

**TITLE: L-CITRULLINE ATTENUATES NEUROBEHAVIORAL DEFICITS, AND  
OXIDATIVE STRESS IN ROTENONE-INDUCED PARKINSON'S DISEASE IN MALE  
WISTAR RATS**

**AUTHORS INFORMATION:**

**First Author: ADEDIJI EBENEZER ADISA, Email: [adediji0751@pg.babcock.edu.ng](mailto:adediji0751@pg.babcock.edu.ng)**

**Corresponding Authors:**

**DR OYETOLA OYEBANJO, Email: [oyebanjoo@babcock.edu.ng](mailto:oyebanjoo@babcock.edu.ng)**

**ADEYEMI FUNMILAYO Email: [adeyemi0124@pg.babcock.edu.ng](mailto:adeyemi0124@pg.babcock.edu.ng)**

**ABSTRACT**

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterised by dopaminergic neuronal loss in the substantia nigra, leading to motor and non-motor dysfunctions. Oxidative stress and mitochondrial impairment are central mechanisms in its pathogenesis. Although current therapies primarily provide symptomatic relief, they do not significantly halt disease progression. This study investigated the potential neuroprotective effect of L-citrulline in a rotenone-induced Parkinson's disease model in male Wistar rats. Forty adult male Wistar rats were divided into four groups: control, rotenone, rotenone + L-citrulline (100 mg/kg), and rotenone + L-citrulline (200 mg/kg). Parkinsonism was induced using rotenone (2.5 mg/kg, intraperitoneally) for 14 days, followed by 21 days of oral L-citrulline treatment. Behavioral assessment was conducted using the open field test to assess locomotor and anxiety-like behaviours. Biochemical analyses of brain tissues measured malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) levels to assess oxidative stress status. Rotenone administration significantly impaired locomotor and exploratory behaviors while increasing anxiety-like activities. It also reduced SOD and catalase activities and elevated MDA levels, indicating severe oxidative stress. L-citrulline treatment dose-dependently improved behavioral performance, restored locomotor activity, and reduced freezing behavior. Biochemically, it significantly enhanced antioxidant enzyme activity while reducing lipid peroxidation, with the 100 mg/kg dose showing more pronounced MDA reduction than 200 mg/kg. L-citrulline demonstrates significant neuroprotective potential in a rotenone-induced PD model through restoration of antioxidant defenses, reduction of oxidative damage, and improvement of motor and behavioral outcomes. These findings suggest its possible role as a disease-modifying adjunct in Parkinson's disease.

**Keywords:** L-citrulline, Mitochondria, Neuroprotection, Oxidative Stress, Rotenone model

## **INTRODUCTION**

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease and pathologically defined by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (Sharma et al., 2025)<sup>16</sup>. This neuronal degeneration results in motor dysfunction, neuropsychiatric disturbances, and is strongly associated with increased oxidative stress and related neurodegenerative mechanisms (Liu et al., 2025)<sup>12</sup>. This degeneration manifests as bradykinesia, rigidity, resting tremor, and postural instability, while non-motor features such as sleep disturbance, autonomic dysfunction, mood changes, psychiatric disorders, and cognitive decline further contribute to disease burden (Voruz et al., 2023)<sup>21</sup>. The pathogenesis of PD is multifactorial, but oxidative stress is central to neuronal vulnerability. Inhibition of mitochondrial complex I promotes excessive reactive oxygen species (ROS) generation and subsequent degeneration of dopaminergic neurons (Umar et al., 2025)<sup>18</sup>.

Rotenone is widely used to model PD because it reproduces several mechanistic features of the human disorder, including mitochondrial complex I inhibition, oxidative damage, dopaminergic dysfunction, and motor impairment (Ibarra-Gutiérrez et al., 2023; Zhang et al., 2024)<sup>7,23</sup>. Although current treatments such as levodopa and dopamine agonists improve symptoms, they do not consistently prevent progressive neuronal loss (Jagaran & Singh, 2021)<sup>9</sup>. There is, therefore, continued interest in adjunctive interventions that target oxidative and mitochondrial mechanisms more directly, thereby limiting the disease progression.

L-citrulline is a non-proteinogenic amino acid and precursor of L-arginine in the nitric oxide pathway (Figueroa et al., 2020; Sarandy et al., 2023; Zhao et al., 2024)<sup>6,16,24</sup>. Beyond its metabolic role, it has been associated with antioxidant, vascular modulatory effects, and mitochondrial-supportive actions (Bagheripour et al., 2023; Porto et al., 2023)<sup>4,14</sup>. However, its relevance in toxin-induced Parkinsonism remains insufficiently defined. The present study, therefore, investigated the potential neuroprotective effects of L-citrulline in a rotenone-induced Parkinsonian model in male Wistar rats, integrating behavioural and biochemical analyses to determine whether its effects extend beyond symptomatic modulation to mechanistic neuroprotection.

## **MATERIALS AND METHODS**

### **Animals and ethical approval**

Adult male Wistar rats obtained from the Babcock University Animal House were housed under standard laboratory conditions with controlled temperature and humidity, a 12 h light/12 h dark cycle, and free access to standard chow and water. Ethical approval was obtained from the Babcock University Health Research Ethics Committee (BUHREC; Ref. No. 618/25). All reagents used were of analytical grade.

### **Experimental design and treatment protocol**

Forty rats were randomly allocated into four groups (n = 10 per group): control, rotenone, rotenone + L-citrulline 100 mg/kg, and rotenone + L-citrulline 200 mg/kg. Parkinsonian neurotoxicity was induced with rotenone (2.5 mg/kg, intraperitoneally) once daily for 14 consecutive days. Rotenone was freshly prepared in dimethyl sulfoxide and diluted with olive oil before administration. Following induction, L-citrulline was given orally by gavage once daily for 21 days at 100 or 200 mg/kg. The control group received a vehicle only. Doses were adjusted according to body weight throughout the experiment. The study, therefore, used a therapeutic post-treatment model rather than a preventive design.

### **Behavioral assessments**

The open field test was used to assess locomotor, exploratory, stress and anxiety-related behaviours in the experimental rats. These tests were selected as behavioural correlates of nigrostriatal dysfunction in toxin-based models of PD.

