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Full-Length Research Article

Luteolin Offers Novel Therapeutic Regimen in Rotenone-Induced Parkinson's Disease via Modulation of TNFα/FXMRP/Serotonin/Tyrosine Hydroxylase Signaling Pathway

Oyagbemi A.A.[1], Awogbindin I.O.[2], Salami A.T.[3], *Ajibade T.O.[1], Awoyomi O.V.[4], Esan O.O.[5], Oyagbemi T.O.[4], Adeogun A.V.[1], Igado O.I.[6], Onukak C.E.[1], Jaja I.F.[7a, 7b], Omobowale T.O.[5], Ola-Davies O.E.[1], Saba A.B.[7], Adedapo A.A.[7], Oguntibeju O.O.[8], Yakubu M.A.[9], Nwulia E.[10]

Departments of [1]Veterinary Physiology & Biochemistry, [3]Physiology, [5]Veterinary Medicine, [6]Veterinary Anatomy and [8]Veterinary Pharmacology & Toxicology, University of Ibadan, Ibadan, Nigeria.
Gastrointestinal Physiology and Inflammation Research Unit, Department of Physiology, University of Ibadan, Nigeria
[2]Division of Medical Sciences, University of Victoria, Victoria, BC, Canada.
[4]Federal College of Animal Health and Production Technology, Ibadan, Nigeria.
[7a]Department of Livestock and Pasture Science, University of Fort Hare, Alice 5700, South Africa
[7b]Department of Agriculture and Animal Health, University of South Africa, Roodepoort, Johannesburg 1709, South Africa
[9]Phytomedicine and Phytochemistry Group, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa.
[10]Department of Environmental & Interdisciplinary Sciences, College of Science, Engineering & Technology, Vascular Biology Unit, Center for Cardiovascular Diseases, COPHS, Texas Southern University, Houston, Texas, USA.
[11]Department of Psychiatry and Behavioural Sciences, College of Medicine, Howard University Hospital, Howard University, Washington, District of Columbia, USA.

Summary: Exposure to pesticides and to the complex I inhibitor rotenone has been shown to recapitulate features of Parkinson's disease, including selective nigrostriatal dopaminergic degeneration and α-synuclein-positive cytoplasmic inclusions. Sixty mice were randomly divided into six groups (n=10) and orally treated for 28 consecutive days as follows; group 1: vehicle (10 mL/kg), group 2- vehicle + rotenone (10 mg/kg p.o. in 0.5% carboxymethyl cellulose (CMC), group 3 – rotenone + 100 mg/kg Luteolin, group 4 – rotenone + 200 mg/kg Luteolin, group 5 - 100 mg/kg Luteolin and group 6 - 200 mg/kg Luteolin, respectively. At the end of the experiment, neurobehavioral studies were carried out. Brain tissues were harvested for biomarkers of oxidative stress, , histology, and immunohistochemistry for Tumour Necrosis Factor alpha (TNF-α), Fragile X Mental Retardation Protein (FXMRP), serotonin, and tyrosine hydroxylase. Rotenone toxicity significantly increased oxidative stress biomarkers and acetylcholinesterase activity and decreased the antioxidant defence system. Significant reduction in motor coordination and movement disorder, together with vacuolation (demyelination) and atrophy of neurons, was observed in ROT-untreated mice. Treatment of mice with Luteolin lowered oxidative stress biomarkers and neuroinflammation, increased Fragile X mental retardation Protein expression, improved serotonin and tyrosine hydroxylase expression, and restored neuronal ultrastructure.

Keywords: *Rotenone, Luteolin, oxidative stress, inflammation, tyrosine hydroxylase*

**Authors for correspondence: toajibade@gmail.com; Tel: +234-9071842142*

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INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by degeneration of the substantia nigra pars compacta and by accumulation of α-synuclein in Lewy bodies (Santulli *et al*., 2022). One of the major historic milestones in PD ethiopathogenesis include the identification of Lewy bodies as intracytoplasmic inclusion bodies by Frederick Lewy in 1912 and the discovery of dopamine deficiency. Furthermore, the pioneering work of Arvid Carlsson and Oleh Hornykiewicz starting in 1957 also established the link between dopamine deficiency and PD. Interestingly, the work of Arvid Carlsson and Oleh

Hornykiewicz was supported by the proof of concept demonstrating clinical rescue in the first trial in PD patients with intravenous levodopa in 1961 and the introduction of high dosage levodopa therapy by George Cotzias in 1967 (Fahn, 2018).

Parkinson's sickness was first depicted by Dr. James Parkinson in 1817 as a "shaking palsy" (DeMaagd and Philip, 2015). Parkinson's disease is characterised by bradykinesia, rest tremor, unbending nature, and postural instability (Armstrong and Okun, 2020; Scanga *et al*., 2023). Sleep disorders have been recognized as the most common non-motor symptoms in PD and are associated with reduced cognition and health-related quality of life (Scanga *et al*., 2023). Kartik *et al*. (2023) reported motor and non-motor impairments in PD in old age. Human postmortem studies have revealed that PD patients exhibit neuronal loss in the substantia nigra pars compacta, locus coeruleus, and other neuronal populations (Simon *et al*., 2020). It has been estimated that the number of PD case will double from about 7 million in 2015 to about 13 million in 2040, with the resultant PD Pandemic in the future by the Global Burden of Disease Study (Dorsey, 2018). For now, the aetiology of PD remains uncertain and elusive.

Currently, there have been no effective treatment regimens or specific diagnostic biomarkers for PD (Bhattacharyya *et al*. 2023). Recently, protein dyshomeostasis has been identified as the hallmark of many age-related neurodegenerative disorders, including PD, as described by Kulkarni *et al*. (2023). Pharmacological treatment of PD aims to improve functional mobility and increase life expectancy in PD patients. This includes dopaminergic supplements with levodopa, catechol-O-methyltransferase (COMT) inhibitors, anticholinergic agents, dopaminergic agonists, and inhibitors of Monoamine oxidase B (MAO-B) (Marino *et al*., 2020). Alpha-synuclein monoclonal antibodies have been employed as disease-modifying therapies to minimise accumulation and progression of aggregated, toxic α-synuclein (Brys *et al*., 2019; Jankovic *et al*., 2018; Jankovic, 2019). It has been extensively documented that oxidative stress, neuroinflammation, mitochondrial dysfunction critical factors involved in the pathophysiology and pathogenesis of PD (Jung and Kim, 2018).

