Artikel Asli/*Original Article*

Anti-Inflammatory Activity of Ethyl Acetate Extract of *Zingiber zerumbet* on Paraquat-Induced Parkinsonism in Sprague-Dawley Rats

Aktiviti Antiradang Ekstrak Etil Asetat *Zingiber zerumbet* terhadap Parkinsonisme-Aruhan Parakuat dalam Tikus Sprague-Dawley

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ABSTRACT

Zingiber zerumbet possesses anti-inflammatory properties that may offer neuroprotection against neurodegenerative diseases such as Parkinson's disease (PD). This study investigates the neuroprotective effects of *Z. zerumbet* ethyl acetate extract in paraquat (PQ)-induced Parkinsonian rat model. Fifty male Sprague-Dawley rats were divided into five groups: negative control (normal saline), untreated (PQ only), positive control (N-acetyl cysteine + PQ), and two treated groups receiving *Z. zerumbet* extract (200 mg/kg and 400 mg/kg). PQ (10 mg/kg, i.p.) was administered for five days, while *Z. zerumbet* extract was given orally for 19 days. Neuroinflammatory markers (NF-κB, iNOS, IL-1β, and α-synuclein) were quantified, and histological analysis was performed to assess neuronal integrity. PQ significantly increased NF-κB, iNOS, IL-1β, and total protein levels while reducing α-synuclein and neuronal counts. *Z. zerumbet* treatment, particularly at 400 mg/kg, significantly lowered NF-κB, iNOS, and IL-1β levels ($p < 0.05$) and preserved α-synuclein. Histological analysis showed significantly higher neuronal counts ($p < 0.05$) in treated groups, indicating neuroprotection. *Z. zerumbet* extract, particularly at 400 mg/kg, provides neuroprotection in PQ-induced Parkinsonism by reducing neuroinflammation, preserving α-synuclein, and maintaining neuronal integrity, supporting its potential as a therapeutic agent for PD.

Keywords: *Zingiber zerumbet*, Parkinson's disease, paraquat, neuroinflammation

ABSTRAK

Zingiber zerumbet memiliki sifat antiradang yang berpotensi memberikan neuroperlindungan terhadap penyakit neurodegeneratif seperti penyakit Parkinson (PD). Kajian ini menyelidik kesan neuroperlindungan ekstrak etil asetat *Z. zerumbet* dalam model tikus Parkinsonisme yang diaruh parakuat (PQ). Lima puluh ekor tikus jantan Sprague-Dawley dibahagikan kepada lima kumpulan: kawalan negatif (larutan salina normal), tidak dirawat (PQ sahaja), kawalan positif (N-asetil sisteina + PQ), dan dua kumpulan rawatan yang menerima ekstrak *Z. zerumbet* (200 mg/kg dan 400 mg/kg). PQ (10 mg/kg, i.p.) diberikan selama lima hari, manakala ekstrak *Z. zerumbet* diberikan secara oral selama 19 hari. Penanda neuroinflamasi (NF-κB, iNOS, IL-1β, dan α-sinuklein) dikuantifikasi, dan analisis histologi dijalankan untuk menilai integriti neuron. PQ secara signifikan meningkatkan aras NF-κB, iNOS, IL-1β dan protein jumlah, serta mengurangkan α-sinuklein dan bilangan neuron. Rawatan dengan *Z. zerumbet*, khususnya pada dos 400 mg/kg, secara signifikan menurunkan aras NF-κB, iNOS, dan IL-1β ($p < 0.05$) serta memelihara α-sinuklein. Analisis histologi menunjukkan peningkatan signifikan dalam bilangan neuron ($p < 0.05$) dalam kumpulan yang dirawat, menunjukkan kesan neuroperlindungan. Ekstrak *Z. zerumbet*, terutamanya pada dos 400 mg/kg, memberikan neuroperlindungan terhadap Parkinsonisme yang diaruh PQ dengan mengurangkan neuroinflamasi, memelihara α-sinuklein, dan mengekalkan integriti neuron, sekali gus menyokong potensinya sebagai agen terapeutik bagi penyakit Parkinson.

Kata kunci: *Zingiber zerumbet*, penyakit Parkinson, parakuat, neuroinflamasi

INTRODUCTION

Parkinson's disease (PD) is a progressive, age-related neurodegenerative disorder characterised by resting tremors, rigidity, bradykinesia, and postural instability (Agostini et al. 2024; Revankar et al. 2024). The pathological hallmark of PD is the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), which project to the dorsal striatum, leading to motor deficits (Revankar et al. 2024). Clinical symptoms

typically manifest when dopaminergic neuronal loss surpasses a critical threshold, estimated at 70–80% depletion of striatal nerve terminals and 50–60% loss of SNpc perikarya (Heng et al. 2023). Under normal physiological conditions, dopaminergic neurons in the SNpc release dopamine, which modulates motor control via the nigrostriatal pathway (Ni & Ernst 2022). However, current pharmacological treatments, such as levodopa, primarily provide symptomatic relief and are associated with significant side effects, including dyskinesia and motor fluctuations, while failing to halt disease progression (Hong et al. 2025). Consequently, there is growing research interest in identifying neuroprotective agents, including medicinal plants, that may delay disease onset, slow progression, or mitigate neurodegeneration.