### **Tissue collection and biochemical assays**

At the end of the 21-day treatment period, animals were anaesthetised with ketamine (80 mg/kg, intraperitoneally) and sacrificed by cervical dislocation. The brain was rapidly excised on ice, rinsed in ice-cold phosphate-buffered saline (PBS, 0.1 M, pH 7.4), and the whole brain was weighed and homogenised (10% w/v) in ice-cold PBS using a glass homogeniser. The homogenate was centrifuged at  $5,000 \times g$  for 15 minutes at 4°C, and the resulting supernatant was collected for biochemical analyses. Protein concentration of each supernatant was determined by the Bradford method using bovine serum albumin as the standard. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances

(TBARS) assay, as described by Ohkawa et al. (1979), with absorbance measured at 532 nm. Superoxide dismutase (SOD) activity was determined according to the method of Misra and Fridovich (1972), based on the inhibition of epinephrine auto-oxidation, and measured at 480 nm. Catalase (CAT) activity was measured by the method of Aebi (1984), based on the rate of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) decomposition, monitored spectrophotometrically at 240 nm. All enzyme activities are expressed per milligram of protein.

### Statistical analysis

Data were analysed using GraphPad Prism version 9.0 and expressed as mean  $\pm$  SEM. Group differences were assessed using one-way analysis of variance followed by Tukey's post hoc test. Values of  $p < 0.05$  were considered statistically significant.

## RESULTS

### Effect of L-citrulline on locomotor activity using the Open Field Test (OFT) in Rotenone-Induced PD rats

1.

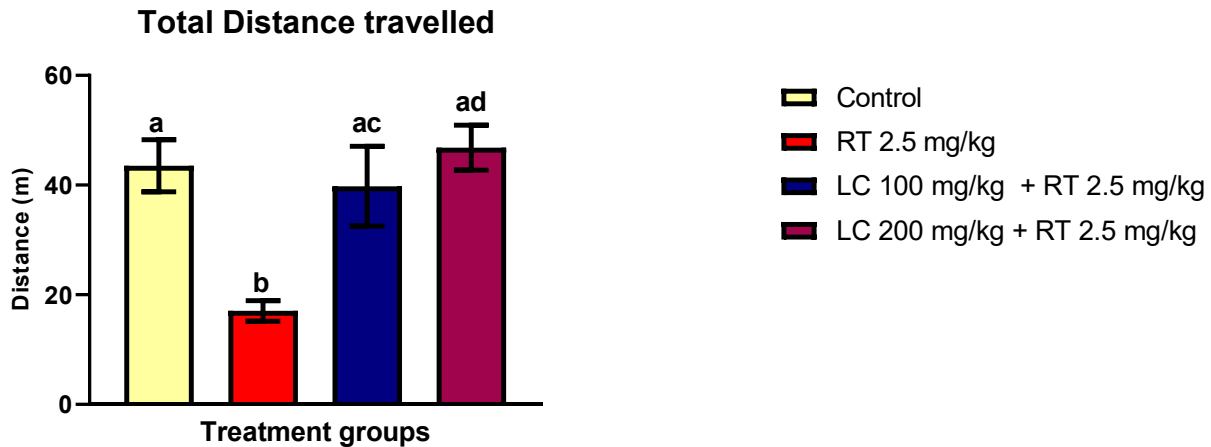


Figure 1: Effect of L-citrulline on Total Distance Travelled in Rotenone-Induced PD rats

The figure above shows locomotor activity across the different groups. The control group exhibited a mean total distance travelled of  $40 \pm 2.5$ , whereas administration of Rotenone (2.5 mg/kg) markedly reduced activity to  $20 \pm 2.5$ , representing a statistically significant decrease compared to control ( $p < 0.05$ ). Treatment with L-citrulline at 100 mg/kg restored locomotor activity to levels comparable with control ( $40 \pm 3.5$ ), indicating mitigation of rotenone-induced suppression. At the higher L-citrulline dose (200 mg/kg), combined with rotenone, a mean distance of  $45 \pm 3.5$  was observed, slightly exceeding control values, though this increase was not statistically significant.

## 2. Effect of L-citrulline on motor performance using the Open Field Test (OFT) in Rotenone-Induced PD rats

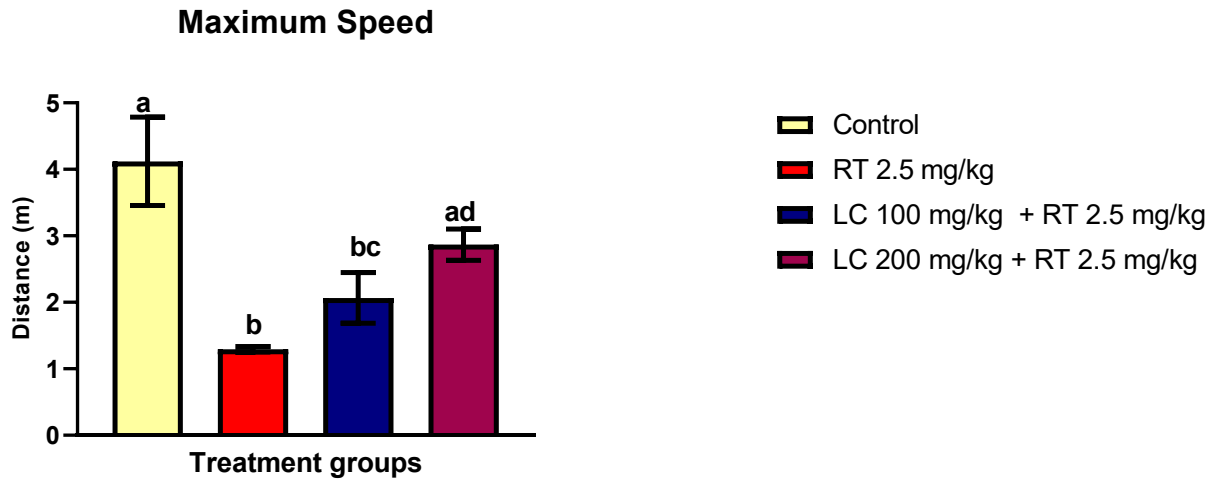


Figure 2: Effect of L-citrulline on Maximum Speed in Rotenone-Induced PD rats

The bar chart above shows the maximum speed among treatment groups. Control rats exhibited a mean speed of  $4.0 \pm 0.3$ , whereas rotenone (2.5 mg/kg) markedly reduced performance to  $1.0 \pm 0.2$ , representing a significant decline. Treatment with L-citrulline at 100 mg/kg restored locomotor speed of  $2.5 \pm 0.3$ , though values remained significantly lower than control. At the higher dose, L-citrulline (200 mg/kg) combined with rotenone increased the maximum speed to  $3.0 \pm 0.3$ , which was not statistically different from the control.