Moreover, different therapeutic regimens have been deployed for the management of PD. Dopamine replacement therapy remains the first line strategy in PD treatment with attendant side-effects such as fluctuations in motor response and dyskinesia (Bargiotas & Konitsiotis, 2013). Body of evidence has recommended medicinal plants, fruits, vegetables, and plant-derived phytochemicals including flavonoids to have beneficial effects on memory and learning (Patel *et al*. 2022; Spohr *et al*. 2022; Mhalhel *et al*., 2023; Onukak *et al*., 2025). In the last few decades, attention has been shifted to natural polyphenols for the management of neurodegenerative diseases (Emran *et al*., 2022; Lü *et al*., 2023). Polyphenols have been documented to exert neuroprotective effects by targeting multiple mechanisms, including anti-inflammatory, antioxidant, and metal-chelating activities in the central nervous system (CNS), as previously reported (Tsarouchi *et al*., 2022; Rodrigues-Costa *et al*., 2023). They also have the capacity to inhibit the formation of misfolded α-synuclein aggregates and to reduce mitochondrial dysfunction-induced oxidative stress and the inflammatory response (Gallardo-Fernández *et al*., 2022; Yamamoto *et al*., 2023). The antioxidant activity of polyphenols is known to contribute to the activation of the Nuclear Factor-Erythroid 2-related Factor 2 (Nrf2)/Antioxidant Responsive Element (ARE) as reported elsewhere (Costa *et al*., 2017).

Epigallocatechin-3-gallate (EGCG), a major flavanol in tea, has been reported to exert neuroprotective effects in various PD models (Sergi, 2022; Wang *et al*., 2023). Intake of the anthocyanin pelargonidin has been reported to dose-dependently attenuate behavioural and structural abnormalities, due to 6-OHDA toxicity and to decrease lipid (Roghani *et al*., 2010; Panchal *et al*., 2022; Ursu *et al*., 2022). The glycoside of quercetin − rutin, has also been shown to protect against 6-OHDA-induced neurotoxicity in experimental rat model (Enogieru *et al*., 2018; Christmann *et al*., 2021; Rodríguez-Arce and Saldías, 2021). Myricitrin, derived from the root bark of Myrica cerifera, and its aglycon, flavonol myricetin have also been shown to exert neuroprotective effects (Zhang *et al*., 2020; Bai *et al*., 2022; Banerjee *et al*., 2022; Azlan *et al*., 2023). Oxidative damage has been reported in the midbrain, olfactory bulb, striatum and cortex together with extensive microglial activation seen in both the SNc and striatum in rats treated with rotenone (Azimullah *et al*., 2023; Essam & Kandil, 2023). Hence, the search for novel therapeutic agents with fewer side effects is essential.

Luteolin, 3', 4', 5, 7-tetrahydroxyflavone, belongs to a group of naturally occurring compounds called flavonoids that are found widely in the plant kingdom. Vegetables and fruits such as celery, parsley, broccoli, onion leaves, carrots, peppers, cabbages, apple skins, and chrysanthemum flowers have been reported rich in luteolin (Li *et al*., 2019; Nabavi *et al*., 2015; Choi *et al*., 2017). The neuroprotective effects of Luteolin have been reported (El-Nashar *et al*., 2022; Rahimpour *et al*., 2023). This study is designed to investigate the reversal effects of Luteolin on PD-induced α-synuclein and Parkin over-expressions and reduction in the activity of tyrosine hydroxylase and density of dopamine transporter.

MATERIALS AND METHOD

Chemicals: Luteoline, rotenone, carboxymethyl cellulose (CMC Trichloro acetic acid (TCA), sodium hydroxide, O-dianisidine, and hydrogen peroxide (H_2O_2), xylenol orange (XO), potassium hydroxide, reduced glutathione (GSH), oxidized glutathione (GSSG), NaF, thiobarbituric acid (TBA), 1,2-dichloro-4-nitrobenzene, were purchased from Sigma (St. Louis, MO, USA). Normal goat serum, Biotinylated, and antibody 2-step plus Poly-HRP Anti Mouse/Rabbit IgG Detection System with DAB solution were purchased from Elabscience Biotechnology®, China), anti- Fragile X mental retardation protein1 monoclonal Antibody (E-AB-15046; 1:500 Dilution), Tyroinse hydroxylase Monoclonal Antibody (Cat Number: E-AB-70206; 1:500 Dilution), Serotonin Polyclonal Antibody (Cat Number: E-AB-16233; 1:200 Dilution), and TNF alpha Polyclonal Antibody (Cat Number: E-AB-40015; 1:200 Dilution). All other chemicals used for this study were of analytical grade.

Experimental Design and Induction of Parkinson Disease: Sixty mice were randomly divided into six groups (n=10) and orally treated for 28 consecutive days with the following; group 1: vehicle (10 mL/kg), group 2- vehicle + rotenone (10 mg/kg p.o. suspended in 1% chloroform reconstituted in 0.5% carboxymethyl cellulose (CMC), group 3 – rotenone + 100 mg/kg Luteolin, group 4 – rotenone + 200 mg/kg Luteolin, group 5 - 100 mg/kg Luteolin and group 6 - 200 mg/kg Luteolin, respectively.

Neurobehavioral tests

Hanging wire grip test: The hanging wire grip test was performed to evaluate the grasping ability of the forelimb and coordination of movement. The rats were placed with their forelimbs suspended from a wire 0.3 cm in diameter, 40 cm in length**,** and 45 cm above soft ground (Kim *et al.,* 2017). The time spent holding the wire was recorded during 2 daily trials for each rat. A maximum cut-off latency time of 120 sec was recorded.

Preparation of serum*:* The mice were anaesthetised with xylazine/ ketamine (v/v) at 0.1 mL per 100 g of mice and administered intramuscularly. Retro–orbital venous puncture was employed for blood collection into plain tubes. The blood samples were allowed to clot, and then centrifuged at 4, 000 *g* for 15 minutes. Serum was separated with a Pasteur pipette into another plain tube and then stored at 4ºC until needed.

Brain Sample Collection and Preparation: At termination of the study, the mice were humanely handled, brain tissues were harvested for biochemical assay and immunohistochemistry.

Neuronal Post-mitochondrial Fractions preparation: Brain samples were quickly excised, rinsed in iced-cold phosphate-buffered saline, blotted with filter paper, weighed, cut into bits and homogenised with homogenising buffer (0.1M phosphate buffer, pH 7.4) using a Teflon homogeniser. The homogenate obtained was centrifuged at 10,000 *g* for 10 min with a cold centrifuge at −4ºC to obtain post-mitochondrial fractions (PMFs). The supernatants (PMFs) obtained were used for biochemical assays.
The mice were handled in accordance with animal handling ethics. All procedures were performed in accordance with the recommendations of the NIH Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th ed. Washington (DC): National Academies Press (US).

Biochemical Assays

Determination of neuronal antioxidant defence status: The Superoxide dismutase (SOD) assay was performed according to the method of Misra and Fridovich (1972), with slight modifications as described by Oyagbemi *et al*. (2023). The glutathione peroxidase (GPx) activity was also measured according to Beutler *et al*. (1963), glutathione S-transferase (GST) was estimated by the method of Habig *et al.* (1974) using 1-chloro-2,4-dinitrobenzene as substrate, and the reduced glutathione (GSH) content was estimated by the method of Ellman (1959).

