



Neuroprotective effects of zinc oxide nanoparticles of epigallocatechin-3-gallate on rotenone-induced cerebellar neurotoxicity and histological alterations in rats

Isaac Olawale Babatunde^{1,4}, Olu Israel Oyewole¹, Ebenezer Idowu O. Ajayi¹, George Emmanuel Taiwo², Johnson Olaleye Oladele^{3,4}

¹Department of Biochemistry, Faculty of Basic and Applied Sciences, Osun State University, Osogbo, Nigeria

²Department of Physiology, Faculty of Basic Medical Sciences, Osun State University, Osogbo, Nigeria

³Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX 77845, USA

⁴Phytochemistry and Phytomedicine Research Unit, Royal Scientific Research Institute, Osogbo, Osun State, Nigeria

Corresponding author's email: isaac.babatunde@uniosun.edu.ng

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ABSTRACT

Rotenone, an environmental neurotoxicant, induces neurotoxicity by promoting mitochondrial function impairment, redox inflammation, and neurodegeneration. The study examined the neuroprotective efficacy of zinc oxide nanoparticles of epigallocatechin-3-gallate (ZnONP-EGCG) in cerebellar neurotoxicity mediated by rotenone in rats. A total of fifty-four male adult Wistar rats (180 – 220 g) were randomized to nine groups: Control, RTN (2.5 mg/kg, rotenone); RTN + Sinemet®, and groups treated with ZnONP-EGCG at doses of 10, 20, or 40 mg/kg, administered either alone or alongside rotenone once daily for 14 days. Post-treatment analyses of cerebellar homogenate were performed to assess biomarkers of oxidative stress, inflammation, neurotransmission, and cellular integrity, as well as histopathological examination. Rotenone administration significantly altered cerebellar redox homeostasis as it decreased the endogenous antioxidant enzyme activity (glutathione-S-transferase, glutathione peroxidase, catalase, and superoxide dismutase), with a concomitant increase in lipid peroxidation. It also markedly decreased neurotransmitter (dopamine and γ -aminobutyric acid) levels, intensified inflammatory signaling by elevating inflammatory cytokines (interleukin-6 and tumor necrosis factor- α) levels, and mediators (C-reactive protein, cyclooxygenase-2, and nitric oxide levels, as well as the activity of myeloperoxidase); whereas it increased DNA fragmentation, decreased glucose and total protein levels, and caused histopathological alterations in the cerebellar neuron architecture. Conversely, treatment with ZnONPs-EGCG restored antioxidant enzyme activities, suppressed lipid peroxidation and the inflammatory cascade, preserved dopaminergic and GABAergic neurotransmission, normalized cerebellar metabolic alterations, maintained cellular integrity, and minimized DNA damage and cerebellar histopathological alterations. Overall, these findings support ZnONPs-EGCG as a promising nanotherapeutic strategy for mitigating cerebellar neurotoxicity associated with environmental exposures.

Keywords: ZnONPs-EGCG, Oxidative stress, Rotenone, Cerebellar, Neurotoxicity

1.0 INTRODUCTION

Human and animal exposure to the pesticide rotenone, at chronic low doses, has been linked to neurotoxicity and neurodegeneration, which are considered risk factors for Parkinson's disease (PD) (Grisotto *et al.*, 2025). Rotenone, a pesticide derived from a plant source, is used widely as a piscicide and molluscicide in aquaculture and as a broad-spectrum insecticide in homes, gardens, and agriculture. Consumption of fish harvested by prior rotenone treatment, use of contaminated water, ingestion of rotenone-containing plants, accidental skin contact, or inhalation during formulation of rotenone-containing pesticides are parts of various routes of exposure to animals and humans (Tat *et al.*, 2024; Lunghi *et al.*, 2025). Due to the lipophilic nature of rotenone, it can easily penetrate the blood-brain barrier and specifically inhibits mitochondrial complex 1 of dopaminergic neurons, thus impairing electron transport and oxidative phosphorylation processes, which enhances the

production of Reactive Oxygen Species (ROS), declines the cellular antioxidant system, activates inflammatory cascade reactions, disrupts synaptic function, and disrupts neurobiochemical homeostasis. Collectively, these result in neuronal cell death (Riad *et al.*, 2025; Rubio *et al.*, 2025) and are attributes of a reliable model for induced neurotoxicity and Parkinsonian features in rats.

Aside from the substantia nigra and motor cortex, which are mainly affected by rotenone, studies have linked this neurotoxicant exposure to cerebellar toxicity and degeneration (Fikry *et al.*, 2022; Onyeleonu *et al.*, 2025). The cerebellum functions principally in motor control and coordination, and also supports cognition and emotion; impairment of which results in cerebral dysfunction, which is implicated in the physiology of PD and other neurological diseases (Onyeleonu *et al.*, 2025). Due to high metabolic demands and a dense population of Purkinje neurons,

the cerebellum is vulnerable to rotenone-mediated oxidative stress, neuroinflammation, neurotransmitter dysregulation, and structural alterations, characterized by Purkinje cell loss and granular layer degeneration (Onyeleonu *et al.*, 2025).

One intriguing catechin found in green tea is epigallocatechin-3-gallate (EGCG), a bioactive polyphenolic compound identified as a promising catechin capable of exerting multifaceted effects against many interconnected mechanisms involved in neurodegeneration (Islam *et al.*, 2025). Suppression of neuronal toxicity and enhancement of cognitive function through its antioxidant and anti-inflammatory properties have been documented in many *in vitro* studies (Shen *et al.*, 2024; Al-Zharani *et al.*, 2025). These findings highlight EGCG as a potential neuroprotective candidate, but its usage is limited owing to poor bioavailability (< 5%) as it is rapidly metabolized by the liver and intestine (Sun *et al.*, 2024). Based on this limitation, several researchers have suggested nanoformulation strategies to enhance its delivery, bioavailability, and therapeutic efficacy (Wang *et al.*, 2022).

Recently, biomedical applications of zinc oxide nanoparticles have drawn more attention due to their multifaceted effects in targeting neurodegeneration, as well as their biocompatibility, ease of cellular uptake, and delivery to brain cells (Tang *et al.*, 2024). Therefore, functionalizing ZnONPs with phytoactive compounds like EGCG, as dual-function nanoparticles, might be beneficial in attenuating cerebellar neurotoxicity and neurodegeneration. In previous studies, ZnONPs-EGCG had been synthesized and characterized. Reports from its *in-vivo* application have shown it to be a potent anticancer drug that is non-cytotoxic to normal human cells but cytotoxic to human cancer lines such as PC-3 and CCL-75 (Samutprasert *et al.*, 2018). It has additionally been documented to exert antimicrobial and antioxidant effects (Suresh *et al.*, 2015). Furthermore, research has pointed out the role of ZnONPs and phytoactive compounds in combating oxidative damage and neuronal degeneration in brain tissues, including the cerebellum (Adelakun *et al.*, 2024; El-Beltagy *et al.*, 2026).

