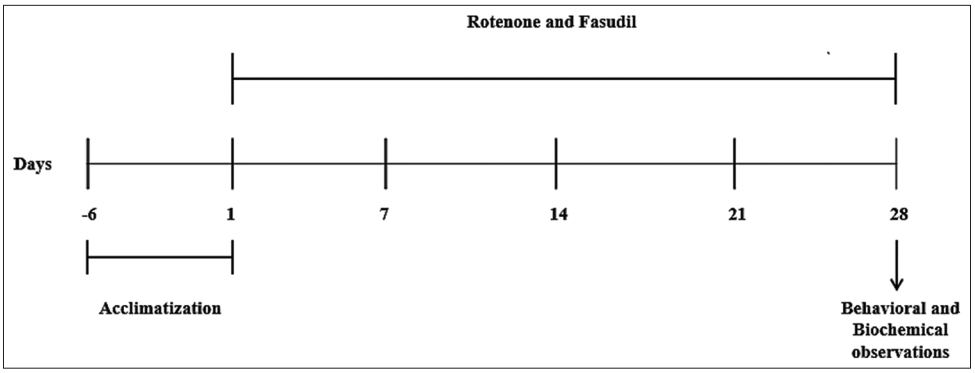

Figure 1: Illustration showing the detailed timeline of the experimental procedure

time taken to transverse a narrow beam with two endpoints was recorded to evaluate hind-limb impairment, and motor coordination was assessed accordingly.[34]

Footprint analysis test

Gait assessment was performed by measuring the stride length and base width of both sets of paws. To collect footprints, the rats' forepaws and hindpaws were painted with red and green-colored dye, and they were allowed to walk on a white sheet placed on the runway floor (100 × 10 × 20 cm). The footprints were analyzed by measuring two parameters: (i) Base width of both limbs and (ii) stride length of both limbs. For more accurate observations, a series of 3 consecutive trials was selected.[35]

Open field test

This test evaluated the rodents' locomotion, emotional state, and exploratory activity. Each animal was placed in the center of a specially constructed open-field box (dimensions: 53 × 53 × 60 cm), with its base partitioned into nine equally sized squares. Behavioral characteristics were recorded, including total distance travelled, immobility time, number of rearing, and number of lines crossed by hind limbs. Under efficient lighting conditions, all the selected parameters were recorded by using a camera to ensure precise observational data.[36]

Rota rod test

It is the most widely used behavioral test for evaluating motor coordination. Before the actual studies were performed, all rats were trained over 5 days to get familiar with the apparatus. This apparatus consists of a rotating, non-slippery rod that is electrically powered by a motor and rotates at speeds ranging from 4 to 40 rpm. Experimental animals were placed on the rod in the opposite direction to the rotating rod. Rats were allowed to sustain on the rotating rod for a maximum of 60 s while the floor sensors detected fall latency. For more accurate observations, a series of 3 consecutive trials was selected.[36]

Grip strength test

It is a commonly used method for monitoring the neuromuscular strength of animals. In this protocol, the animal was suspended by its forepaws on the center of a horizontally oriented metal wire (90 cm in length and 1 mm in diameter) to assess NM strength. The grip strength of the animal was evaluated based on the following scale: 0 – fell off immediately; 1 – hung using only forepaws; 2 – hung with forepaws + attempted to climb; 3 – hung with forepaws + one or both hind paws; 4 – hangs with all four limbs + tail wrapped around the wire; and 5 – escaped the wire and reached the support surface.[37,38]

Locomotor activity test

This test utilized an actophotometer apparatus to measure the locomotor activity of the animals. This apparatus consists of a rectangular cage equipped with infrared sensors or photoelectric cells along the wall. Each animal was placed in the apparatus, and the sensors detected the interruptions in the light beam as it crossed the photo beam. These beam interruptions were monitored over a duration of 5 min, and the total count of beam breaks was used to quantify locomotor activity.[39,40]

Biochemical estimations

Estimation of DA

To determine rats' brain DA level, 0.2 mL of the sample (aqueous phase) was mixed with 0.05 mL of 0.4 M HCl, 0.1 mL of ethylenediaminetetraacetic acid (EDTA) buffer (pH 6.9), and 0.1 mL of Na_2SO_3 solution. This mixture was kept aside for 1.5 min to facilitate the oxidation process, followed by the addition of 0.1 mL of CH_3COOH solution. After 6 min of boiling at 100°C, the solution was cooled to room temperature. At 350 nm, the absorbance of DA was measured.[41]