Determination of neuronal markers of oxidative stress: Hydrogen peroxide (H_2O_2) generation was determined using the method described by Wolff (1994). Malondialdehyde (MDA) as a product of lipid peroxidation was determined using the method described by Varshney and Kale (1990). Serum myeloperoxidase (MPO) was determined by the method described by Xia and Zweier (1997). The brain protein carbonyl content was determined using the method described by Reznick and Packer (1994). The acetylcholinesterase (AChE) activity was evaluated with the method of Ellman (1959).

Determination of serum total protein and nitric oxide: Serum Nitric oxide was determined using the method described by Olaleye *et al.* (2007), while serum total protein was determined by Biuret's method as described by Gornal *et al.* (1949).

Histological evaluation: Mid-sagittal cut of the whole brain was harvested, while the left hemisphere was sectioned for histological evaluation and fixed in 10% formalin. The fixed tissues were immersed in paraffin wax, sectioned, and mounted on slides for histopathological evaluation after staining with Hematoxylin, Eosin and Nissl as previously described by Drury and Wallington (1976).

Immunohistochemistry (IHC) protocol: Immunohistochemistry was done as described by Oyagbemi *et al.* (2019) Antibodies against anti- Fragile X mental retardation protein1 monoclonal Antibody (E-AB-15046; 1:500 Dilution), Tyroinse hydroxylase Monoclonal Antibody (Cat Number: E-AB-70206; 1:500 Dilution), Serotonin Polyclonal Antibody (Cat Number: E-AB-16233; 1:200 Dilution) with slight modification using 2-step plus Poly-HRP Anti Mouse/Rabbit IgG Detection System with DAB solution (Catalog number: E-IR-R217 from Elabscience Biotechnology®, China). Slides were removed, allowed to dry and a DPX mountant was applied. Sections were observed with light microscope (Leica LAS-EZ®) using Leica software application suite version 3.4 equipped with a digital camera.

Statistical analysis
All values were expressed as mean ± standard deviation (SD) and the test of significance between two groups was estimated with Student's t-test. The One-Way Analysis of Variance (ANOVA) with Turkey's post-hoc test of Graph pad prism 5.0 was also carried out with p-Values < 0.05 considered statistically significant (Fleiss *et al.,* 2003).

RESULTS

Neurobehavioral studies
The pole and the hanging wire tests revealed a significant reduction in the grasping ability of the forelimb and coordination of movement of ROT-untreated mice (Figures 1A & 1B). Treatment with LUT (100 mg/kg and 200 mg/kg) significantly improved muscular strength and the coordination of movement.

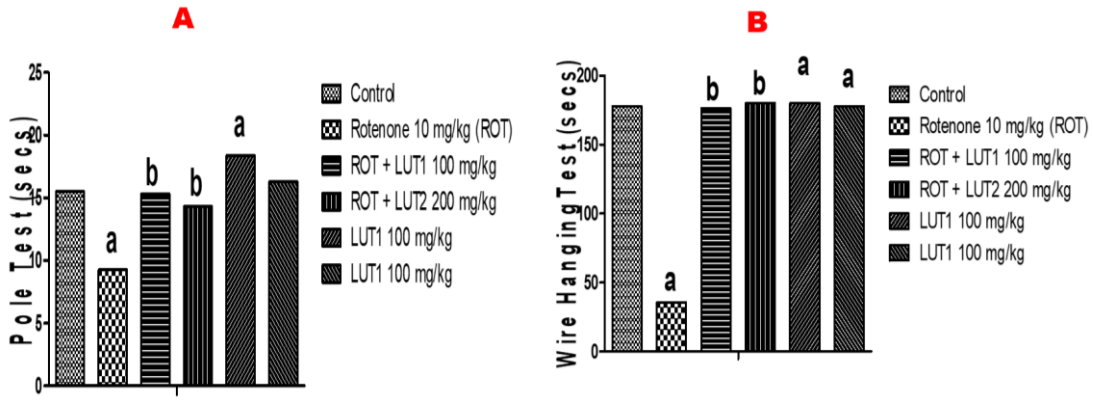

Figure 1.
The effect of luteolin on rotenone-induced Parkinson's disease in rats on neurobehavioral studies. Group A (Control), Group B (Rotenone (ROT; 10 mg/kg), Group C (ROT + Luteolin (LUT1 100 mg/kg), Group D (ROT + Luteolin (LUT2 200 mg/kg), Group E (LUT1 100 mg/kg), & LUT2 200 mg/kg). Superscript (a) indicates a significant difference when compared to the control, while (b) indicates a significant difference when compared to Rotenone alone. Values are presented as Mean ± SD (n = 5).

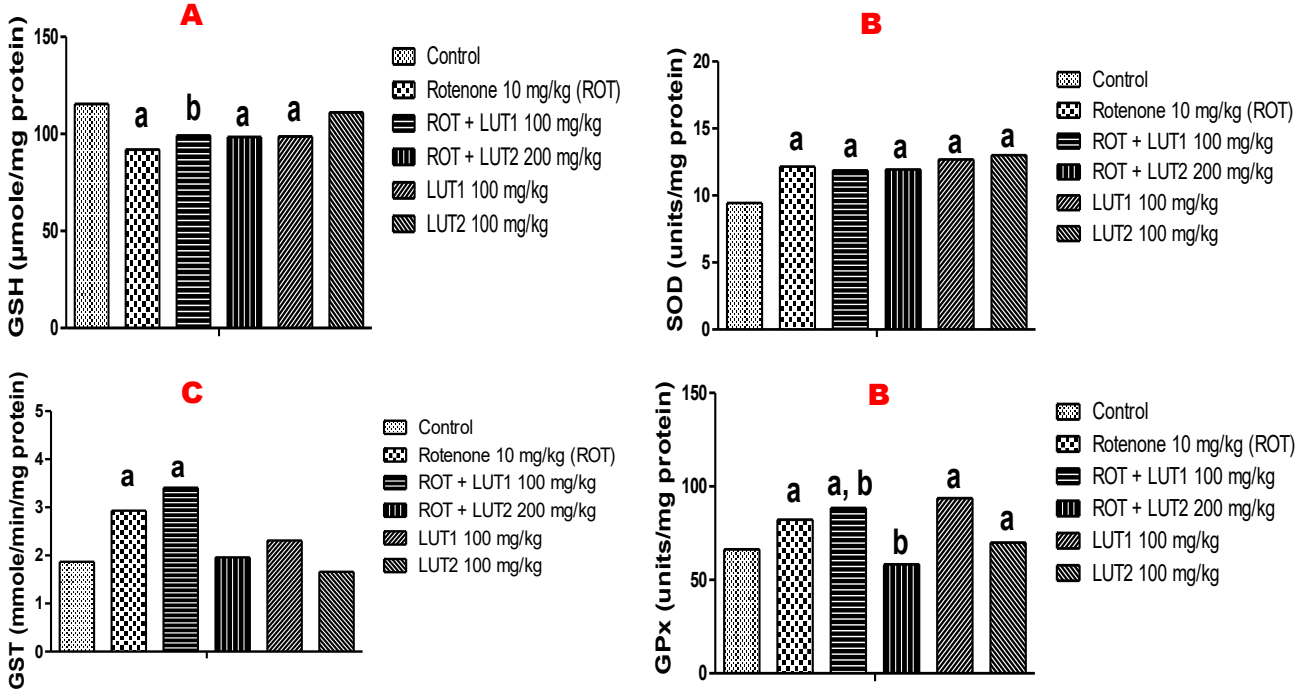

Figure 2.
The effect of luteolin treatment on rotenone-induced Parkinson's disease in rats on brain antioxidant status. Group A (Control), Group B (Rotenone (ROT; 10 mg/kg), Group C (ROT + Luteolin (LUT1 100 mg/kg), Group D (ROT + Luteolin (LUT2 200 mg/kg), Group E (LUT1 100 mg/kg), & LUT2 200 mg/kg). Superscript (a) indicates a significant difference when compared to the control, while (b) indicates a significant difference when compared to Rotenone alone. Values are presented as Mean ± SD (n = 5).