Despite these promising findings, limited information exists regarding the neuroprotective effects of ZnONPs-EGCG against rotenone-induced cerebellar neurotoxicity. Therefore, the present study was designed to investigate the neuroprotective impact of ZnONPs-EGCG on rotenone-induced cerebellar damage in Wistar rats. Specifically, the study aimed to evaluate their effects on antioxidant defense systems, neurotransmitters, neurobiochemical markers, inflammatory cytokines, and histopathological changes in cerebellar tissues. Findings from this research may

provide further insight into the therapeutic potential of nanoformulated EGCG in the management of cerebellar neurotoxicity and neurodegeneration.

2.0 MATERIALS AND METHODS

2.1 Materials

Epigallocatechin-3-gallate (98%) was obtained from Kan Phytochemical PVT Ltd, India. Total protein and Glucose (S.L.) kits were obtained from AGAPPE Diagnostic Ltd, India. Zinc acetate, dimethyl sulfoxide, adrenaline, KCl, Na₂HPO₄ · 2H₂O, NaH₂PO₄ · 2H₂O, and glutathione were obtained from Sigma- Aldrich, Inc. (St. Louis, USA). Rat ELISA kits for 5-hydroxytryptamine (5-HT), Ellman's reagent, COX-2, TNF-, and IL-6 were purchased from Mormed Medical Equipment (Nanjing, India). Rotenone and corn oil used were products of Forever Analytical (China) and Central Drug House Ltd (India), respectively.

2.2 Synthesis and characterization of Zinc nanoparticles of EGCG

The phytochemical-mediated approach was used for the synthesis of ZnONPs-EGCG, where the phytoactive compound, epigallocatechin-3-gallate, was used to mediate the synthesis of ZnONPs. The approach basically requires the reduction of zinc ions from any precursor salt (e.g., zinc acetate) to ZnONPs by EGCG. With slight modifications, the synthesis followed the combined methods of Jeyaleela *et al.* (2020) and Faisah *et al.* (2021). In brief, zinc acetate solution (0.02 M) and EGCG solution (0.1 M) were first prepared separately using distilled water. EGCG solution was added in drops to a conical flask containing the zinc acetate solution (4:1, v/v), placed on a magnetic stirrer at 40°C. The resultant colored colloid was continuously stirred for 1 hour. The UV-Vis spectroscopy analysis of the solution was done by scanning with a spectrophotometer (Thermo Scientific Evolution 220) over a range of 200–800 nm. The process was repeated three times, and consistent peaks were obtained. Thereafter, the nanoparticle formed, which appeared as a suspension, was centrifuged for 10 minutes at 4000 rpm to obtain a pellet, which was dried in a water bath at 50°C and stored for biochemical analyses. The process was scaled up for larger production.

2.3 In-vivo Study

2.3.1 Laboratory Animals

In all, fifty-four (54) male Wistar rats weighing 180 - 220 g were employed for this investigation. The University of Ilorin Animal House was the source from which the experimental rats were procured. Two weeks before the experiment, the rats were transported to the Central Animal House of the Osun State University, Osogbo, for acclimatization to laboratory conditions. The rats were kept in clean cages and maintained at standard atmospheric conditions (45-50% humidity

and temperature of $22 \pm 3^{\circ}\text{C}$) for a 12-hour daylight/night cycle. The rats were placed on standard chow and water *ad libitum*.

Ethical approval was obtained for the study from the Health Planning, Research and Statistics Department, Ministry of Health, Osogbo, Osun State, Nigeria (OSHREC/PRS/2026/842/01/232). The animals were handled by adhering to the Care and Guide protocol for laboratory animals of NIH (NIH Publication No. 85–23, amended 1985) and guidelines established by the College of Health Sciences, Osun State University, Osogbo, Nigeria.

Cerebellar neurotoxicity was induced in rats *via* intraperitoneal administration of 2.5 mg/kg rotenone for 14 consecutive days, adhering to the prior methods of Riad *et al.* (2025). The standard drug, Sinemet[®], and varying doses of the nanoparticles were administered orally. Dose selection for the standard drug and ZnONPs-EGCG was based on previous reports by Hassan *et al.* (2025) and Yeni *et al.* (2024), respectively. Corn oil containing 2% dimethyl sulfoxide (DMSO) served as a vehicle for the drug, rotenone, and nanoparticles.

Experimental Design

The rats were randomized into 9 groups (7 per group) and treated as follows:

Control (Normal): received 0.2 ml of corn oil (containing 2% DMSO, intraperitoneally)

RTN: received 2.5 mg/kg rotenone (RTN)

RTN + NPT1: received 2.5 mg/kg (RTN) + 10 mg/kg of ZnONPs-EGCG

RTN + NPT2: received 2.5 mg/kg RTN + 20 mg/kg of ZnONPs-EGCG

RTN + NPT3: received 2.5 mg/kg RTN + 40 mg/kg of ZnONPs-EGCG

RTN + Sinemet[®]: received 2.5 mg/kg RTN + Sinemet[®] (Levodopa[®]/Carbidopa[®] -- 250/25 mg/kg)

NPT1: received 10 mg/kg ZnONPs-EGCG

NPT2: received 20 mg/kg ZnONPs-EGCG

NPT3: received 40 mg/kg ZnONPs-EGCG

The rats received all treatments for 14 days, and 24 hours following the last treatment, they were sacrificed.

2.4 Harvesting of Brain and Preparation of the Cerebellar Homogenate

The rats were sacrificed by cervical dislocation after being weighed and euthanized. The brains of rats were excised, weighed, placed on ice, and further dissected to remove the cerebellum under the guidance of a trained anatomist. The cerebellum was minced into pieces on ice, then homogenized in 0.1 M phosphate buffer (pH 7.4) at a ratio of 1:4. Thereafter, to extract the post-mitochondrial fraction, the homogenate was centrifuged at $12,000 \times g$ for 20 minutes (Oladele *et al.* 2025). For biochemical analyses, the supernatant was

kept at -20°C . Also, two cerebellar samples from each group were used for histopathological examination, and these were preserved by submerging in 10% neutral buffered formalin (pH 6.8).

2.5 Biochemical Markers Assessment

2.5.1 Measurement of Cerebellar Neuronal Antioxidant Enzyme Activity

The cerebellar supernatant's neuronal antioxidant enzyme activity was estimated as follows: As previously reported by Misra and Fridovich (1972), superoxide dismutase (SOD) activity was evaluated by checking how well it stopped adrenaline from oxidizing in a solution of alkaline pH. In brief, a cuvette was filled with 2.5 ml of carbonate buffer (0.05 M, pH 10.2) and a 10% cerebellum aliquot. The absorbance of the resultant mixture was then measured at 480 nm at 30-second intervals over 150 seconds to determine the SOD activity after 0.3 ml of freshly prepared adrenaline solution (0.3 mM) was rapidly added.