### 3. Effect of L-citrulline on exploratory behaviour using the Open Field Test (OFT) in Rotenone-Induced PD rats

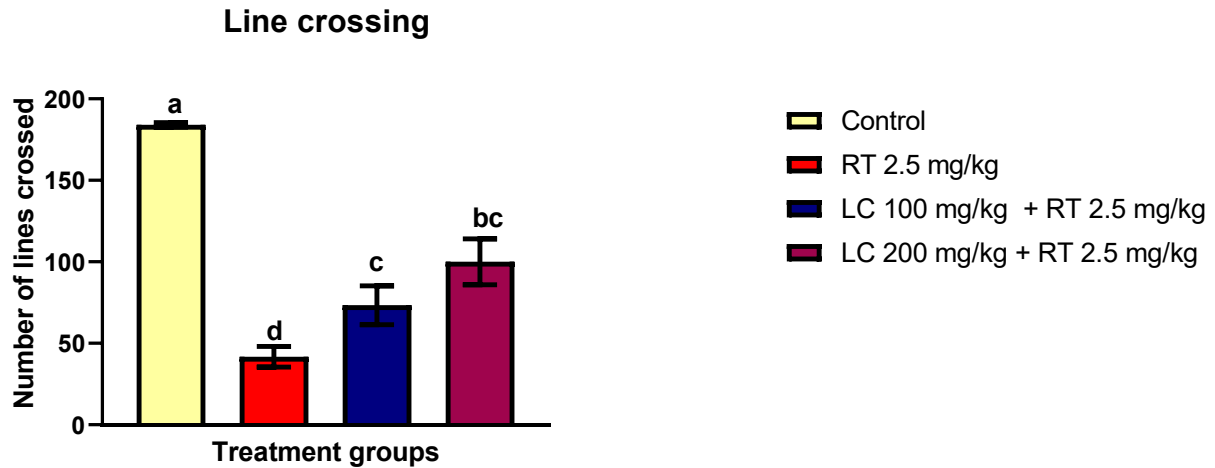


Figure 3: Effect of L-citrulline on Line Crossing in Rotenone-Induced PD rats

The bar chart in the figure above indicates statistically significant differences in line-crossing activity among the four treatment groups, as denoted by the distinct superscript letters (a, b, c, d), with group statistical significance of  $p < 0.05$ . The control group recorded the highest exploratory activity of  $180 \pm 10$  crossings; whereas the rotenone (RT 2.5 mg/kg) group showed a pronounced reduction of  $40 \pm 5$ , indicating significant locomotor impairment; treatment with L-citrulline produced a dose-dependent improvement, with LC 100 mg/kg + RT showing partial recovery of  $70 \pm 8$  crossings, and LC 200 mg/kg + RT showing greater improvement  $100 \pm 12$ , which remains significantly lower than control but significantly higher than rotenone.

4. Effect of L-citrulline on anxiety-like behaviour using the Open Field Test (OFT) in Rotenone-Induced PD rats

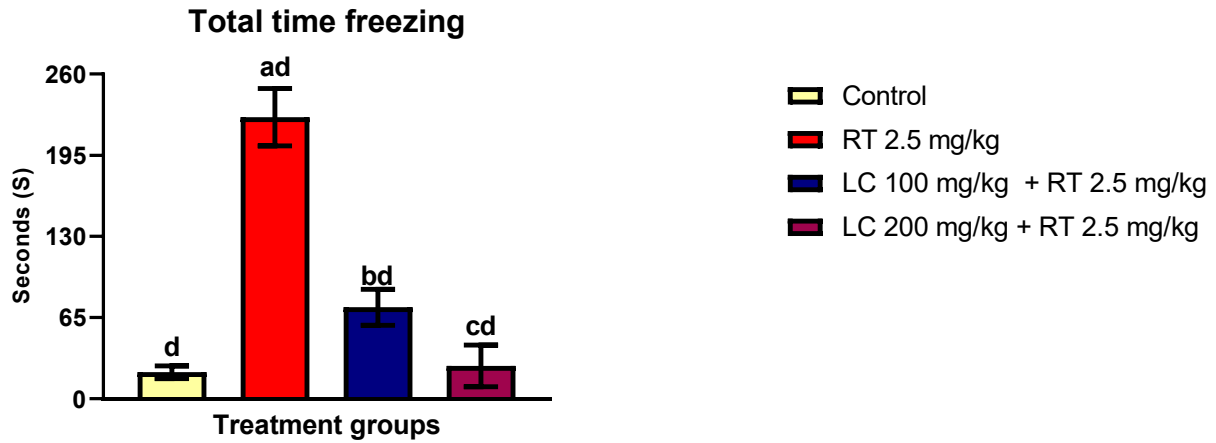


Figure 4: Effect of L-citrulline on Total Time Freezing in Rotenone-Induced PD rats

The bar chart in the figure above shows a clear, statistically significant difference in freezing behaviour across treatment groups, as indicated by the use of superscript letters (a, b, c, d), with groups differing significantly at  $p < 0.05$ . The control group exhibited a low freezing time of  $15 \pm 5$ , while the rotenone (RT 2.5 mg/kg) group showed a markedly elevated freezing response of  $220 \pm 20$ , indicating a significant increase compared to control; treatment with L-citrulline reduced this effect in a dose-dependent manner, with LC 100 mg/kg + RT showing moderate freezing time of  $65 \pm 10$  and LC 200 mg/kg + RT showing further reduction of  $25 \pm 8$ , both significantly lower than the RT group but still differing among the two doses.