***In vivo* antioxidant defence status:** In Figure 2A, there was a noticeable significant ($p < 0.05$) reduction in reduced glutathione (GSH) content in ROT-intoxicated mice in comparison to the control and other treated groups. The antioxidant activity of LUT was demonstrated with a significant ($p < 0.05$) increase in GSH content of ROT-treated mice (Figure 2A). In figure 2B, ROT intoxication caused a significant ($P<0.05$) increase in superoxide dismutase (SOD) activity. Similarly, in ROT-treated rats with LUT (100 and 200 mg/kg) and LUT only LUT (100 and 200 mg/kg), we recorded a significant ($P < 0.05$) in SOD activity across treatment groups (Figure 2B). The activity of glutathione S-transferase (GST) increased significantly in ROT-untreated rats and ROT-treated with LUT (100 mg/kg) in comparison to the control (Figure 2C). However, ROT-treated with LUT (200 mg/kg) and TUL alone (200 mg/kg) did not cause any significant changes in the GST activity. In another experiment, the ROT-treated mice, we recorded a significant ($P<0.05$) increase in glutathione peroxidase (GPx) activity when compared to the control as indicated in figure 2D. Again, ROT-treated rats with LUT (200 mg/kg) and LUT alone (200 mg/kg) showed a significant reduction in GPx activity relative to the ROT-untreated mice and the control, respectively (Figure 2D).

Biomarkers of oxidative stress and neurotoxicity
In this study, oxidative stress biomarkers were assessed as indicators of neurodegeneration. The malondialdehyde (MDA) content, Hydrogen peroxide (H_2O_2) generation, and protein carbonyl levels were significantly elevated in ROT-

untreated mice in comparison to the control (Figures 3A, 3B, & 3C). The anti-oxidative property of LUT (100 mg/kg) was demonstrated with a significant reduction in MDA, H_2O_2, and protein carbonyl levels.

Data from Figure 3D a significant ($p<0.05$) increase in AchE activity in ROT-untreated mice. Co-treatment with LUT (100 and 200 mg/kg) caused a significant ($P<0.05$) decrease in AChE activity relative to ROT-untreated mice. Neuroinflammation was evident in ROT administered rats with a significant ($p<0.05$) increase in brain nitric oxide (NO) content (Figure 3E). The anti-inflammatory and neuro-modulatory actions of LUT (100 and 200 mg/kg) was demonstrated with a significant ($p<0.05$) reduction in brain NO content in a dose-dependent manner (Figure 3E).

Histology

The histology of the brain showed a moderate vacuolation (demyelination) and atrophy of the neurons in ROT-untreated rats (Figure 4). There were diffuse atrophy of neurons and a moderate gliosis were observed in ROT-treated with LUT (100 mg/kg) and LUT alone (100 mg/kg). However, there were no observable lesions in mice treated with 200 mg/kg of LUT (Plate 1).

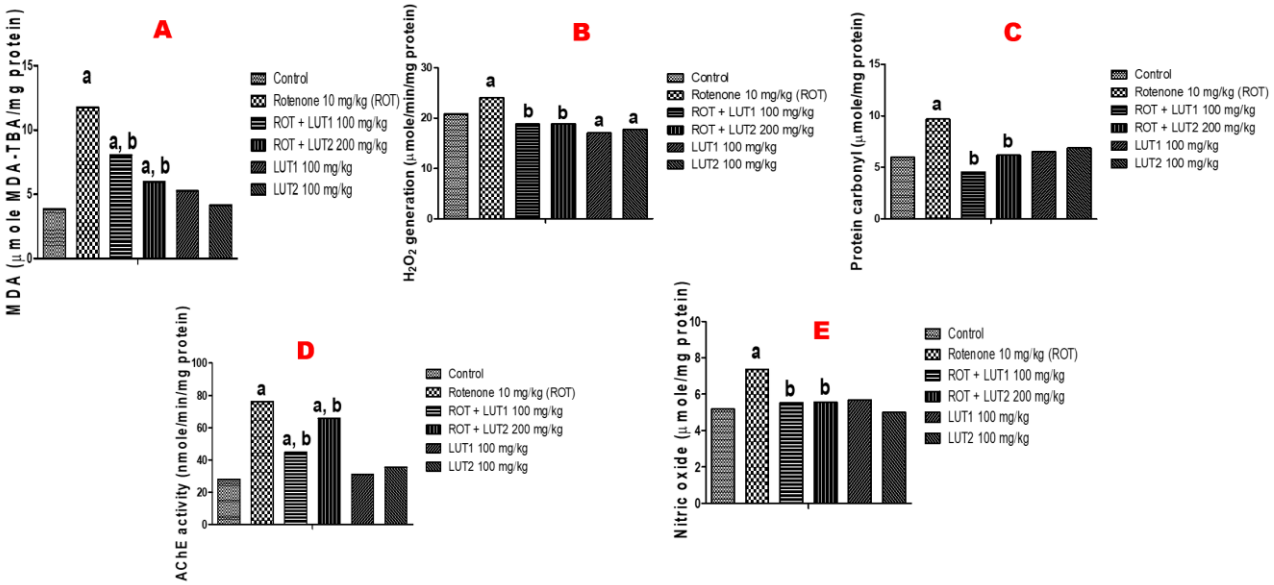

Figure 3.
The effect of luteolin treatment on rotenone-induced Parkinson's disease in rats on oxidative stress biomarkers and neurotoxicity. Group A (Control), Group B (Rotenone (ROT; 10 mg/kg), Group C (ROT + Luteolin (LUT1 100 mg/kg), Group D (ROT + Luteolin (LUT2 200 mg/kg), Group E (LUT1 100 mg/kg), & LUT2 200 mg/kg). Superscript (a) indicates a significant difference when compared to the control, while (b) indicates a significant difference when compared to Rotenone alone. Values are presented as Mean ± SD (n = 5).