Catalase (CAT) activity was assessed spectrophotometrically following the earlier method of Hamza and Hadwan (2020), which entails measuring the unconsumed hydrogen peroxide left by endogenous catalase present in cerebellar supernatant. Briefly, 1 ml of cerebellar supernatant and 2ml of H_2O_2 (10 mM) prepared in the presence of phosphate buffer (7.4, 50 mM) were transferred into a tube, vortexed, and incubated (37°C , 2 min). Thereafter, treated with 6 ml of working reagent (containing anilinium sulfate, hydroquinone, and ammonium molybdate), incubated, and analyzed at 550 nm.

Glutathione peroxidase (GPx) activity assessment was in tandem with Rotruck *et al.* (1973) methods, which involves concurrent oxidation of reduced glutathione with decomposition of peroxides (H_2O_2). Briefly, at 37°C for 3 min, the working solution (containing sodium azide, reduced glutathione, phosphate buffer, hydrogen peroxide, and cerebellar supernatant) was incubated. The reaction was then stopped by adding trichloroacetic acid and centrifuging. Thereafter, 1 ml of Ellman's reagent and 2 ml of K_2HPO_4 were combined with the acquired supernatant, which resulted in a colorful product whose absorbance was measured at 412 nm.

The formation of chromogenic glutathione conjugate, which absorbed UV light at a wavelength of 340 nm, was used for the estimation of the enzymatic activity of Glutathione-S-transferases (GST). Succinctly, 30 μl of GSH (0.1 M) and cerebellar aliquot alongside 2.79 ml phosphate buffer (0.1 M, pH 6.5) were transferred into a test tube already filled with 150 μl of CDNB (20 mM). The reaction mixture was kept on stand for 1 min before the absorbance was read against a blank (Habig *et al.*, 1974)

2.5.2 Assessment of Cerebellar Neuronal Lipid Peroxidation Level

Principally, this involved interacting membrane lipid oxidative degradable products with thiobarbituric acid, forming colored products whose absorbance was read at 532 nm (Varshney and Kale, 1990). Briefly, 0.4 ml of cerebellar supernatant in buffer was mixed with trichloroacetic acid (30%, 0.5 ml), thiobarbituric acid (0.75%, 0.5 ml), and incubated (45 min, 80 °C), leading to the formation of malondialdehyde obtained as a clear supernatant whose absorbance was measured at 532 nm against a blank.

2.5.3 Determination of Cerebellar Dopamine and γ -Aminobutyric Acid (GABA)

Quantitative assessment of the neurotransmitters dopamine and GABA in the cerebellar supernatant was conducted using the ELISA technique. For each assay, their ELISA kit's instructional protocol was followed.

2.5.4 Assessment of Glucose and Total Protein Levels

To assess the cerebellar neuronal metabolic damage and tissue integrity, the total protein and glucose levels were determined spectrophotometrically following their AGAPPE kit instructional protocol.

2.5.5 Quantification of Fragmented DNA in Cerebellar Supernatant

The percentage of fragmented DNA level in cerebellar supernatant was measured using a diphenylamine (DPA)-based colorimetric method (Al-Okbi *et al.*, 2011). DNA was first extracted from the cerebellar pellet and supernatant by treatment with 10% Trichloroacetic acid (TCA). The extracted DNA from both was treated with DPA reagents and kept at 4 °C for 48 hours. At wavelengths of 578 and 600 nm, the absorbance of the resulting mixture was read for the supernatant and pellet, respectively. Thereafter, the optical density ratio of the supernatant to the pellet was compared to determine the percentage (%) of fragmented DNA.

2.5.6 Assessment of Cerebellar Neuronal Inflammatory Markers

The ELISA method was employed for quantifying levels of rat interleukin-6 (IL-6), C-reactive protein (CRP), tumor necrosis factor-alpha (TNF- α), and the activity of cyclooxygenase-2 (COX-2) in the cerebellar supernatant. ELISA kits for each assay were used by strictly adhering to the manufacturer's prescribed methodology.

Nitric oxide (NO), an indicator of neuronal oxidative stress/nitrosative damage and neuroinflammation, was assessed in the cerebellar supernatant through the Griess reaction with nitrites, which leads to the production of detectable chromogenic products (Green *et al.*, 1982). To do this, 0.4 ml of Griess reagent was first transferred into a test tube, followed by the addition of 0.4 ml of cerebellar aliquot. The absorbance of the resulting mixture was read after incubating at 25 °C for 20 min.

2.5.7 Histopathology

/eosin stain for microscopic analysis.

2.5.8 Statistical Analyses

One-way ANOVA with Tukey's post hoc multiple-comparison test was used to compare all experimental groups after analyzing the data obtained for this study. Version 8 of GraphPad Prism software (San Diego, CA) was used for data analysis. The results were expressed as mean $\pm$ standard deviation (SD). Values with $p < 0.05$ were considered to be statistically significant.

3.0 RESULTS

3.1 Optical Analysis of ZnONPs-EGCG

The characteristic absorption band ($\lambda_{\max}$) values were found to be ~ 320 nm (Fig. 1). As $\lambda_{\max}$ peaks were obtained for the three trials, which were 318, 320, and 318 nm, with a broad band around 284 and 360 nm, demonstrating reproducible UV-Vis spectra across independent syntheses. The color changed from white to a dusty palette col

The cerebellum was sectioned and treated with hematoxylin

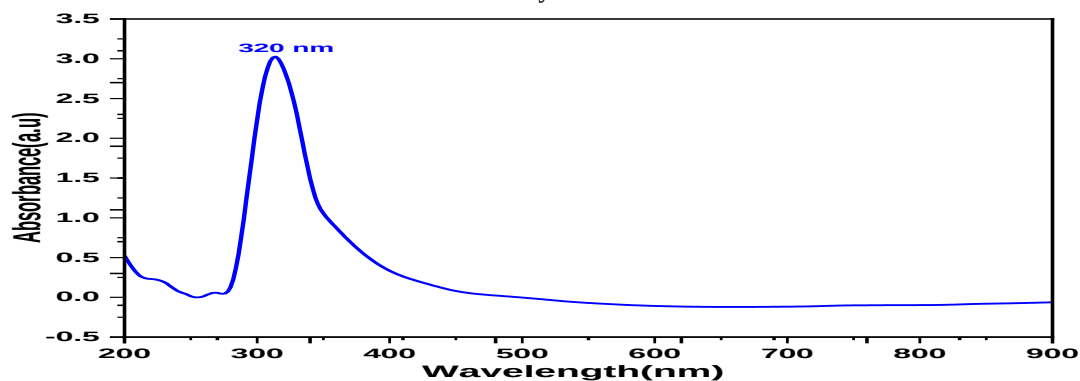
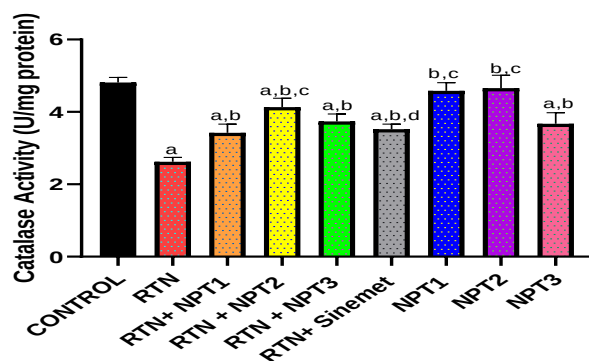
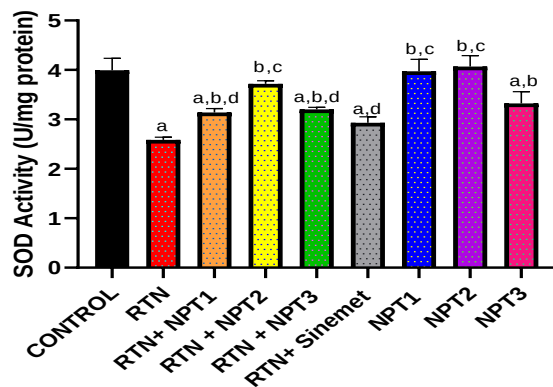