Estimation of SOD activity

To assess SOD activity, 20 µL of brain homogenate was added to a reaction mixture containing 75 mM Tris-HCl buffer (pH 8.2), 30 mM EDTA, and 2 mM pyrogallol. The rate of pyrogallol oxidation was monitored by measuring absorbance at 420 nm over a 3-min period. SOD activity was expressed as units per milligram of protein, with one unit defined as the enzyme quantity required to inhibit 50% of pyrogallol oxidation.[42]

Estimation of catalase (CAT) activity

Together with the 50 µL of brain supernatant, 2 mL of phosphate buffer (pH 7.4), and 1 mL of 30 mM H_2O_2 were also added to the cuvette for estimating CAT concentration. The enzymatic activity was assessed by monitoring the altered absorbance at 240 nm over a 1-min interval and expressed as µM/min./g protein.[43]

Estimation of lipid peroxidation (MDA estimation)

To measure the degree of lipid peroxidation, MDA is used as an indicator. MDA and thiobarbituric acid interact to generate a pink chromogenic adduct, observed at 535 nm, which serves as the basis for the test. During this method, tissue homogenates, including 0.5 mg protein and 20% trichloroacetic acid (0.5 mL) + 0.67% thiobarbituric acid (1 mL) were mixed and incubated for 1 h at 100°C. After cooling, the mixture was centrifuged, and the supernatant was collected and evaluated at 535 nm using a blank that contained all the reagents except the homogenates. MDA level was subsequently calculated and expressed as nM MDA/mg protein.[11]

Striatal mitochondria isolation protocol

The striatal tissue of the brain was excised and thoroughly rinsed with cold 0.9% saline to eliminate blood contamination before mitochondrial extraction. The tissue was homogenized in an isolation buffer containing 230 mM mannitol, 70 mM sucrose, 1.0 mM EDTA, and 10 mM Tris-HCl at pH 7.4. The homogenate was then centrifuged at 700 × g for 10 min at 4°C to remove nuclei and debris. The resulting supernatant was collected and spun at 8000 × g for 10 min at 4°C to obtain the mitochondrial pellet. The mitochondrial pellet was resuspended in the same buffer without EDTA, and the freshly isolated mitochondrial suspension was promptly used for the experiment.[44]

Cell viability assessment

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was used to assess mitochondrial metabolic activity in the isolated striatal mitochondrial fraction. An aliquot was placed in a 96-well plate, and 0.5 mg/mL of MTT prepared in phosphate-buffered saline (pH 7.4) was added to each well. The plate was incubated at 37°C for 4 h, during which mitochondrial dehydrogenases in viable cells reduced the yellow, water-soluble MTT to purple, insoluble formazan crystals. After incubation, 150 µL of dimethyl sulfoxide, was added to each well, and the optical density was subsequently recorded at 570 nm using an absorbance-based system. Cell viability was expressed as a percentage of the control group, indicating the metabolic activity under each condition.[45]

Statistical analysis

All data are expressed as mean ± standard error of the mean. Statistical evaluation was performed using GraphPad Prism version 5.01. One-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls *post hoc* test, was used to assess group differences. A $P < 0.05$ was considered statistically significant.

RESULTS

FSD restores behavioral alterations during the narrow beam walk test in ROT -treated rats

Figure 2 shows how FSD influences motor coordination and balance in rats during the narrow beam walk test, measured by the time to reach the target box (a), the number of left hind paw slips (b), and the number of right hind paw slips (c) in ROT-intoxicated animals. One-way ANOVA revealed significant differences in transfer latency ($F [2, 15] = 60.62$, $P < 0.05$) and the number of left hind-paw slips ($F [2, 15] = 98.08$, $P < 0.05$). However, no significant difference was observed in the number of right hind-paw slips among groups. The *post hoc* test showed that FSD improved the time to reach the goal box and decreased left hind paw slips compared to the ROT-intoxicated animals, indicating enhanced motor function.

FSD restores gait impairment during the footprint analysis test in ROT -treated rats

Figure 3 depicts the alterations in the gait pattern by the treatment of FSD, which were evaluated in terms of fore-paw base width (a), hind-paw base width (b), fore-paw stride length (c), and hind-paw stride length (d) in ROT-intoxicated animals. One-way ANOVA showed that there were notable differences in the fore-paw stride length ($F [2, 15] = 40.52$, $P < 0.05$) and hind-paw stride length ($F [2, 15] = 33.43$, $P < 0.05$). However, no significant differences were observed in the base width of both paws. The *post hoc* test indicated that FSD improved the stride lengths of both paws compared to the ROT group but had no effect on their base widths.