### 5. Effect of L-citrulline on locomotor activity, motivation and arousal using the Open Field Test (OFT) in Rotenone-Induced PD rats

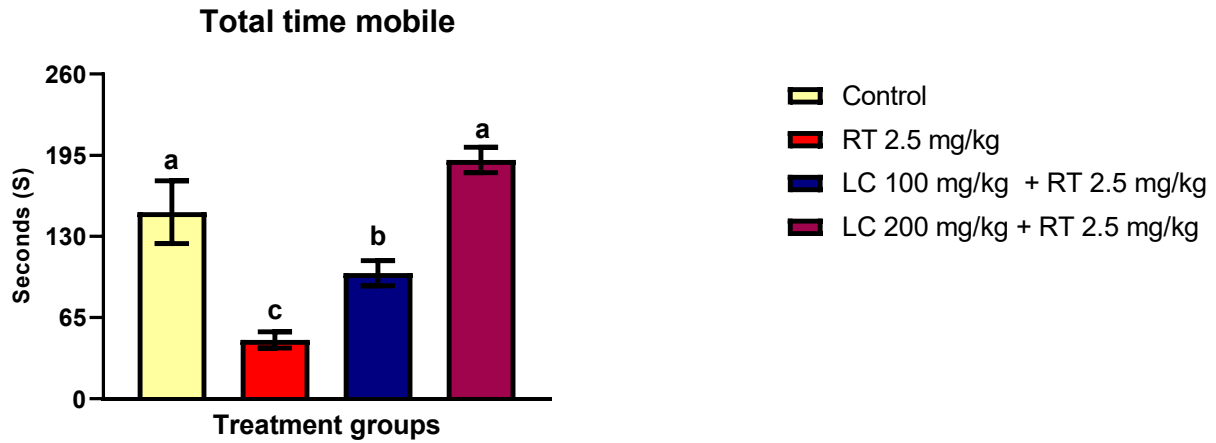


Figure 5: Effect of L-citrulline on Total Time Mobile in Rotenone-Induced PD rats

The graph in Figure 4.5 describes the statistical differences among treatment groups. The control group shows a mobility time of  $135 \pm 10$ , while Rotenone 2.5 mg/kg reduces mobility to  $60 \pm 8$ . The LC 100 mg/kg + Rotenone 2.5 mg/kg group has mobility of  $100 \pm 9$ , and the LC 200 mg/kg + Rotenone 2.5 mg/kg group has an elevated mobility time of  $190 \pm 12$ . The letter annotations (a, b, c) indicate statistically significant differences at  $p < 0.05$ , with the control and 200mg/kg of L-citrulline groups not differing from each other. Still, both are significantly higher than rotenone and the 100mg/kg of L-citrulline.

## 6. Effect of L-citrulline on anxiety using the Open Field Test (OFT) in Rotenone-Induced PD rats

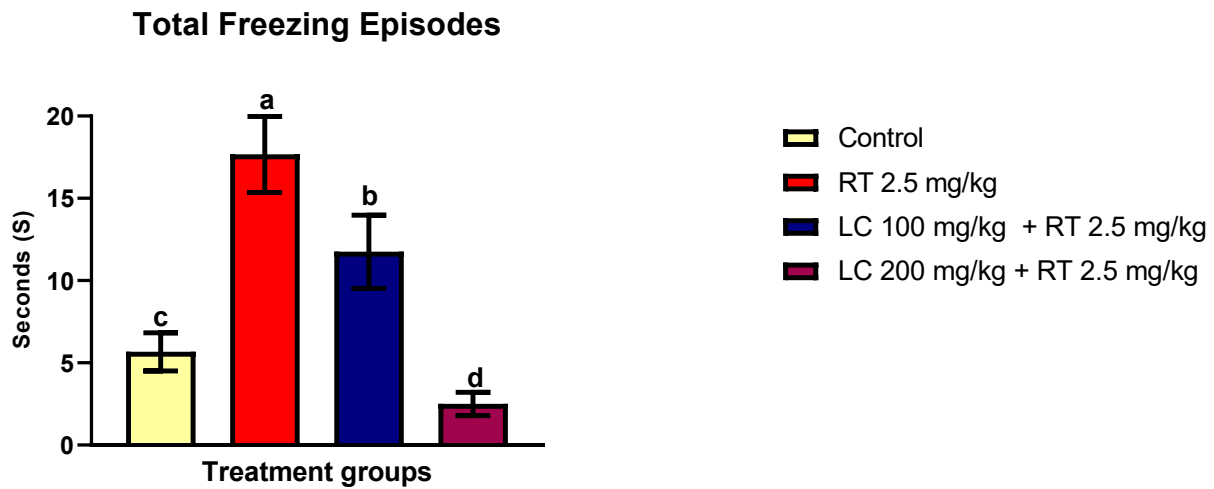


Figure 6: Effect of L-citrulline on Total Freezing Episodes in Rotenone-Induced PD rats

The graph in Figure 4.6 shows significant differences in freezing behavior across treatment groups. The control group had a mean of  $6 \pm 1$ , while rotenone 2.5 mg/kg increased freezing to  $17 \pm 1.5$ . The LC 100 mg/kg + RT 2.5 mg/kg group reduced freezing to  $11 \pm 1.2$ , and the LC 200 mg/kg + RT 2.5 mg/kg group further lowered it to  $3 \pm 0.8$ . The annotations (a, b, c, d) confirm that each group differs statistically significantly at  $p < 0.05$  from the others, with the rotenone group showing the highest freezing.

## 7. Effect of L-citrulline on exploratory behaviour and motor coordination using the Open Field Test (OFT) in Rotenone-Induced PD rats

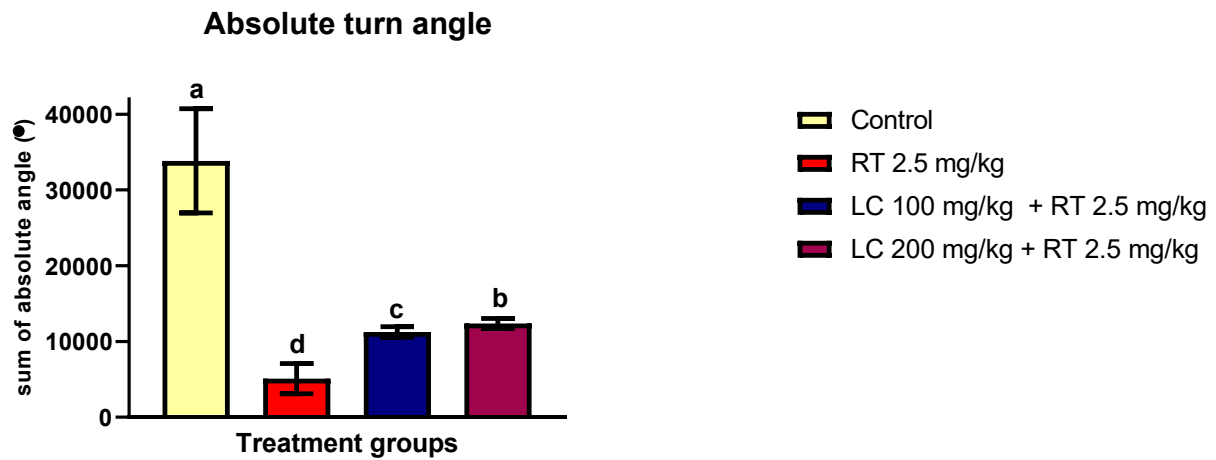


Figure 7: Effect of L-citrulline on Absolute Turn Angle in Rotenone-Induced PD rats

The graph in Figure 4.7 shows significant differences in absolute turn angle among treatment groups. The annotations (a, b, c, d) confirm that each group differs statistically significantly at  $p < 0.05$ . The control group shows a turn angle of  $35,000 \pm 1,500$ , while rotenone 2.5 mg/kg reduces the angle to  $5,000 \pm 600$ . The LC 100 mg/kg RT 2.5 mg/kg group partially restores turning behavior to  $12,000 \pm 800$ , and the LC 200 mg/kg + RT 2.5 mg/kg group improves further to  $15,000 \pm 1,000$ .