Fig. 1: Electronic absorption spectrum of ZnONPs-EGCG in the UV region with $\lambda_{\max}$ of 320 nm

3.2 Neuronal Antioxidant Defense Enzyme Activities in the Cerebellum of Experimental Rats

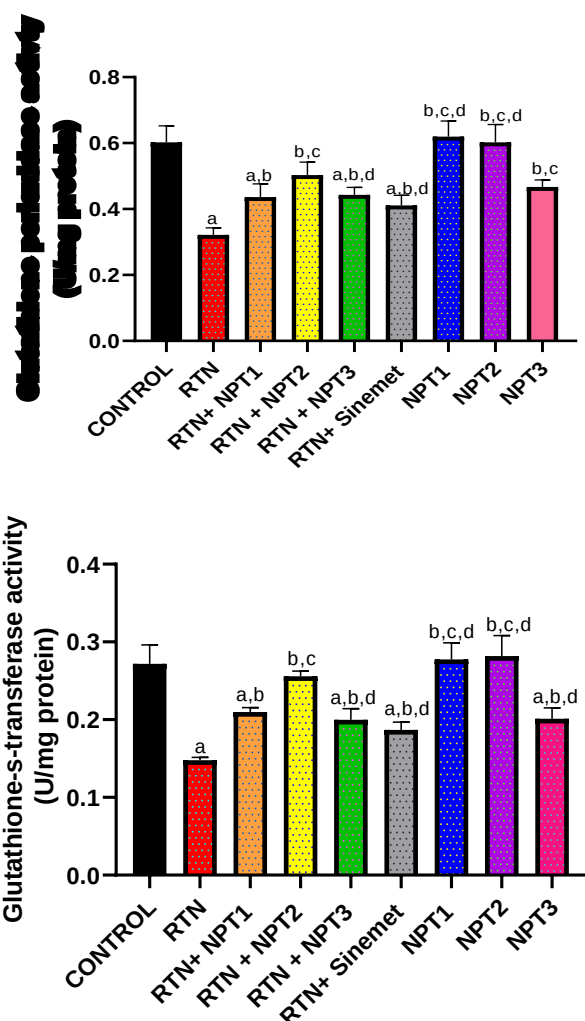
The activities of cerebellar endogenous antioxidant enzymes: Superoxide dismutase (SOD, Fig. 2a), catalase (CAT, Fig. 2b), glutathione peroxidase (GPx, Fig. 2c), and glutathione-S-transferase (GST, Fig. 2d) were significantly ($p < 0.05$) decreased in rats intoxicated with rotenone only in this study. However,

treatment with either Sinemet® or ZnONPs-EGCG significantly ($p < 0.05$) increases their activities. It is worth noting that treatment with 10 and 20 mg/kg of nanoparticles exhibited a dose-dependent increase in the antioxidant enzymatic activities as compared to the RTN groups. However, the effect was reduced in rats administered 40 mg/kg.



(a)

(b)



(c)

(d)

Fig. 2: Cerebellar SOD, CAT, GPx, and GST activities in the experimental rats

The results are displayed as mean $\pm$ SD; n=5 per group. Superscripts **a, b, c, & d** denote those statistically significant differences ($p < 0.05$) when compared with control, RTN-treated only, RTN+NPT1, and RTN+NPT2, respectively.

3.3 Cerebellar Lipid Peroxidation Levels in the Experimental Rats

Fig. 3 illustrates the level of lipid peroxidation assessed as malondialdehyde (MDA) in the experimental rat

cerebellum. The rats exposed to rotenone only without treatment, i.e., the RTN group, had significantly higher ($p < 0.05$) cerebellar MDA levels compared to the control group. However, Treatment with ZnONPs-EGCG or sinemet® significantly reversed the observed rise in cerebellar MDA levels. Notably, rats treated with 20 mg/kg ZnONPs-EGCG showed the highest mitigation of lipid peroxidation.

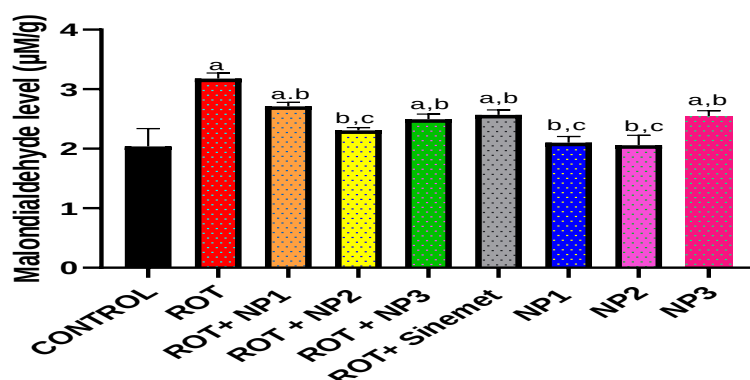


Fig. 3: Cerebellar MDA levels in the experimental rats

The results are displayed as mean $\pm$ SD, with $n=5$ per group. Superscripts **a**, **b**, & **c** denote those statistically significant differences ($p < 0.05$) when compared with control, RTN-treated only, and RTN+NPT1, respectively.

3.4 Cerebellar Dopamine and GABA Levels Across Experimental Groups

The levels of neurotransmitters, dopamine and GABA, in the cerebellum of the experimental rats were depicted in Fig.4. Rotenone intoxication markedly

decreases the cerebellar dopamine and GABA contents when compared to the control group. Nevertheless, co-administration with either ZnONPs-EGCG or Sinemet® significantly reversed the decline in the cerebellar content of these neurotransmitters when compared to the RTN group. Importantly, co-administration with 10 - 40 mg/kg of ZnONPs-EGCG dose-dependently enhanced these neurotransmitter contents in the cerebellum. With 20 mg/kg of ZnONPs-EGCG offering the highest effect.