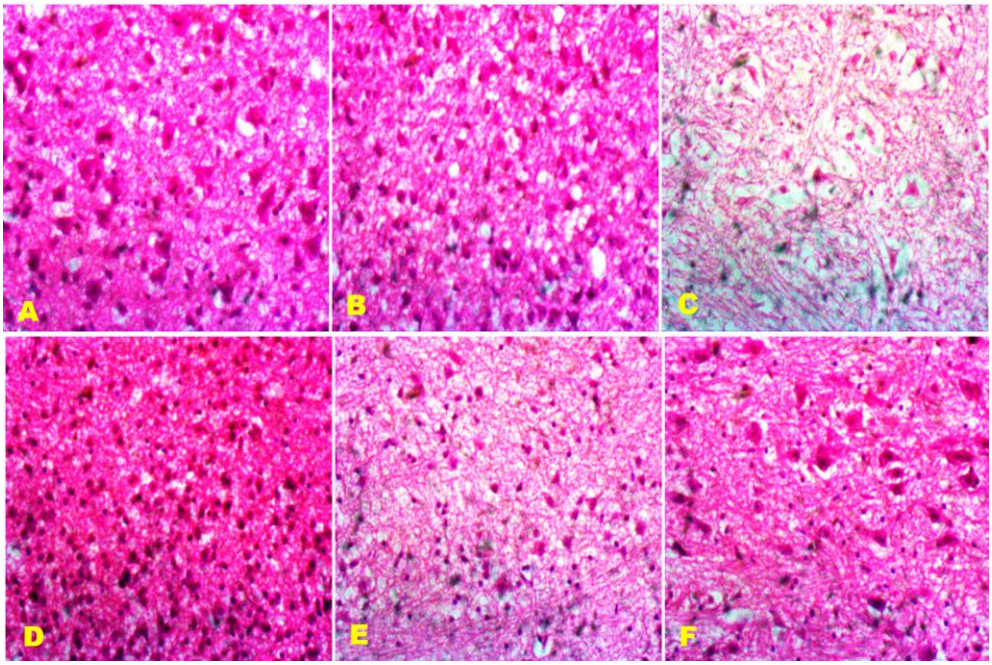

Plate 1.
Histology of the brain. Group A (Control), Group B (Rotenone (ROT; 10 mg/kg), Group C (ROT + Luteolin (LUT1 100 mg/kg), Group D (ROT + Luteolin (LUT2 200 mg/kg), Group E (LUT1 100 mg/kg), & LUT2 200 mg/kg). Group A: There is no observable lesion. Group B: There is moderate vacuolation of nerve fibres (demyelination) and atrophy of neurons. Group C: There is diffuse atrophy of neurons and moderate gliosis. Group D: There is no observable lesion. Group E: There is diffuse atrophy of neurons and moderate gliosis. Group F: There is no observable lesion. Slides stained with Heamtoxylin & Eosin (H&E). Magnification at X 400

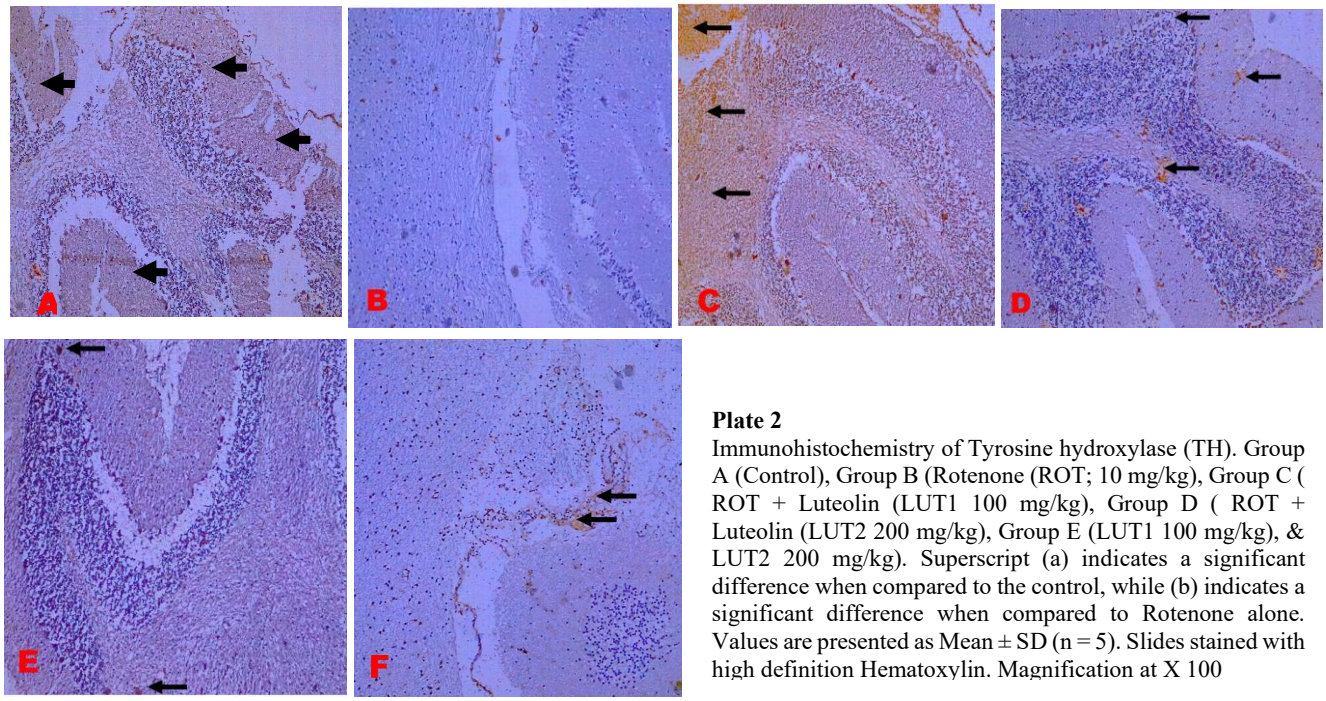

Plate 2
Immunohistochemistry of Tyrosine hydroxylase (TH). Group A (Control), Group B (Rotenone (ROT; 10 mg/kg), Group C (ROT + Luteolin (LUT1 100 mg/kg), Group D (ROT + Luteolin (LUT2 200 mg/kg), Group E (LUT1 100 mg/kg), & LUT2 200 mg/kg). Superscript (a) indicates a significant difference when compared to the control, while (b) indicates a significant difference when compared to Rotenone alone. Values are presented as Mean ± SD (n = 5). Slides stained with high definition Hematoxylin. Magnification at X 100