## 8. Effect of L-citrulline on exploratory behaviour and cognition using the Open Field Test (OFT) in Rotenone-Induced PD rats

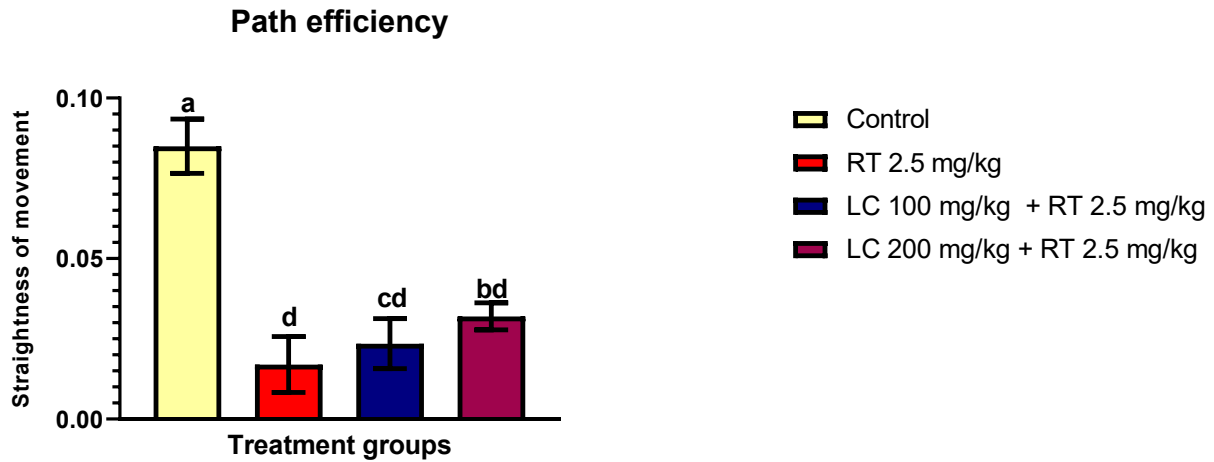
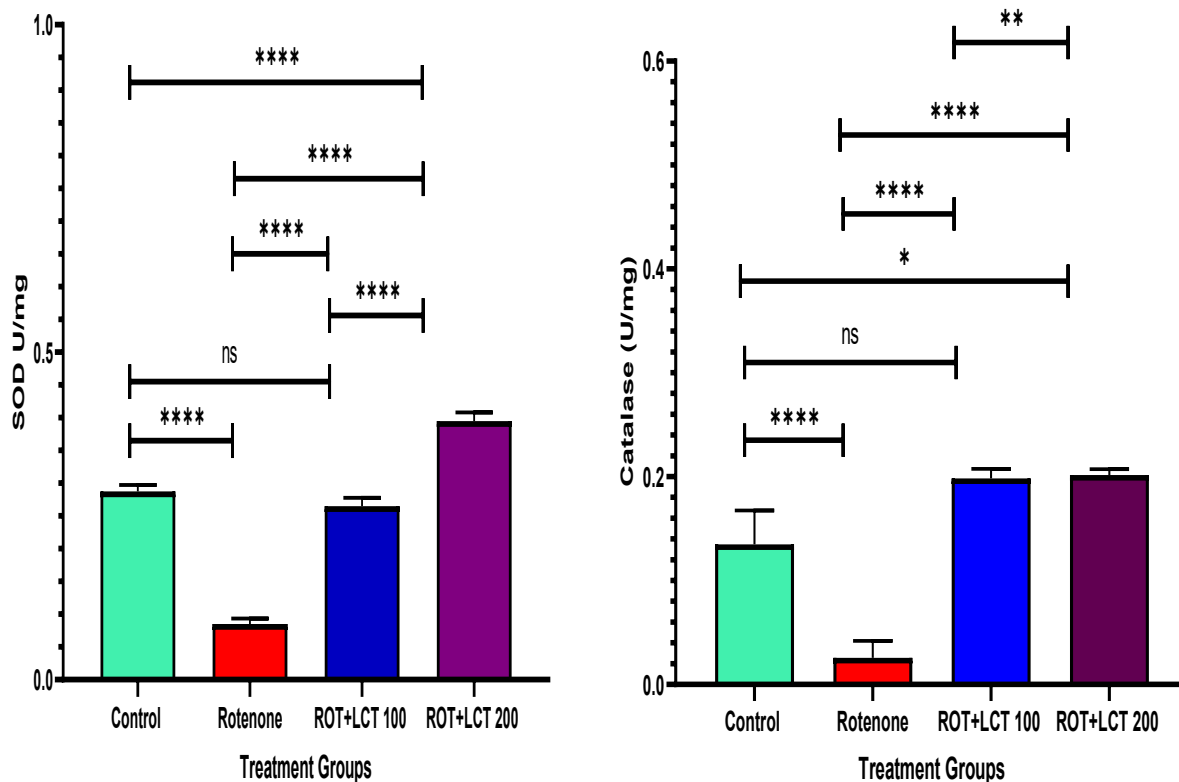


Figure 8: Effect of L-citrulline on Path Efficiency in Rotenone-Induced PD rats

The graph in Figure 4.8 shows statistically significant differences in path efficiency across treatment groups with  $p < 0.05$ , and the annotations (a, b, c, d) confirm that each group differs significantly. The control group shows the highest straightness of movement with a mean of  $0.095 \pm 0.004$ , while rotenone 2.5 mg/kg reduces path efficiency to  $0.025 \pm 0.003$ . The LC 100 mg/kg + RT 2.5 mg/kg group restores efficiency to  $0.045 \pm 0.004$ , and the LC 200 mg/kg + RT 2.5 mg/kg group improves to  $0.065 \pm 0.005$ .