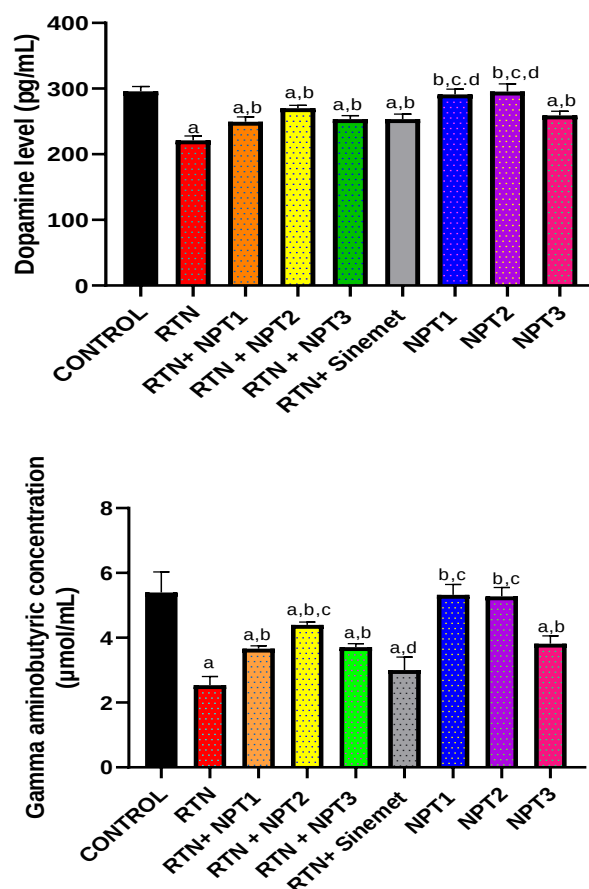


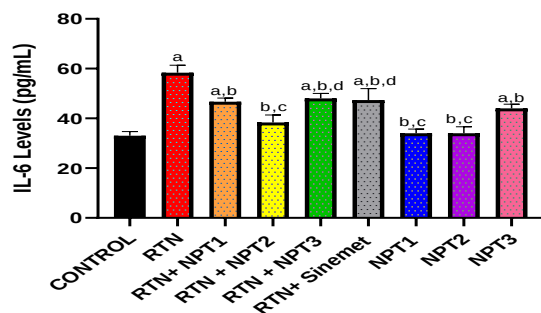
Fig. 4: Dopamine and GABA levels in the cerebellum of the experimental rats

The results are displayed as mean $\pm$ SD, with $n=5$ per group. Superscripts **a**, **b**, & **c** indicate statistically significant differences ($p < 0.05$) when compared with control, RTN-treated only, and RTN+NPT1, respectively.

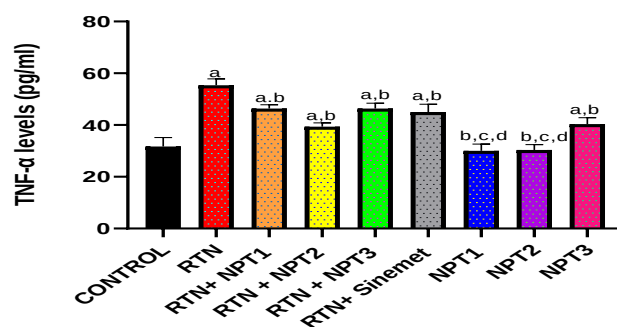
3.5 Cerebellar Pro-inflammatory Cytokines and Mediator Profiles

Fig. 5 depicts the pro-inflammatory cytokines (IL-6, TNF- α) and mediators (COX-2, CRP, MPO, and NO) profiles in the cerebellum of experimental rats. The rats exposed to rotenone only had significantly higher levels relative to the rotenone-intoxicated group only, while 40 mg/kg elicits no further significant decreasing effect of pro-inflammatory cytokines (IL-6 and TNF- α) and mediators (CRP, COX-2, and NO), as well as MPO activity in their cerebellar homogenate, compared

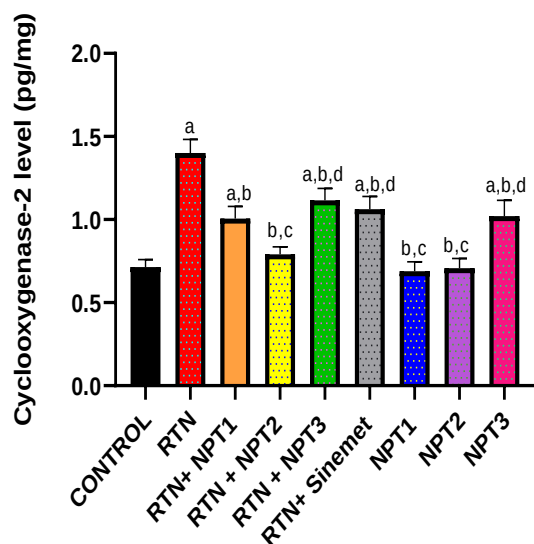
to the control group. Nevertheless, ZnONPs-EGCG or Sinemet® co-treatment significantly reversed the elevated levels/activities of these pro-inflammatory cytokines and mediators. Notably, co-treatment with 10 mg/kg ZnONPs-EGCG significantly reverses the level/activity of these pro-inflammatory markers exposed to rotenone only had significantly higher levels relative to the rotenone-intoxicated group only, while 40 mg/kg elicits no further significant decreasing effect of pro-inflammatory cytokines (IL-6 and TNF- α) and mediators (CRP, COX-2, and NO), as well as MPO activity in their cerebellar homogenate, compared



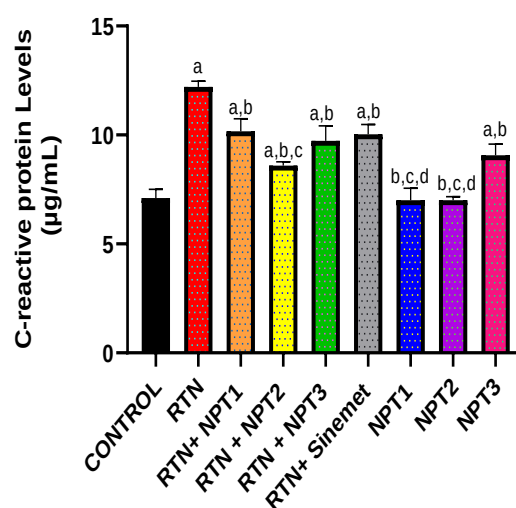
(a)



(b)



(c)



(d)