FSD restores the alterations by ROT during the open-field test

Figure 4 illustrates the effect of FSD on the following activities: Total distance travelled (a), immobility period (b), number of rearing (c), and the number of lines crossed by the hind-limb (d) during the open field test in ROT intoxicated animals. All treated groups significantly impacted the total

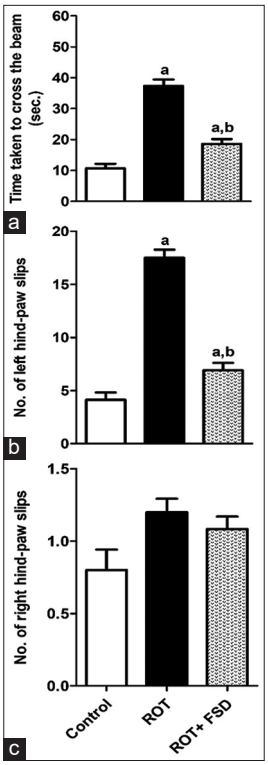

Figure 2: Effect of fasudil on the rotenone (ROT)-induced changes in transfer latency time to reach the target box (a), no. of left hind paw slips (b), and no. of right hind paw slips (c) of the animals during behavioral paradigms. All values are mean ± standard error of the mean ($n = 6$). $^{a}P < 0.05$ compared to Control, $^{b}P < 0.05$ compared to ROT (one-way analysis of variance followed by student–Newman–Keuls *post hoc* test)

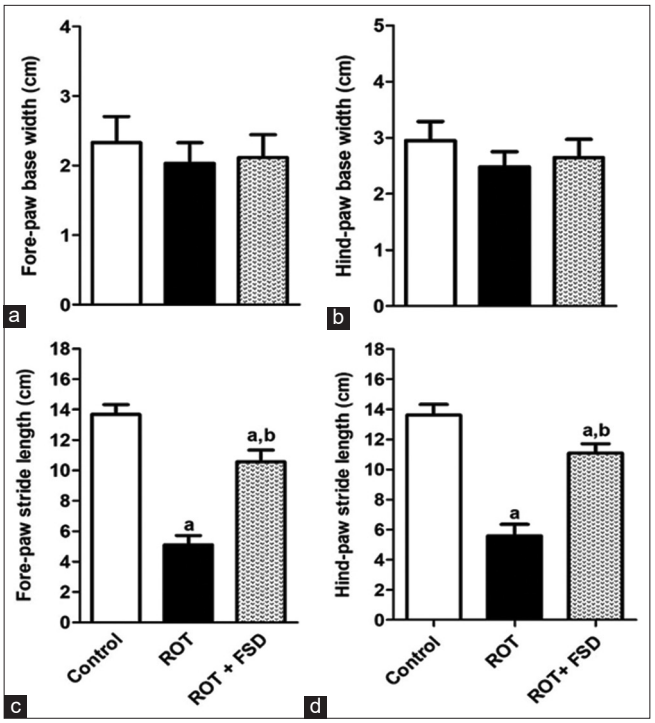

Figure 3: Effect of fasudil on the rotenone (ROT)-induced changes in the fore-paw base width (a), hind-paw base width (b), fore paw stride length (c), and hind-paw stride length (d) of the animals during behavioral paradigms. All values are mean ± standard error of the mean ($n = 6$). $^{a}P < 0.05$ compared to control, $^{b}P < 0.05$ compared to ROT (one-way analysis of variance followed by student–Newman–Keuls *post hoc* test)

distance traveled (F [2, 15] = 54.64, $P < 0.05$), immobility period (F [2, 15] = 59.92, $P < 0.05$), number of rearing (F [2, 15] = 58.70, $P < 0.05$), and the number of lines crossed by the hind limb (F [2, 15] = 61.67, $P < 0.05$). *Post hoc* test analysis revealed that FSD treatment increased the distance travelled, number of rearing, and lines crossed by the hind limb, while reducing immobility time, compared to ROT-intoxicated animals.