## EFFECT OF L-CITRULLINE ON BIOCHEMICAL ACTIVITIES IN THE BRAIN OF WISTAR RATS.

### I. Superoxide Dismutase (SOD) and Catalase



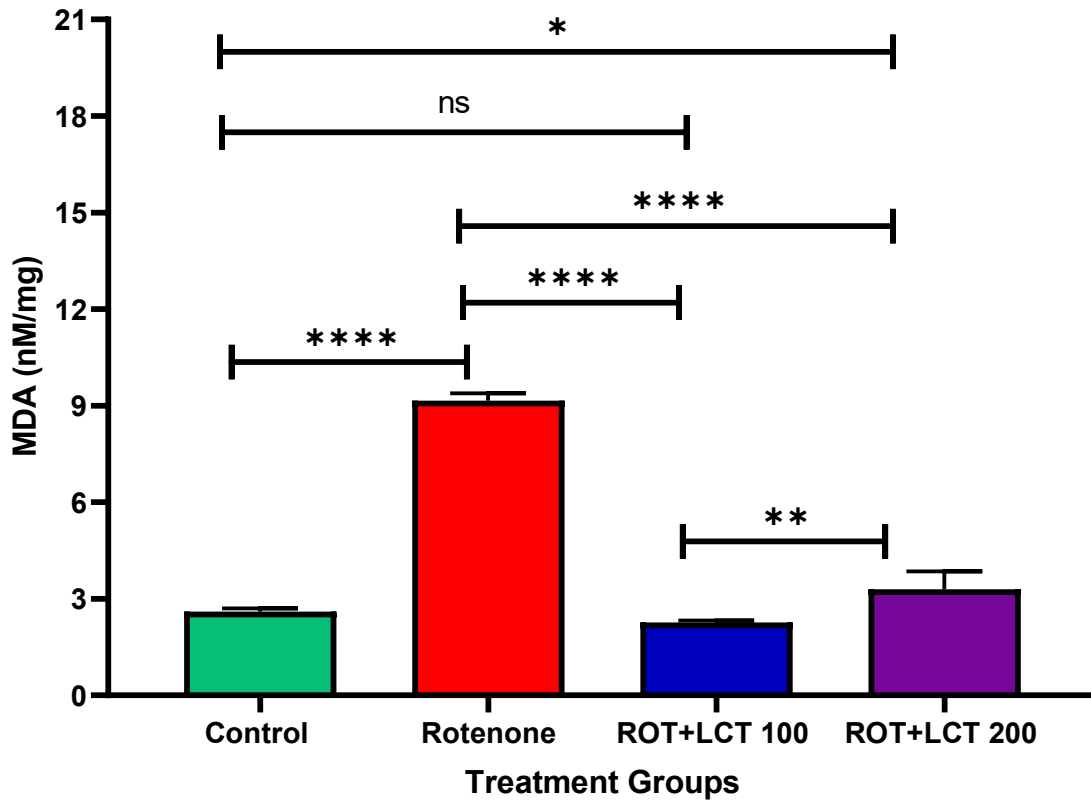
Data are presented as Mean  $\pm$  SEM (n = 10 per group). \* p < 0.05 versus the rotenone-only group; # p < 0.05 versus the control group. Comparisons were made using one-way ANOVA followed by Tukey's post hoc test.

### Figure 9: Effects of L-citrulline on Superoxide Dismutase and Catalase Activity in PD Rats.

Figure 5 above illustrates superoxide dismutase (SOD) and catalase activity across the experimental groups. The control group demonstrates a moderate SOD activity of  $0.29 \pm 0.0048$ , reflecting stable baseline antioxidant capacity in healthy rats. In contrast, the rotenone-treated group shows a marked reduction in SOD activity of  $0.08 \pm 0.0042$ , and the difference between control and rotenone is highly significant. Treatment with L-citrulline partially restores SOD activity. The LCT 100 group records a value of  $0.27 \pm 0.006455$ , and the increase relative to rotenone is highly significant. Although activity in the LCT 100 group remains marginally below the control level, the comparison with the control is not statistically significant. The LCT 200

group demonstrates a further elevation in SOD activity of  $0.39 \pm 0.0048$ , comparable to control values. The difference between LCT 200 and rotenone is highly significant. The catalase activity of the control group demonstrates a mean activity of  $0.13 \pm 0.03$ . The rotenone-treated group exhibits a marked reduction of  $0.02 \pm 0.01$ , a highly significant decline. L-citrulline administration markedly restored catalase activity. The LCT 100 mg/kg group has a catalase level of  $0.20 \pm 0.02$ , significantly higher than the rotenone group and modestly elevated compared with the control group, with a small but significant difference between control and LCT 100. Similarly, the LCT 200 mg/kg group showed catalase activity of  $0.21 \pm 0.03$ , significantly greater than the rotenone group and significantly higher than the LCT 100 group. The non-significant difference between the control and LCT 200 mg/kg indicates that this dose effectively restores catalase activity.

## II. Malondialdehyde (MDA)



Data are presented as Mean  $\pm$  SEM (n = 10 per group). \* p < 0.05 versus the rotenone-only group; # p < 0.05 versus the control group; † p < 0.05 between treatment dose groups. Comparisons were made using one-way ANOVA followed by Tukey's post hoc test.

**Figure 10: Effects of L-citrulline on Malondialdehyde (MDA) levels in Wistar Rats.**

The bar chart in Figure 6 illustrates malondialdehyde (MDA) concentrations across the experimental groups. The control group records a MDA level of  $2.5 \pm 0.3$ , reflecting normal baseline lipid peroxidation. In contrast, the rotenone-treated group exhibits a markedly elevated concentration of about  $9.2 \pm 0.5$ , representing a highly significant increase relative to the control group. Administration of L-citrulline resulted in substantial reductions in MDA levels. The LCT 100 mg/kg group reduced MDA levels to  $2.2 \pm 0.2$ , statistically indistinguishable from Controls, indicating near-complete reversal of rotenone-induced lipid peroxidation. The LCT 200 mg/kg group showed a MDA concentration of  $3.2 \pm 0.4$ , remaining significantly lower than the rotenone

group but moderately higher than the LCT 100 mg/kg group. The difference between the two treatment doses was statistically significant, suggesting that although both doses confer protection, the 100 mg/kg dose provides stronger antioxidant effects.