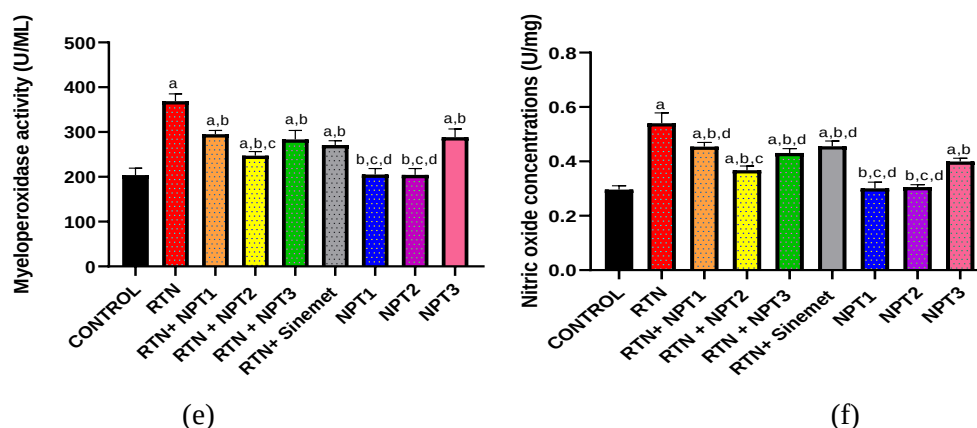


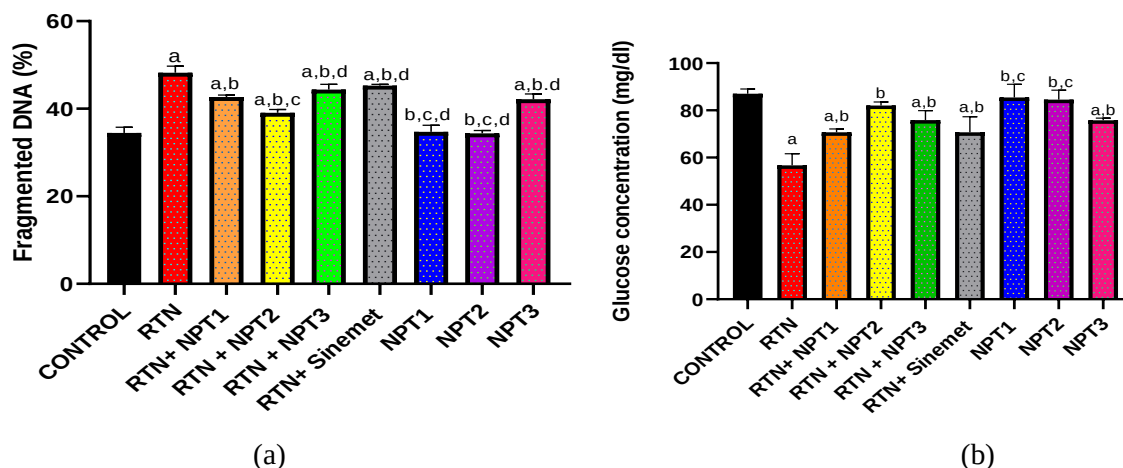
Fig. 5: Profiles of cerebellar pro-inflammatory cytokines and mediators in the experimental rats

The results are displayed as mean $\pm$ SD; n=5 per group. Superscripts **a, b, c, & d** denote those statistically significant differences ($p < 0.05$) when compared with control, RTN-treated only, RTN+NPT1, and RTN+NPT2, respectively.

3.6 Percentage of Fragmented DNA and Levels of Glucose and Total Proteins in the Cerebellum of Experimental Rats

The effect of rotenone on cerebellar content of fragmented DNA expressed as a percentage, glucose, and total neuronal proteins is shown in Fig. 6 below. The results showed that rotenone intoxication markedly increased the percentage of fragmented DNA and decreased the concentrations of glucose and total proteins in the cerebellum relative to the control group.

Conversely, treatment with ZnONPs-EGCG or Sinemet® alongside rotenone markedly decreased the percentage of fragmented DNA and increased the glucose and total protein concentrations in the brain cerebellum of rats when compared to the rotenone-intoxicated group (i.e., RTN only). Notably, the effects of 10 and 20 mg/kg of ZnONPs-EGCG co-treatment were dose-dependent for these parameters, but treatment alone with 10 and 20 mg/kg of ZnONPs-EGCG modulates the levels of these parameters. Similarly, co-treatment with 40 mg/kg also significantly decreased the percentage of fragmented DNA and increased glucose and total protein concentration, but was less than that of 20 mg/kg.



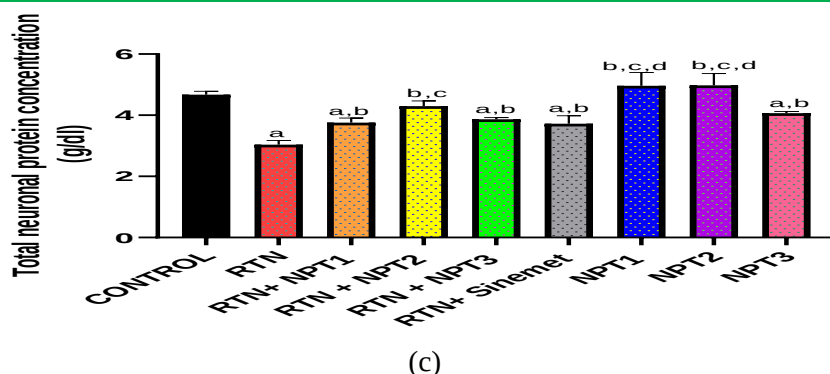


Fig. 6: Levels of fragmented DNA, glucose, and total protein in the cerebellum of experimental rats

The results are displayed as mean $\pm$ SD; n=5 per group. Superscripts **a**, **b**, **c**, & **d** denote those statistically significant differences ($p < 0.05$) when compared with control, RTN-treated only, RTN+NPT1, and RTN+NPT2, respectively

3.7 Cerebellar Histological Evaluation

Representative H&E-stained sections of the cerebellum are presented in Plate 1. The control cerebellum shows intact trilaminar cytoarchitecture, with the Purkinje layer preserved as an orderly, continuous monolayer of large, flask-shaped neurons (black arrows) bearing rounded vesicular nuclei, conspicuous nucleoli, and abundant basophilic cytoplasm. The molecular and granular layers appear normal.

The rotenone-only group (RTN only) exhibited severe cortical derangement concentrated within the Purkinje layer. The monolayer was disrupted and discontinuous, with gaps indicating cell dropout (red arrows). Surviving neurons were irregularly spaced and showed unequivocal degeneration: shrunken,

angular perikarya with condensed, hyperchromatic, pyknotic nuclei.

In RTN + NPT1 rats, Purkinje pathology was mildly demonstrable, with residual degenerating neurons showing shrunken perikarya, pyknotic nuclei, and perineuronal vacuolar spaces. Raising the dose (RTN + NPT2) produced clear improvement, with the Purkinje layer re-established as a substantially continuous row of viable neurons (black arrows) and no degenerative profiles. The high-dose group (RTN + NPT3) showed only mild changes, namely irregular neuronal spacing and molecular-layer vacuolation.

Co-administration of the standard drug afforded substantial protection, restoring a largely continuous monolayer of viable, flask-shaped neurons (black arrows), though with mild vacuolation and Purkinje cell shrinkage.

Rats given the nano extract alone at low and medium doses (NPT1, NPT2) displayed normal trilaminar cytoarchitecture with a preserved Purkinje monolayer (black arrows); the high dose (NPT3 only) showed some degenerative changes (red arrows).