FSD restores ROT -induced behavioral alteration during the rota rod, grip strength, and locomotory activity test

The effect of FSD on changes in retention time (A), grip strength (B), and locomotor activity (C) was evaluated in Figure 5 during behavioral performance tests in ROT-intoxicated animals. One-way ANOVA demonstrated significant differences in retention time (F [2, 15] = 34.88, $P < 0.05$), grip strength (F [2, 15] = 52.50, $P < 0.05$), and locomotor activity (F [2, 15] = 53.85, $P < 0.05$). *Post hoc* analysis indicated that ROT-intoxicated animals exhibited decreased motor functions, shown by reductions in retention time, grip strength, and locomotor activity compared to the control group. In addition, daily FSD treatment significantly improved these behavioral activities that were altered in the ROT-intoxicated animals.

FSD restores ROT -induced changes in striatal DA level

The effect of FSD on the level of striatal DA concentration is depicted in Figure 6 in ROT-intoxicated animals. Statistically, a significant difference was observed in striatal DA levels among all treatments (F [2, 15] = 46.28, $P < 0.05$). The *post hoc* test indicated that ROT injection significantly reduced striatal DA levels compared to the control group. In addition, daily treatment with FSD restored DA levels compared to the ROT group.

FSD mitigates ROT -induced OS markers

Figure 7 depicts the effect of FSD on OS markers, including SOD (a), CAT (b), and MDA (c) in ROT-intoxicated animals. Statistical data showed significant differences in the activities of SOD (F [2, 15] = 47.89, $P < 0.05$), CAT (F [2, 15] = 53.89, $P < 0.05$), and MDA (F [2, 15] = 66.86, $P < 0.05$) among all experimental groups. *Post hoc* analysis revealed that ROT-treated rats had reduced SOD and CAT levels, along with

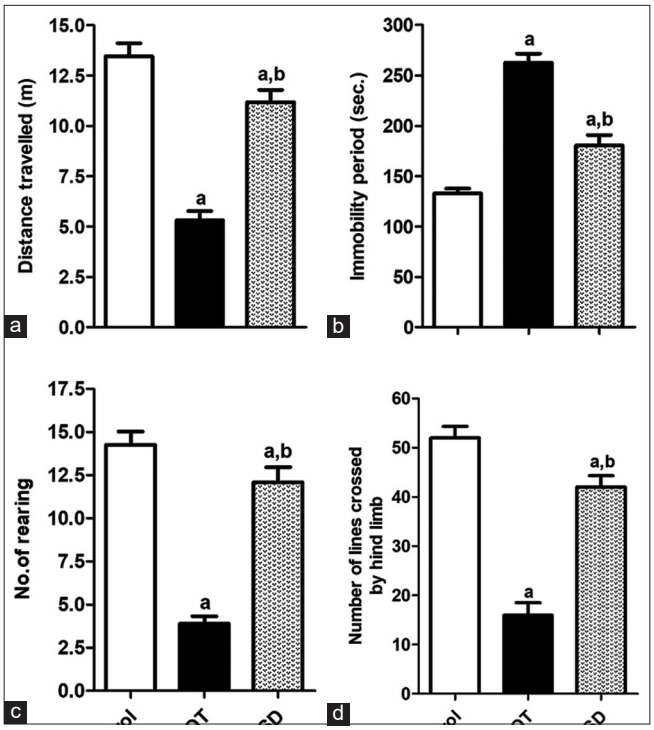

Figure 4: Effect of fasudil on the rotenone (ROT)-induced changes in total distance traveled (a), immobilityperiod (b), no. of rearing (c), and the no. of lines crossed by the hind-limb (d) of the animals during behavioral paradigms. All values are mean ± standard error of the mean ($n = 6$). $^{a}P < 0.05$ compared to Control, $^{b}P < 0.05$ compared to ROT (one-way analysis of variance followed by student–Newman–Keuls *post hoc* test)

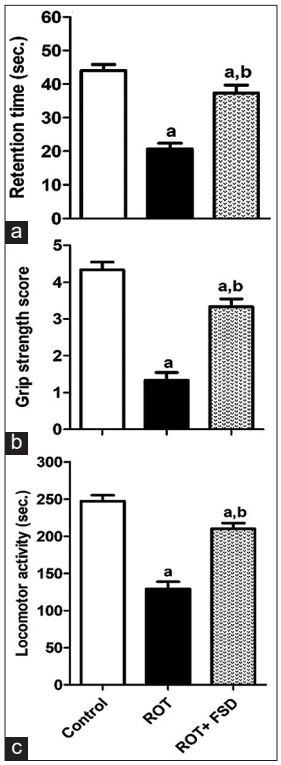

Figure 5: Effect of Fasudil on the rotenone (ROT)-induced changes in retention time (a), grip strength score (b), andlocomotor activity (c) of the animals during behavioral paradigms. All values are mean f standard error of the mean ($n = 6$). $^{a}P < 0.05$ compared to Control, $^{b}P < 0.05$ compared to ROT (one-way analysis of variance followed by student–Newman–Keuls *post hoc* test)

increased MDA levels, compared to the control group. Daily FSD administration significantly elevated SOD and CAT activities and decreased MDA concentration compared to the ROT group.