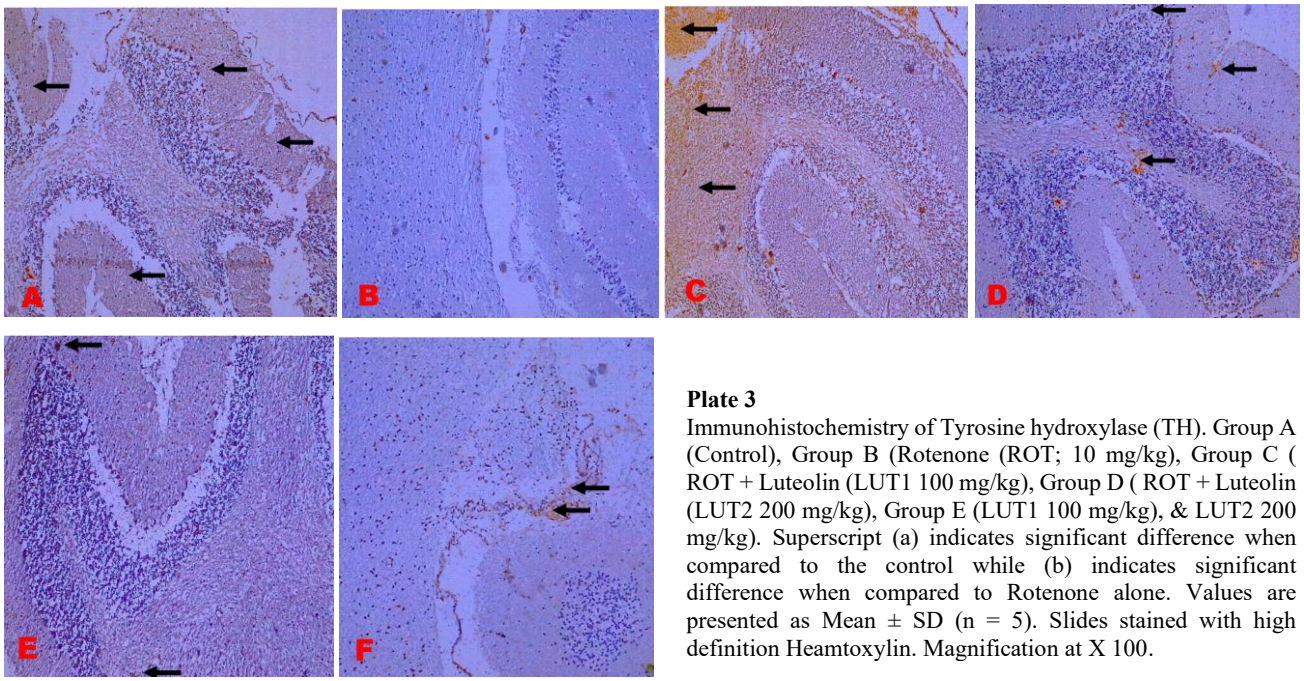

Plate 3
Immunohistochemistry of Tyrosine hydroxylase (TH). Group A (Control), Group B (Rotenone (ROT; 10 mg/kg), Group C (ROT + Luteolin (LUT1 100 mg/kg), Group D (ROT + Luteolin (LUT2 200 mg/kg), Group E (LUT1 100 mg/kg), & LUT2 200 mg/kg). Superscript (a) indicates significant difference when compared to the control while (b) indicates significant difference when compared to Rotenone alone. Values are presented as Mean ± SD (n = 5). Slides stained with high definition Heamtoxylin. Magnification at X 100.

Immunohistochemistry

Immunohistochemistry revealed an increase in the expression of TNF-α in ROT-untreated mice (Figure 5). However, the expression of TNF-α was not visible in ROT-treated LUT (100 mg/kg and 200 mg/kg) and LUT only (100 mg/kg and 200 mg/kg) as indicated in Plate 2. The immune-positive reactions of serotonin were less noticeable in ROT-untreated mice (Figure 6). On the other hand, higher serotonin expression was observed in the control, ROT-treated with LUT (100 mg/kg and 200 mg/kg), and LUT-only (100 mg/kg and 200 mg/kg) groups compared with ROT-untreated mice (Plate 3). The expression of tyrosine hydroxylase immunoreactivity was lower in ROT-untreated rats (Plate 4). Further, mice intoxicated with ROT and co-treated with LUT (100 mg/kg and 200 mg/kg) showed higher tyrosine hydroxylase immunopositive staining (Plate 4). The immunohistochemical expression of Fragile X Mental Retardation Protein (FXMRP) in ROT-untreated was higher than in the control, LUT (100 mg/kg and 200 mg/kg), and LUT only (100 mg/kg and 200 mg/kg), as indicated in Plate 5.

DISCUSSION

Parkinson's disease (PD) is caused by progressive degeneration of dopamine (DA) neurons in the Substantia Nigra pars compacta (SNpc), resulting in the deficiency of DA in the striatum (Yuan *et al*. 2010). This is anomaly is also accompanied with the gradual appearance of α-synuclein (α-syn)-containing neuronal protein aggregates (Tan *et al.,* 2020). Several studies have reported using ROT as an experimental model of Parkinson's disease (Musheghyan *et al.,* 2023; Xu *et al.,* 2023).