Toxin-induced models are widely employed to study PD pathogenesis and evaluate potential therapies. Among the most used neurotoxins are paraquat (PQ), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and rotenone, all of which induce PD-like pathology in animal models (Thirugnanam & Santhakumar 2022). MPTP undergoes metabolic conversion to its toxic derivative, 1-methyl-4-phenylpyridinium (MPP+), which selectively enters dopaminergic neurons via the dopamine transporter (DAT), leading to mitochondrial complex I inhibition and oxidative stress (Guo & Liu 2025). In contrast, rotenone, a pesticide known for its ability to induce PD-like neurodegeneration, does not rely on DAT for neuronal uptake but directly inhibits mitochondrial complex I, resulting in ATP depletion and neuronal death (Balakrishnan et al. 2021). Due to its structural similarity to MPP+, PQ was initially hypothesised to enter dopaminergic neurons through DAT. However, unlike MPTP, PQ does not depend on DAT for uptake. Instead, it crosses the blood-brain barrier via the neutral amino acid transporter, inducing dopaminergic toxicity through oxidative stress and neuroinflammation (Silva et al. 2024). These findings suggest that PQ represents a distinct PD model, offering insights into the interplay between oxidative stress, neuroinflammation, and dopaminergic degeneration.

Zerumbone is a sesquiterpene phytochemical derived from *Zingiber zerumbet* Smith, an edible ginger species native to Southeast Asia, commonly known in Malaysia as “lempoyang.” This species, belonging to the *Zingiberaceae* family, has been traditionally utilised in herbal medicine for its anti-inflammatory, antioxidant, and anticancer properties (Supiandi et al. 2024). Recent studies have also explored its pharmacological potential, highlighting its anti-leukaemic and antiviral activities (Mohd Salleh et al. 2024). Within the *Zingiberaceae* family, several bioactive compounds have demonstrated neuroprotective properties, making *Z. zerumbet* a promising candidate for PD research.

A previous study demonstrated that the ethyl acetate extract of *Z. zerumbet* attenuated oxidative stress and reduced tremor severity in a PQ-induced Parkinsonian rat model (Ibrahim et al. 2018). The extract significantly lowered oxidative stress markers, such as malondialdehyde (MDA) and protein carbonyl levels, while enhancing the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Additionally, behavioural assessments indicated improvements in motor function. While these findings underscore the antioxidant effects of *Z. zerumbet*, its potential role in modulating neuroinflammation in PD remains poorly understood. Since neuroinflammation accelerates dopaminergic neurodegeneration in PD, further research into the anti-inflammatory properties of *Z. zerumbet* is warranted. Therefore, this study aims to evaluate the neuroprotective effects of the ethyl acetate extract of *Z. zerumbet* rhizome in a PQ-induced rat model of PD, with a particular focus on its anti-inflammatory properties.

METHODOLOGY

PLANT MATERIALS

The fresh rhizomes of *Z. zerumbet* were collected from Temerloh, Pahang. The specimen was first validated and deposited in the Herbarium, Faculty of Science and Technology (FST), Universiti Kebangsaan Malaysia (UKM), under the voucher number UKMB-29952. The rhizomes were scrubbed, cleaned, finely chopped using a dry blender, and then air-dried at room temperature for three days.

PLANT EXTRACTION

The air-dried chopped rhizomes of *Z. zerumbet* (7 kg) were sequentially soaked at room temperature in 100% *n*-hexane for 72 hours. The hexane extract was filtered, and the rhizome residue was re-soaked in fresh hexane for another 72 hours. This cycle was repeated three times. The residue from the final hexane extraction was subsequently soaked in 100% fresh ethyl acetate, followed by 100% methanol, using the same procedure. The resultant solvent extracts (filtrates) from each stage were evaporated to dryness using a rotary evaporator to yield crude extracts of hexane, ethyl acetate, and methanol. All crude extracts were stored at 4°C until further use. Before use, the *Z. zerumbet* ethyl acetate extract was dissolved in 100% dimethyl sulfoxide (DMSO), diluted in phosphate-buffered saline (PBS; pH 7.4), aliquoted, and stored as a stock solution at -20°C before being used for oral supplementation (Ibrahim et al. 2018).