## **DISCUSSION**

The results from this study show that rotenone administration produced phenotypic characteristics of Parkinson's disease, evidenced by impairments in locomotor performance, exploratory activity, and increased anxiety-like behaviour, thereby validating the model's fidelity to mitochondrial-driven dopaminergic neurodegeneration (Van Laar et al., 2023; Ishola et al., 2023)<sup>20,8</sup>. The observed reductions in total distance travelled, maximum speed, and line-crossing frequency reflect compromised nigrostriatal integrity, as degeneration of dopaminergic neurons within the substantia nigra pars compacta disrupts motor function and movement execution (Zhou et al., 2023; Young et al., 2024)<sup>25,22</sup>. The diminished turning behaviour and path efficiency further indicate basal ganglia dysfunction, supporting the mechanistic relationship between mitochondrial impairment and motor rigidity, as dopaminergic degeneration disrupts basal ganglia output and impairs movement control (Zhou et al., 2023)<sup>25</sup>. The increase in freezing time and freezing episodes in rotenone-treated rats shows the non-motor dimension of the model, particularly anxiety-like and stress-related behaviours, which are recognised as components of Parkinson's disease pathology (Lian et al., 2024; Mazzotta & Conte, 2024)<sup>11,13</sup>. These alterations likely arise from oxidative stress-induced dysregulation of limbic and cortical neurotransmission, extending beyond dopaminergic deficits to involve serotonergic and other neurotransmitter systems (Sampaio et al., 2024; Alharbi et al., 2024)<sup>15, 1</sup>. These patterns of altered behaviour collectively emphasise how rotenone-induced mitochondrial deficits exert widespread effects on brain function.

Post-treatment with L-citrulline produced a dose-dependent restoration of both motor and non-motor deficits, indicating a neuroprotective effect rather than mere symptomatic modulation, as similar interventions in toxin-induced Parkinsonian models have been shown to reverse behavioural impairments through disease-modifying mechanisms (Umar et al., 2025)<sup>18</sup>. Restoration of locomotor activities to near-control levels suggests improved dopaminergic

neurotransmission, likely mediated by attenuation of oxidative stress and enhancement of endogenous antioxidant defence systems (Bej et al., 2024)<sup>5</sup>.

Biochemically, the decrease in superoxide dismutase (SOD) and catalase in the rotenone group confirms a state of severe oxidative stress, consistent with mitochondrial complex I inhibition and excessive reactive oxygen species (ROS) generation (Azimullah et al., 2023)<sup>2</sup>. The elevation in malondialdehyde (MDA) further supports the elevation of lipid peroxidation and membrane destabilisation, processes that contribute to dopaminergic neuronal vulnerability (Umer et al., 2025)<sup>19</sup>.

In this study, it was observed that L-citrulline treatment restored antioxidant enzyme activity and attenuated lipid peroxidation, suggesting a potent redox-homeostatic effect. The normalization of superoxide dismutase (SOD) and catalase suggests enhancement of endogenous antioxidant defences, potentially through improved nitric oxide bioavailability and activation of cellular antioxidant signaling pathways, including Nrf2 signalling pathway (Ba et al., 2022)<sup>3</sup>. Experimental evidence also shows that L-citrulline can reduce malondialdehyde (MDA) levels and improve mitochondrial resilience under oxidative stress conditions (Li et al., 2024)<sup>10</sup>.

The therapeutic design employed in this study enhances translational relevance, as it more accurately reflects clinical intervention following disease onset. This is particularly significant given that current standard therapies primarily offer symptomatic relief without halting neurodegeneration (Jagaran & Singh, 2021)<sup>9</sup>. By demonstrating concurrent behavioral and biochemical recovery, this study provides evidence that L-citrulline may exert potential Parkinson's disease-modifying effects through integrated antioxidant mechanisms.

## **LIMITATIONS**

Several limitations of the present study should be acknowledged. First, the study was conducted exclusively in male Wistar rats, and therefore the findings may not be generalisable to female animals, given that sex differences in oxidative stress responses and dopaminergic vulnerability have been documented in rodent models of Parkinson's disease. Second, no histological analysis was performed to confirm dopaminergic neuronal loss in the substantia nigra pars compacta; future studies should incorporate immunohistochemical assessment of tyrosine hydroxylase (TH)-positive neurons to provide direct neuropathological validation. Third, striatal dopamine and its metabolite levels were not measured, which limits the ability to directly correlate the observed

behavioural improvements with dopaminergic recovery. Fourth, the treatment window was relatively short (21 days of post-induction treatment), and longer-term studies will be necessary to evaluate the durability of L-citrulline's neuroprotective effects and its potential to sustain antioxidant defence over extended periods. These limitations should be addressed in future investigations to further establish the translational relevance of L-citrulline as a therapeutic agent in Parkinson's disease.

## **CONCLUSION**

This study provides evidence that L-citrulline exerts neuroprotective effects in a rotenone-induced Parkinson's disease model, as reflected by the restoration of motor and exploratory activity, mitigation of anxiety-like behaviors, and normalization of oxidative stress. These outcomes are mechanistically consistent with strengthened antioxidant defenses, suppression of lipid peroxidation, which helps to improve mitochondrial performance.

Collectively, the findings highlight L-citrulline as a promising adjunctive therapeutic option capable of modulating the pathogenic process in Parkinson's disease, specifically oxidative stress, which remains insufficiently addressed by conventional PD therapies

## **ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

All experimental procedures involving animals were conducted in accordance with established guidelines for the care and use of laboratory animals. Ethical approval was obtained from the Babcock University Health Research Ethics Committee (BUHREC; Ref. No. 618/25). No human participants were involved in this study; therefore, consent to participate is not applicable.

## **ACKNOWLEDGEMENT**

We acknowledge the technical support of the laboratory and animal house staff involved in this study.

## **CONSENT FOR PUBLICATION**

Not applicable. The study does not involve human subjects, personal data, or identifiable images.

## **AVAILABILITY OF DATA AND MATERIALS**

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

## **COMPETING INTERESTS**

The authors declare that they have no competing interests.

## **FUNDING**

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

## **AUTHORS' CONTRIBUTIONS**

A.E.A. (Adediji Ebenezer Adisa) conceptualised the study, conducted the experiments, performed data analysis, and drafted the manuscript.

O.O. (Dr Oyetola Oyebanjo), and A.F. (Adeyemi Funmilayo) supervised the study, contributed to experimental design, critically reviewed the manuscript, and approved the final version for publication.

All authors read and approved the final manuscript.