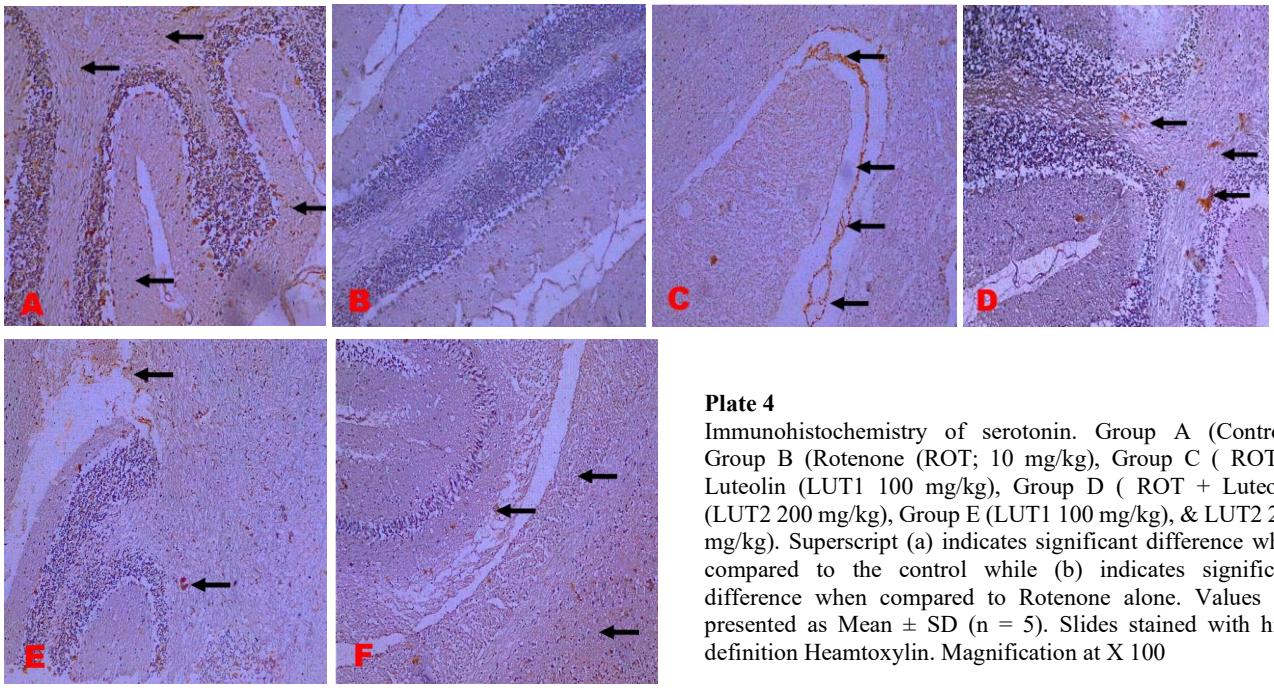

Plate 4
Immunohistochemistry of serotonin. Group A (Control), Group B (Rotenone (ROT; 10 mg/kg), Group C (ROT + Luteolin (LUT1 100 mg/kg), Group D (ROT + Luteolin (LUT2 200 mg/kg), Group E (LUT1 100 mg/kg), & LUT2 200 mg/kg). Superscript (a) indicates significant difference when compared to the control while (b) indicates significant difference when compared to Rotenone alone. Values are presented as Mean ± SD (n = 5). Slides stained with high definition Heamtoxylin. Magnification at X 100

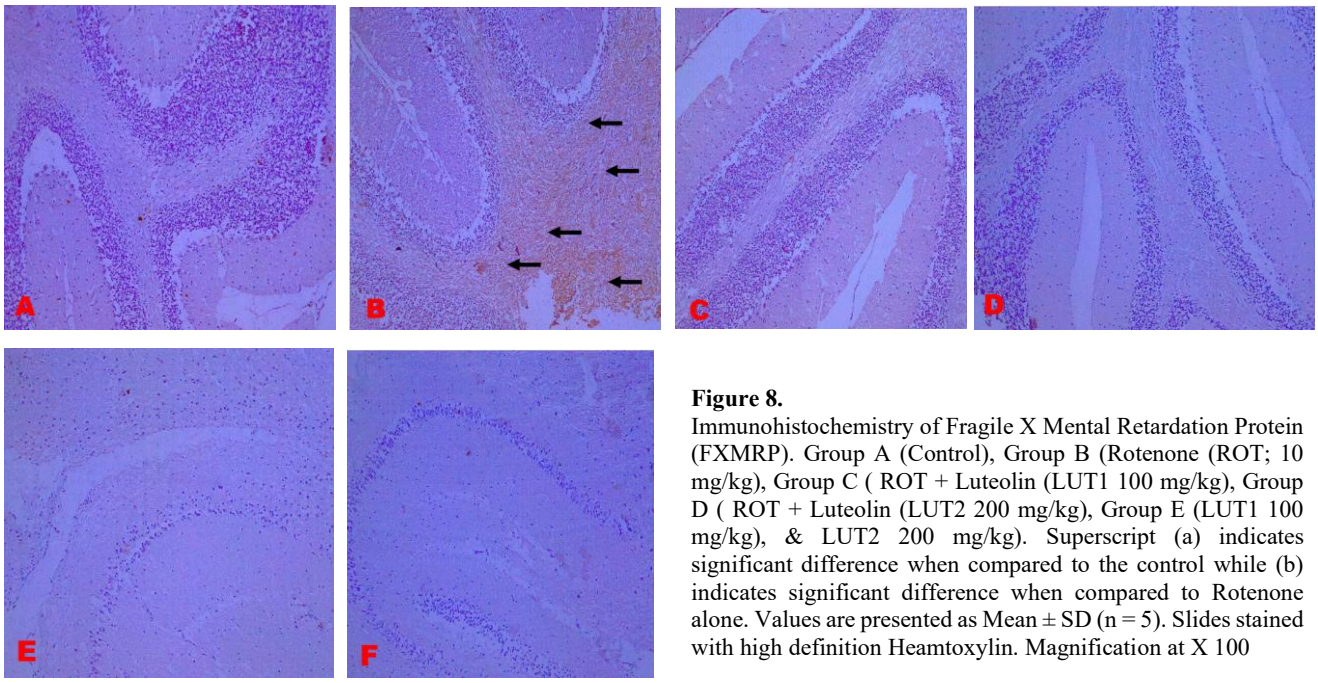

Figure 8.
Immunohistochemistry of Fragile X Mental Retardation Protein (FXMRP). Group A (Control), Group B (Rotenone (ROT; 10 mg/kg), Group C (ROT + Luteolin (LUT1 100 mg/kg), Group D (ROT + Luteolin (LUT2 200 mg/kg), Group E (LUT1 100 mg/kg), & LUT2 200 mg/kg). Superscript (a) indicates significant difference when compared to the control while (b) indicates significant difference when compared to Rotenone alone. Values are presented as Mean ± SD (n = 5). Slides stained with high definition Heamtoxylin. Magnification at X 100

The pathogenesis of ROT-induced Parkinson's disease involves loss of dopaminergic neurons in the substantia nigra, motor impairment, and α-synuclein-positive cytoplasmic inclusions (Sherer *et al.,* 2003; von Wrangel *et al.,* 2015).

From the present study, ROT intoxication induced Parkinson-like syndrome in exposed mice. This was indicated as the grasping ability of the forelimb was markedly reduced and dysfunctional coordination of movement with concomitant loss of muscular strength. The results of the present study are in consonance with previous studies on ROT-induced Parkinson's disease (Ishola *et al.,* 2023). We observed that co-treatment with LUT (100 and 200 mg/kg) significantly attenuated oxidative stress markers, along with a noticeable improvement in SOD, GST, and GPx activities. This finding supports the previously reported antioxidant capacity of LUT in the management of neurodegenerative disease conditions (Kothawade *et al.,* 2023).

Oxidative and free radical generation has been linked to several diseases including neurodegenerative disease conditions such as PD, multiple sclerosis, and Alzheimer's disease (Korovesis *et al.,* 2023; Kumar *et al.,* 2023; Olufunmilayo *et al.,* 2023). The *in vivo* antioxidant defence system is known to be compromised with a reduction in the intracellular defence system (GSH) and other key antioxidant enzymes in neurodegenerative diseases (Roy *et al.,* 2023). It was apparent from our results that ROT-induced oxidative stress as manifested in the heightened or

exaggerated increase in the biomarkers of oxidative stresses viz: MDA content, H_2O_2 generation, and protein carbonyl content with concomitant increase in antioxidant enzymes. The observable improvement in antioxidant enzymes might be related to adaptive response through Nrf2-Keap1 pathway. The Nrf2-Keap1 pathway has been reported in several research findings from which toxicant-induced oxidative stress has been implicated to stimulate this pathway (Song *et al.,* 2021). The Nrf2 is always located in the cytoplasm associated with Keap1 (Song *et al.,* 2021). However, exaggerated oxidative stress conditions have been documented to cause translocation of Nrf2 from the cytoplasm to the nucleus, where it binds to the Antioxidant Response Element (ARE) (Wang *et al.,* 2022). We therefore speculate that ROT-induced oxidative stress could facilitate the binding of the Nrf2 to ARE with resultant increase in the expression of genes associated with Protein carbonyl is a good candidate biomarker for protein oxidation especially tyrosine residues in proteins (Fedorova *et al.,* 2014). Oxidation of proteins and protein cross-linking have been linked with accelerated ageing as well as in Alzheimer's disease (AD), PD, and amyotrophic lateral sclerosis (ALS) as previously reported (Beal, 2002). The observed increase in protein carbonyl content could be associated with reported ageing in PD (Sharma *et al*. 2020). The combination oxidative stress and protein oxidation could be referred to as hallmarks of loss of muscular activity and ageing (Tanase *et al.,* 2016). The attenuation of protein oxidation with LUT was an indication that LUT could be employed as an anti-ageing agent.