FSD restores ROT -induced mitochondrial dysfunction

The effect of FSD on mitochondrial cell viability in the striatum of ROT-intoxicated rats is shown in Figure 8. Statistical analysis revealed a significant difference in cell viability among the experimental groups ($F [2, 15] = 66.86$, $P < 0.05$). *Post hoc* comparisons suggested that ROT administration significantly decreased cell viability compared to the control group. However, daily treatment with FSD significantly improved mitochondrial cell viability relative to the ROT group, indicating a protective effect of FSD against ROT-induced mitochondrial dysfunction.

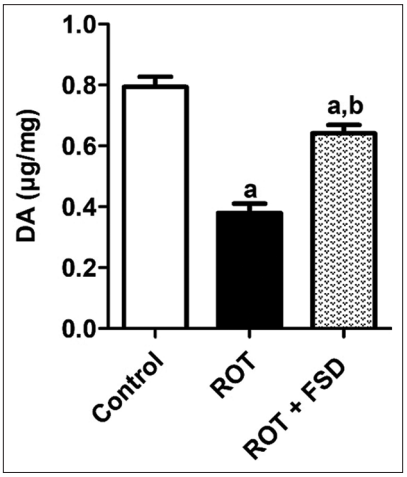

Figure 6: Effect of Fasudil on the rotenone (ROT)-induced changes in striatal DA level of the animals during biochemical paradigms. All values are mean ± SEM (N = 6). $^{a}p < 0.05$ compared to Control, $^{b}p < 0.05$ compared to ROT (one-way ANOVA followed by Student–Newman–Keuls Post-hoc test).

DISCUSSION

The present study demonstrates, for the first time, the neuroprotective activity of FSD in a rat model of ROT-induced PD. FSD administration not only mitigated behavioral deficits associated with ROT-induced PD-like symptoms but also effectively attenuated dopaminergic toxicity in the striatum caused by ROT during the protocol. These improvements are particularly noteworthy because OS and mitochondrial dysfunction are widely recognized

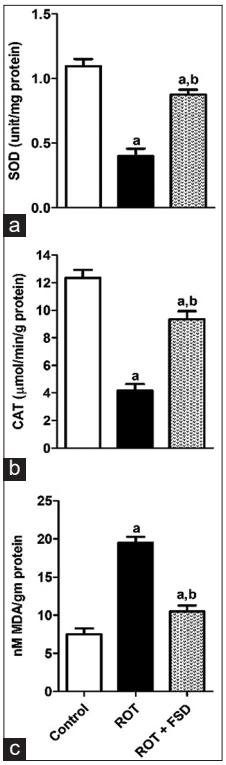

Figure 7: Effect of Fasudil on the rotenone (ROT)-induced changes in SOD (A), CAT (B), and MDA (C) of the animals during biochemical paradigms. All values are mean ± SEM (N = 6). [a]p < 0.05 compared to Control, [b]p < 0.05 compared to ROT (one-way ANOVA followed by Student–Newman–Keuls Post-hoc test)

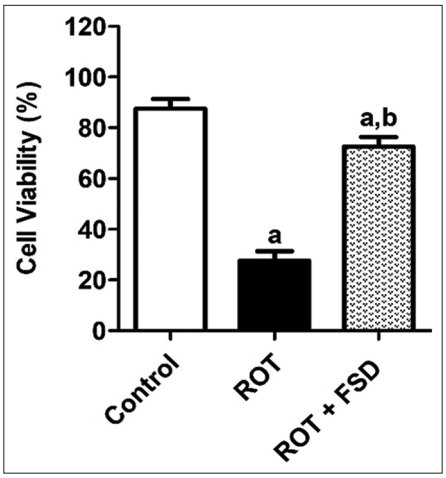

Figure 8: Effect of Fasudil on the rotenone (ROT)-induced changes in mitochondrial cell viability of the animals during biochemical paradigms. All values are mean ± SEM (N = 6). [a]p < 0.05 compared to Control, [b]p < 0.05 compared to ROT (one-way ANOVA followed by Student–Newman–Keuls Post-hoc test).

as significant factors in the degeneration of dopaminergic neurons. In this context, FSD treatment significantly reduced oxidative burden, suggesting its role in reestablishing redox homeostasis in ROT-intoxicated animals.