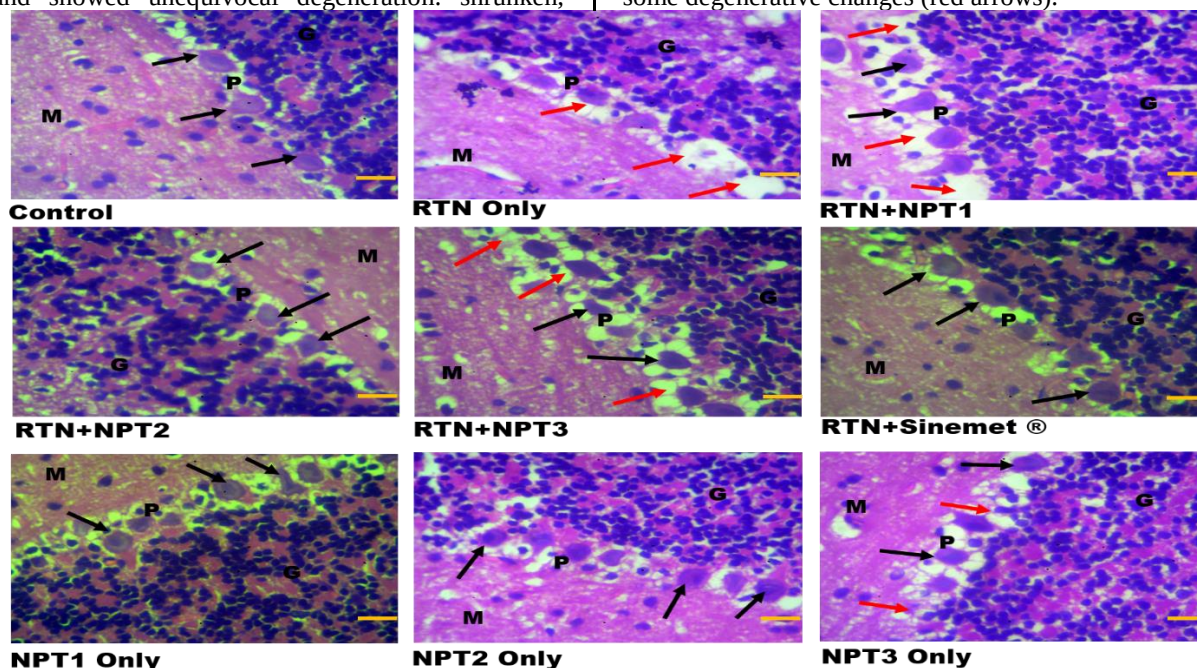


Plate 1: Representative photomicrographs of the cerebellar cortex of adult Wistar rats across the nine experimental groups. The cerebellar cortex shows its three constituent laminae: the molecular layer (M), the Purkinje cell layer (P), and the granular layer (G). Black arrows indicate Purkinje cell somata of normal morphology — large, flask-shaped neurons with vesicular nuclei, prominent nucleoli and Nissl-rich basophilic cytoplasm, arranged in a regular monolayer. Red arrows indicate degenerating Purkinje neurons, characterised by shrunken angular perikarya, condensed hyperchromatic (pyknotic) nuclei and conspicuous perineuronal vacuolar spaces; Haematoxylin and eosin (H&E). Scale bar = 25 μ m.

4.0 DISCUSSION

In this study, we investigated the cerebellar toxicity mediated through exposure to the neurotoxicant, rotenone, by examining its effect on the cerebellar redox-inflammatory markers, neurotransmitter homeostasis, and histopathological architecture in a rat model. Also, zinc oxide nanoparticles of epigallocatechin-3-gallate were synthesized, and its neuroprotective efficacy in mitigating rotenone-induced cerebellar toxicity and histopathological damage was evaluated.

The rotenone-induced neurotoxicity model has long established oxidative stress as a critical factor that not only drives the generation of ROS and free radicals, leading to oxidative damage in dopaminergic neurons, but also contributes to other pathological mechanisms (Riad *et al.*, 2025). In this study, rotenone intoxication impaired cerebellar redox homeostasis as it significantly decreased the enzymatic activities of the cerebellar antioxidants (SOD, CAT, GST, and GPx). This has been attributed to the inhibitory effect of rotenone on mitochondrial complex 1 of cerebellar neurons (Purkinje cells), leading to electron leakage, which promotes excessive generation of ROS and free radicals that overwhelm the endogenous antioxidant defense system, or to oxidative attack on antioxidant enzyme structure (Mohammed *et al.*, 2024). Findings from this study corroborate the report of Fikry *et al.* (2022) and Onyeleonu *et al.* (2025), as rotenone significantly decreased the cerebellar antioxidant system in the Parkinson's disease rat model. However, co-treatment with ZnONPs-EGCG markedly enhanced the cerebellar antioxidant enzyme activities, indicating the ability of these dual-functionalized nanoparticles to improve the antioxidant enzyme potentials, mitigating rotenone-induced oxidative insult. The antioxidant enzyme activity enhancement displayed by ZnONPs-EGCG in this study aligned with those reported for curcumin-conjugated zinc oxide nanoparticles (Fahmy *et al.*, 2024).

The brain, a central regulatory organ of the nervous system, contains neuronal cells, which immensely utilize oxygen due to high energy demand and a lack of energy storage mechanisms, making their membrane

lipids, which are high in polyunsaturated fatty acids, more vulnerable to oxidative damage (i.e., lipid peroxidation), and this further contributes to oxidative stress and inflammation (Adewale *et al.*, 2023; Babatunde *et al.*, 2025; Oladele *et al.*, 2025). In this study, rotenone intoxication markedly increased cerebellar membrane lipid peroxidation, as indicated by heightened MDA levels, thus indicating a state of compromised structural integrity and degeneration of the cerebellar membrane, which might lead to the leakage of neuronal proteins, enzymes, and neurotransmitters (Oladele *et al.*, 2025). However, the elevated MDA levels were markedly reversed by the co-administration of Sinemet® and the varying doses of ZnONPs-EGCG. It is worth noting that the anti-lipid-peroxidative activity of 20 mg/kg ZnONPs-EGCG appeared to be optimal among the varying doses of nanoparticles chosen. The anti-lipid-peroxidative effects of the nanoparticles of EGCG might be due to their ability to directly scavenge ROS, thereby preventing the initiation of lipid peroxidation in neuronal membranes, or their ability to enhance neuronal antioxidant enzymes activities, as observed in this study. A similar anti-lipid-peroxidative effect was reported by Zaki *et al.* (2024) for ZnONPs of resveratrol against levofloxacin-induced testicular oxidative stress in rats.