The histology revealed a moderate vacuolation (demyelination) and atrophy of the neurons in ROT intoxicated mice. Research findings have shown that ROT intoxication could enhance neurotoxicity and associated pathology in the brain (Zahra *et al.,* 2020; Jing *et al.,* 2021; Singh *et al.,* 2023). Surprisingly, diffuse atrophy of neurons and a moderate gliosis were observed in ROT-treated with LUT (100 mg/kg) and LUT alone (100 mg/kg). However, there was no observable lesion in rats treated with 200 mg/kg of LUT. This is an indication that the neuro-protective action of LUT is dose-dependent with 200 mg/kg of LUT being the more potent dosage. In fact, the architecture of the brain was well preserved in mice co-treated with the higher dosage of LUT.

Neuro-inflammation has been linked with pathophysiology and pathogenesis of PD. One of the biomarkers of inflammation TNF-alpha was assessed in this study with immunohistochemistry. It was observed that ROT-untreated mice had higher TNF-α expression compared with the control. TNF-α has been implicated in the pathogenesis of ROT and Aluminium chloride-induced PD, thereby contributing to neuronal apoptosis (Alghamdi *et al.,* 2023; Rajendran *et al.,* 2023; Vastegani *et al.,* 2023). TNF-α is a ligand to death receptor pathway of apoptosis (MacFarlane *et al.,* 2003; Idriss *et al.,* 2000; Wang *et al.,* 2023). The reduction in the expression of TNF-α could be attributed to anti-inflammatory and anti-apoptotic activity of LUT. Several studies have reported the ameliorative effects of LUT on neuronal apoptosis and inflammation (Li *et al.,* 2021; Zhu *et al.,* 2022). Therefore, the mechanism underlying the anti-Parkinson effect of LUT may involve its anti-inflammatory and anti-apoptotic activities.

Fragile X Mental Retardation protein1 (FXMR1) controls the expression and function of numerous neuronal genes related to neuronal excitability and synaptic function (Tan *et al.,* 2020). In fact, FXMR1 is the most frequent cause of inherited intellectual disabilities and autism spectrum disorders, characterised by cognitive deficits and autistic behaviours (Romagnoli *et al.,* 2021). The up-regulation of FXMR1 has been positively correlated with mental retardation with attendant dysfunctional psychomotor activity in late-stage PD (Cabal-Herrera *et al*., 2020). We observed that ROT-untreated rats showed higher FXMR1 expression than ROT-treated rats with LUT (100 mg/kg and 200 mg/kg). This pathway might be a novel mechanism of LUT mitigating ROT-induced PD. Hence, FXMR1 could serve as a molecular therapeutic agent for the management of PD. Previous reports have documented the targeting FXMR1 could provide an insight into resolving PD severity (Chonchaiya *et al.,* 2009; Hall *et al.,* 2010). More so that there is currently no drug that could eliminate PD as most drugs are for palliative treatment (Korczyn, 2004; Koszła *et al.,* 2021).

The synthesis of neurotransmitters is highly regulated and conserved in the mammalian system (Nagatsu *et al.,* 2023). However, tyrosine hydroxylase has been reported to play a key role in the regulation of synthesis of neurotransmitters (Daubner *et al.,* 2011). Tyrosine hydroxylase is the enzyme responsible for catalysing the conversion of the amino acid L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) and the rate-limiting enzyme of catecholamine biosynthesis, which is a critical step in neurotransmitter synthesis, storage, and release (Wang *et al.,* 2023). In PD, the activity and expression of tyrosine hydroxylase have been found to be downregulated, resulting in the loss of dopaminergic neurons and a reduction in the availability of neurotransmitters for proper brain function (Amo-Aparicio *et al.,* 2023). In this study, tyrosine hydroxylase expression was less visible in a rat with ROT-induced PD, which is indicative of the pathological hallmark of tyrosine hydroxylase in PD. A deficiency of tyrosine hydroxylase is known to exacerbate PD and increase its severity (Mani *et al.,* 2023; Neal *et al.,* 2023; Reyes *et al.,* 2023). The observed higher expression of tyrosine hydroxylase in ROT-treated rats with LUT could indicate that LUT as a flavonoid could be involved in *de novo* synthesis of tyrosine hydroxylase. In the last two decades, flavonoids and plant-derived products have been reported for the management of PD as a therapeutic intervention with the ultimate aid of slowing down the progression of PD (de Rus Jacquet *et al.,* 2021; Behl *et al.,* 2022).

From this study, rotenone induced experimental Parkinson-like disease as demonstrated with generation of oxidative stress, reduction of brain antioxidant defense system and serotonin production, loss of muscular activity, neuro-behavioral changes, decline activity of tyrosine hydroxylase, exaggerated increase in acetylcholinesterase activity, fragile x mental retardation protein, and tumour necrosis factor alpha. Rotenone treated mice exhibited mild symptoms of Parkinson disease, improved muscular activity and serotonin. The neuro-protective effect of luteolin against rotenone-induced Parkinson disease could be through its antioxidant, anti-inflammatory together with the modulation of fragile x mental retardation protein and

serotonin production. Therefore, the use of luteolin as a functional food could open a novel therapeutic window for the management of Parkinson's disease and its associated pathology.

Authors' Contributions

All authors contributed to the study conception and design. The authors, Ademola Adetokunbo Oyagbemi and Ifeoluwa O Awogbindin, designed the experiment. Temitayo Olabisi Ajibade, Adeola Temitope Salami, Omolola Victoria Awoyomi, Oluwaseun Esan, Taiwo Olaide Oyagbemi, and Adewunmi Victoria, Adeogun performed laboratory work, biochemical assays, immunohistochemistry, and statistical analysis. Ademola Adetokunbo Oyagbemi, Oluwamayowa Igado, Ishmael Festus Jaja, Temidayo Olutayo Omobowale, Olufunke Eunice Ola-Davies, Adebowale Benard Saba, Adeolu Alex Adedapo, Oluwafemi Omoniyi Oguntibeju, Momoh Audu Yakubu, Evaristus Nwulia supervised the experiment, prepared the manuscript, proofread, and approved the submission.

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