ROT is a neurotoxin that effectively mimics PD-like pathology in the substantia nigra, leading to motor impairments.[46,47] The present research aligns with previous studies, showing that long-term exposure to ROT significantly diminishes motor functions.[46,47] The administration of FSD restored these impaired behaviors in the present experiment, consistent with other reports.[48] This neurobehavioral improvement is likely mediated through inhibition of the ROCK signaling pathway, a mechanism known to be important in maintaining dopaminergic neuronal integrity.

Furthermore, accumulating evidence supports that ROT-induced increase in OS disrupts dopaminergic signaling and accelerates neuronal degeneration.[49] In line with these findings, our study revealed that FSD increased dopaminergic activity in the striatum, which was depleted by ROT exposure, aligning with earlier reports.[28] Past research indicates that FSD not only protects damaged neurons but also promotes neuron outgrowth *in vitro*.[28] Moreover, ROCK inhibition appears to be crucial for the survival of dopaminergic neurons, as observed in other models involving FSD as a potent modulator of ROCK signaling and dopaminergic function.[24,50] These findings suggest that FSD enhances motor function by maintaining dopaminergic levels in ROT-challenged rats through the ROCK pathway.

Disturbance in neuronal redox potential impairs multiplecellular processes, ultimately leading to neuronal death and serving as an essential marker of various neurodegenerative diseases.[51] Substantial evidence suggests that OS is a key factor in damaging cellular components within SNpc in Parkinsonism.[52] Supporting prior evidence, our data demonstrate that ROT administration significantly increases OS.[53] Treatment with FSD significantly reduces this oxidative burden, as evidenced by increased activities of endogenous antioxidant enzymes – SOD and CAT, along with a decrease in MDA levels, similar to other reports on cardiac failure.[54] Therefore, it can be concluded that FSD improves motor functions, dopaminergic activity, and OS through involvement of the ROCK pathway.

In addition to these findings, mitochondrial dysfunction – a primary cause of ROT-induced neurodegeneration – was further validated by the MTT assay. The decreased formazan intensity observed in the ROT group indicates a significant reduction in mitochondrial metabolic activity and overall cell viability in the striatum. Treatment with FSD markedly improved cell viability, demonstrating its potential to preserve mitochondrial functions. These results further support the neuroprotective role of FSD, likely mediated by its ability to restore mitochondrial dynamics and counteract oxidative injury through modulation of the ROCK pathway.

SUMMARY AND CONCLUSION

In this study, ROT-induced Parkinson-like symptoms serve as a platform to test new treatment approaches. FSD, a ROCK inhibitor, notably improves motor functions, reduces OS, and restores DA levels. In addition, MTT assay results indicate that FSD significantly boosts mitochondrial cell viability in the striatum compared to ROT-treated rats, supporting its neuroprotective potential. Therefore, FSD could be a promising pharmacotherapy for PD.

LIST OF ABBREVIATIONS

1. ND- Neurodegenerative disease
2. PD- Parkinson's disease
3. OS- Oxidative stress
4. ROS- Reactive oxygen species
5. SNpc- Substantia nigra pars compacta
6. DA- Dopamine
7. ROT- Rotenone
8. ROCK- Rho-associated protein kinase
9. FSD- Fasudil
10. MPTP- 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
11. 6-OHDA- 6-hydroxydopamine
12. i.p. – intraperitoneally
13. SOD- Superoxide dismutase
14. CAT- Catalase
15. MDA- Malondialdehyde
16. GSH- Reduced glutathione
17. SEM- Standard error of the mean
18. IL-1β- Interleukin-1β
19. IL-6- Interleukin-6
20. TNF-α- Tumor necrosis factor-α
21. MTT- (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide)

AUTHORS'S CONTRIBUTION

The authors confirm contribution to the paper as follows: Study conception and design: V.V.; data collection: A.K.; analysis and interpretation of results: A.K., V.V.; draft manuscript: A.K.; Both authors reviewed the results and approved the final version of the manuscript.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGMENT

The authors thank BioRender for providing the platform to create professional science figures.

DATA AVAILABILITY

This research article contains all the data; we have nothing to reveal.

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Source of Support: Nil. **Conflicts of Interest:** None declared.