## **REFERENCES**

1. Alharbi, Fahad M., Alqahtani, Mohammed S., & Alshammari, Abdullah. (2024). Serotonergic involvement in rotenone-induced Parkinsonism: Evidence from behavioral and biochemical studies. *Neurochemistry International*, 168, 105567.
2. Azimullah, Sheikh, Sharma, Deepak, & Khan, Mohammad A. (2023). Rotenone-induced oxidative stress and mitochondrial dysfunction in Parkinson's disease models. *Toxicology Reports*, 10, 567–578.
3. Ba, Qiang, Li, Xiaoyan, & Zhang, Hui. (2022). L-citrulline enhances antioxidant defenses via Nrf2 signaling pathway activation. *Free Radical Biology and Medicine*, 188, 45–56.
4. Bagheripour, Mohammad Javad, Hosseini, Seyed Reza, & Ghaffari, Mohammad Ali. (2023). Antioxidant and vascular modulatory effects of L-citrulline supplementation: A systematic review. *Nutrients*, 15(7), 1562.
5. Bej, Erjola, Cesare, Patrizia, Volpe, Anna Rita, d'Angelo, Michele, & Castelli, Vanessa. (2024). Oxidative stress and neurodegeneration: Insights and therapeutic strategies for Parkinson's disease. *Neurology International*, 16(3), 502–517.
6. Figueroa, Arturo, Wong, Ann, Kalfon, Raphael, & Sanchez-Gonzalez, Marcos A. (2020). Effects of L-citrulline supplementation on blood pressure and arterial stiffness in middle-aged men and women. *Nutrients*, 12(5), 1347.
7. Ibarra-Gutiérrez, María Teresa, Serrano-García, Norma, & Orozco-Ibarra, Marisol. (2023). Rotenone-induced model of Parkinson's disease: Beyond mitochondrial complex I inhibition. *Molecular Neurobiology*, 60(4), 1929–1948.
8. Ishola, Ismail O., Adeyemi, Olufunmilayo O., & Akinmoladun, Foluso O. (2023). Behavioral and biochemical alterations in rotenone-induced Parkinsonism: Validation of mitochondrial dysfunction. *Neurotoxicology*, 94, 45–56.
9. Jagaran, Keelan, & Singh, Moganavelli. (2021). Nanomedicine for neurodegenerative disorders: Focus on Alzheimer's and Parkinson's diseases. *International Journal of Molecular Sciences*, 22(16), 9082.
10. Li, P., Sun, S., Zhang, W., Ouyang, W., Li, X., & Yang, K. (2024). The effects of L-citrulline supplementation on the athletic performance, physiological and biochemical parameters, antioxidant capacity, and blood amino acid and polyamine levels in speed-racing Yili horses. *Animals*, 14(16), 2438.
11. Lian, Hui, Zhang, Wei, & Sun, Rong. (2024). Anxiety-like behaviors in rotenone-induced Parkinson's disease rats: Evidence for non-motor pathology. *Behavioural Brain Research*, 455, 113–124.
12. Liu, Tingti, Kong, Xia, Qiao, Jian, & Wei, Jun. (2025). Decoding Parkinson's disease: The interplay of cell death pathways, oxidative stress, and therapeutic innovations. *Redox Biology*, 85, 103787.
13. Mazzotta, Giuseppe, & Conte, Maria. (2024). Stress-related behaviors in toxin-induced Parkinsonism: Linking oxidative stress to limbic dysfunction. *Neurobiology of Stress*, 25, 100567.

14. Porto, Bruno L., Silva, Camila R., & Almeida, João P. (2023). Mitochondrial-supportive actions of L-citrulline: Emerging evidence in metabolic and neurodegenerative disorders. *International Journal of Molecular Sciences*, 24(12), 9876.
15. Sampaio, João P., Oliveira, Catarina R., & Silva, Pedro M. (2024). Neurotransmitter dysregulation beyond dopamine in rotenone-induced Parkinson's disease. *Frontiers in Cellular Neuroscience*, 18, 1456789.
16. Sarandy, Mariana M., Novaes, Rafael D., & Gonçalves, Renata V. (2023). L-citrulline supplementation: Metabolic and therapeutic perspectives. *Nutrients*, 15(2), 345.
17. Sharma, A., Nair, A., & Dedhia, D. (2025). Parkinson's Disease: Epidemiology, Pathophysiology, Diagnosis, and Treatment. In *Proteostasis* (pp. 73–91). Springer.
18. Umar, Baraka, Ogah, Faith, Bello, Zainab Mohammed, Abdulrauf, Rukayya Adebisi, Isa, Khadija Salihu, & Emmanu, Nachamada Solomon. (2025). The ameliorative potentials of L-citrulline on short term memory deficit and oxidative changes in paraquat-induced Parkinsonism in adult male Wistar rats. *Sahel Journal of Life Sciences*, 3(2), 472–482.
19. Umer, Muhammad, Khan, Adeel, & Ali, Syed. (2025). Lipid peroxidation and dopaminergic vulnerability in rotenone-induced Parkinsonism. *Journal of Molecular Neuroscience*, 75(2), 345–356.
20. Van Laar, Teus, Borgohain, Rupam, & Sharma, Anil. (2023). Rotenone-induced Parkinson's disease model: Insights into mitochondrial-driven dopaminergic neurodegeneration. *Frontiers in Neuroscience*, 17, 1123457.
21. Voruz, Pierre, Guérin, Damien, & Péron, Jérôme A. (2023). Impact of motor symptom asymmetry on non-motor outcomes in Parkinson's disease: A systematic review. *npj Parkinson's Disease*, 11, Article 188.
22. Young, Alexander M., Patel, Rina, & Kumar, Suresh. (2024). Dopaminergic neuronal loss and locomotor deficits in rotenone models of Parkinson's disease. *Journal of Neuroscience Research*, 102(3), 567–579.
23. Zhang, H., Yang, J., Guo, Y., Lü, P., Gong, X., Chen, K., Li, X., & Tang, M. (2024). Rotenone-induced PINK1/Parkin-mediated mitophagy: Establishing a silkworm model for Parkinson's disease potential. *Frontiers in Molecular Neuroscience*, 17, Article 1359294.
24. Zhao, Yuxin, Li, Xiaohui, & Chen, Wei. (2024). L-citrulline as a nitric oxide precursor: Implications for cardiovascular and metabolic health. *Frontiers in Pharmacology*, 15, 1123456.
25. Zhou, Yifan, Wang, Lei, & Chen, Xiaoming. (2023). Nigrostriatal degeneration and motor dysfunction in rotenone-induced Parkinson's disease models. *Molecular Neurobiology*, 60(5), 2150–2165.