Neuroinflammation, a contributory pathological factor to neuronal toxicity and neurodegeneration, is known to be further enhanced by excessive ROS and free radical generation (Fathi *et al.*, 2022; Mohammed *et al.*, 2024). Rotenone, as a xenobiotic, has been reported to enter the neuron easily and to elicit an inflammatory response via activation of macrophages or excessive ROS generation, which collectively activate the NF- κ B signaling pathway that triggers the secretion of pro-inflammatory cytokines such as IL-1 β , TNF- α , and IL-6, and activities or levels of pro-inflammatory mediators such as CRP, NO, and COX-2 (Onyeleonu *et al.*, 2025). Of note, NO and MPO, aside from being neuroinflammatory biomarkers, are also oxidative stress biomarkers, as their increased cellular levels result in oxidative insults. A similar effect was observed in this study, as rotenone exposure led to elevated levels of IL-6, TNF- α , CRP, NO, COX-2, and MPO activity in the cerebellum. However, a significant reduction in the activity of MPO and levels of IL-6, TNF- α , CRP, COX-2, and NO were observed upon co-administration with ZnONPs-EGCG.

This implies that the EGCG-mediated ZnONPs exhibit anti-inflammatory potential against rotenone-induced cerebellar inflammation in Wistar rats. This neuroinflammation suppression potential may be linked to its ROS/free radical-scavenging and antioxidant enzymes-enhancing potential, as earlier discussed, or its ability to suppress the release of inflammatory cytokines and mediators. The observed anti-inflammatory activity displayed by ZnONPs-



EGCG in this study is similar to those reported for ZnONPs of berberine (El-Salakawy *et al.*, 2025). Rotenone-induced neurotoxicity has been implicated as a key factor that contributes to dopaminergic neuron loss, up to 50-70% (Heng *et al.*, 2023). The loss was reported to disrupt the transmission of other neurotransmitters, such as GABA (Alharbi *et al.*, 2024). The impaired GABAergic transmission exacerbated oxidative stress via neuronal hyperexcitability and excessive glutamate release, thus highlighting the role of GABAergic transmission in neurotoxicity and neurodegeneration (Pellicano *et al.*, 2025). A similar effect was observed in our study, as rotenone intoxication markedly decreased the cerebellar dopamine and GABA levels when compared to the control. This suggests that the targeted destruction of the substantia nigra dopaminergic neurons in the midbrain caused by rotenone affected their regional distributions and, in turn, reduced GABAergic transmission. This suggests their contributory roles in anxiety, tremor, and seizures seen in PD patients, as earlier reported by Alharbi *et al.* (2024). However, treatment with ZnONPs-EGCG markedly increased the dopamine and GABA levels in the cerebellum of rotenone-intoxicated rats. This implies that the EGCG nanoparticles enhanced the survival of dopaminergic neurons and GABAergic transmission in rotenone-intoxicated rats. The dopamine enhancement potential of ZnONPs-EGCG in this study is comparable to that reported for biogenic ZnONPs against rotenone-induced neuroendocrine toxicity (Akintunde *et al.*, 2021).

Neuronal DNA fragmentation is a marker of oxidative DNA damage, apoptosis, and neuronal injury, arising from ROS attack on DNA (Farombi *et al.*, 2019). Also, ROS attack on membrane lipids results in leakage of neuronal molecules like proteins and glucose, thus exposing them to oxidative attack as well as decreased cellular and metabolic utilization (Qin *et al.*, 2026). Aside from these, neurons are obligated glucose users for ATP production. Thus, a reduction in glucose content or utilization signals energy failure and injury. Therefore, DNA fragmentation index, total neuronal protein, and glucose levels collectively seem to be triad factors that may reflect structural integrity, functionality, and metabolic status of neurons. In respect to this, it was observed that rotenone intoxication altered DNA structural integrity, protein homeostasis, and glucose utilization, as there was a marked decline in total protein and glucose levels with a concomitant increase in the percentage of fragmented DNA in the cerebellum of rats, reflecting molecular alterations indicative of cellular injury. However, these molecular alterations were markedly abrogated by treatment with either Sinemet® or ZnONPs-EGCG (20 mg/kg ZnONPs-EGCG showing the most significant response).

Findings from the histopathological examination of the cerebellum further validated the neuronal toxicity and

degenerative impact of rotenone on the Purkinje cell layer. As evident in angular distortion and shrinkage of the flask-shaped perikarya; loss of Purkinje somata; and dark, shrunken, and hyperbasophilic nuclear appearance, featuring pyknosis. All these are indicative of cerebellar injury and degenerative histological changes. The report of these histopathological findings corroborates the report of Onyeleonu *et al.* (2025) and Fikry *et al.* (2022). Albeit, these cerebellar histopathological alterations were attenuated by co-treatment with either ZnONPs-EGCG or sinemet®. Notably, 20 mg/kg of ZnONPs-EGCG shows the most appreciable cerebelloprotective potential among all.

In spite of the cerebellar neuroprotective activity of ZnONPs-EGCG, its usage warrants dose optimization for maximum efficacy and safety of the neurons, which is commonly associated with nanoparticle usage. In this study, low to moderate doses (10 and 20 mg/kg) conferred cerebellar neuroprotection, but optimum efficacy was observed at 20 mg/kg, suggesting it as a dose of good biocompatibility and maximum therapeutic efficacy, while exposure of rats to the highest dose (40 mg/kg) of ZnONPs-EGCG alone elicited adverse effects, as observed in this study, giving great concern. As reflected from the results of this study, 40 mg/kg ZnONPs-EGCG treatment alone induced mild redox-inflammation, neurotransmitter alteration, and energy utilization changes when compared to the other groups treated alone with the nanoparticles. These might be due to nanoparticle aggregation and bioaccumulation, which may trigger them to act as a potential pro-oxidant, causing ROS insult, injury, and inflammation (Mirzaei *et al.*, 2025). Further studies on the toxicological impact on some signaling pathways, gene expression, and safety may be conducted on these otherwise promising phytomediated ZnONPs for translational research.

5.0 CONCLUSION

Findings from this present investigation have shown that exposure of rats to rotenone is connected to cerebellar toxicity, as evident by incidences of oxidative stress, inflammation, neurochemical and histopathological alterations. However, ZnONPs-EGCG, a phytoactive-mediated ZnONPs at a dose of 20mg/kg, can effectively mitigate the incidence of cerebellar neurotoxicity, as it modulates cerebellar antioxidant enzyme activities, inflammatory markers, neurochemical levels, and DNA damage, which was further reflected by near-normalcy in the appearance of cerebellar architecture.

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The authors have no known conflicts of interest to declare or financial or personal relationships that may construed the findings reported in this study.

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The ethical approval for this study was obtained from the Health Planning, Research and Statistics Department, Ministry of Health, Osogbo, Osun State, Nigeria, and the ethical approval number assigned was OSHREC/PRS/2026/842/01/232. The animals used for this study were handled in line with the laboratory animal care and guidelines published by methods of NIH.

Consent to publish or participate

Not applicable

Authors' contributions

Conceptualization: **I.O.B., J.O.O.**; Study design and methodology: **I.O.B., O.I.O., E.I.O.A., J.O.O.**; Investigation, analysis and data collection: **I.O.B., E.I.O.A., G.E.T.**; data analysis, writing and editing: **I.O.B., E.I.O.A., J.O.O., G.E.T.**; **Review and Supervision: O.I.O., E.I.O.A., J.O.O.** All authors have read and approved the final manuscript.

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