EXPERIMENTAL PROTOCOL

This study utilised fifty adult male Sprague-Dawley rats (180–240 g), supplied by the Laboratory Animal Resource Unit at Universiti Kebangsaan Malaysia (UKM). The rats were housed in a controlled environment at 24°C ± 1 with a 12-hour light/dark cycle and had ad libitum access to food and water. The animals were randomly divided into five groups (n=10/group): a negative control group (normal saline, orally), a PQ-treated group (PQ only, intraperitoneal), an NAC + PQ group (20 mg/kg N-acetylcysteine with PQ, NAC administered orally and PQ intraperitoneally), a 200 + PQ group (200 mg/kg *Zingiber zerumbet* with PQ, *Z. zerumbet* administered orally and PQ intraperitoneally), and a 400 + PQ group (400 mg/kg *Z. zerumbet* with PQ, *Z. zerumbet* administered orally and PQ intraperitoneally). The ethyl acetate extract of *Z. zerumbet* was administered orally for 19 consecutive days, while paraquat (PQ, 10 mg/kg) and normal saline were administered intraperitoneally from Day 8 to Day 12 of the treatment regime. Fresh brain tissue containing the substantia nigra pars compacta (SNpc) region was collected for biochemical and histological analysis (Ibrahim et al. 2018). The research timeline is illustrated in Figure 1. The procedures involving the use of laboratory animals were approved by the UKM Animal Ethics Committee (UKMAEC) (approval code: FSK/BIOMED/2013/ASMAH/14-NOV.15566-NOV.-2013-JUNE-2014) and conducted following UKM's ethical guidelines for the use of laboratory animals.

SAMPLE PREPARATION AND TOTAL PROTEIN MEASUREMENT

All rats were sacrificed on Day 20. Anaesthesia was induced using a combination of ketamine, xylazine, and Zoletil (KTX) at a dose of 0.1 mL/200 g, administered intraperitoneally. Following anaesthesia, the rats were decapitated, and brain tissues were collected. The striatum and substantia nigra (SN) were identified and isolated using a chilled rodent brain matrix, as illustrated in Figure 2 (Paxinos & Watson 2007). The isolated brain regions were rinsed with ice-cold potassium chloride (KCl) to remove any remaining blood traces. The SN region was fixed in 10% formalin for 3–4 days for histological analysis, while the striatum was processed for biochemical assays. Striatal tissue was homogenised in 1.15% KCl (pH 7.4, 1:10 w/v) on ice using an Ultra-Turrax T25 homogeniser. The homogenate was then centrifuged at 4,000 rpm for 20 minutes at 4°C to obtain the supernatant, which was stored at -40°C for further analysis. Total protein concentrations in the striatal homogenates were determined using the Bradford method (Bradford 1976).

EVALUATION OF INFLAMMATORY AND DISEASE-RELATED BIOMARKERS

Inflammatory markers, including inducible nitric oxide synthase (iNOS), interleukin-1β (IL-1β), nuclear factor kappa B (NF-κB), and α-synuclein (SNCA), were quantified using enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions. Standard curves were generated using serial dilutions of recombinant standards, and sample absorbance was measured at 450 nm using a microplate reader. All samples and standards were analyzed in duplicate to ensure accuracy. The obtained optical density (OD) values were used to determine cytokine concentrations based on the standard curve. ELISA procedures were performed under controlled conditions to minimize variability.

HISTOLOGY ANALYSIS

To evaluate the potential loss of dopaminergic neurons following PQ treatment and to assess the neuroprotective effects of *Z. zerumbet* extract as an anti-inflammatory agent, neuronal quantification and morphological analysis were performed in the substantia nigra pars compacta (SNpc). Histological assessment involved Nissl staining to examine neuronal integrity and neuroinflammatory changes. Neuronal density was quantified within a defined area (neurons per 10^3 μm²) in the SNpc, while morphological alterations were also assessed.

STATISTICAL ANALYSIS

All data were analyzed using GraphPad Prism 9.3.1 and are presented as mean ± SEM. Group comparisons were conducted using one-way analysis of variance (ANOVA) to assess differences in total protein levels, biochemical markers (NF-κB, iNOS, α-synuclein, and IL-1β), and neuronal count based on Nissl staining. The normality assumption was evaluated, and any violations were addressed accordingly. Statistical significance was set at $p < 0.05$, with Tukey's post-hoc test applied for multiple comparisons.

RESULTS

EFFECTS OF Z. *Zerumbet* ON TOTAL PROTEIN LEVELS IN THE RATS' STRIATUM

Based on Figure 3, total protein levels were significantly higher in the PQ-treated group compared to the normal group. In contrast, the NAC+PQ and *Z. zerumbet* (200+PQ and 400+PQ)

groups exhibited significantly lower total protein levels compared to the PQ-treated group.

EFFECTS OF Z. *Zerumbet* ON INFLAMMATORY AND DISEASE-RELATED BIOMARKERS IN THE RATS' STRIATUM

Figure 4a shows that NF-κB levels were significantly higher in the PQ-treated group compared to the normal group, while the NAC+PQ and *Z. zerumbet*-treated groups (200+PQ and 400+PQ) exhibited significantly lower levels than the PQ-treated group. As shown in Figure 4b, iNOS levels were significantly elevated in the PQ-treated group relative to the normal group. However, only the *Z. zerumbet*-treated groups (200+PQ and 400+PQ) showed a significant reduction in iNOS levels compared to the PQ-treated group. According to Figure 4c, IL-1β levels were significantly lower in the high-dose *Z. zerumbet* (400+PQ) group compared to the PQ-treated group. Lastly, Figure 4d demonstrates that α-synuclein levels were significantly lower in the PQ-treated, NAC+PQ, and low-dose *Z. zerumbet* (200+PQ) groups compared to the normal group, while the high-dose *Z. zerumbet* (400+PQ) group showed no significant difference from the normal group.

NEURON COUNT AND HISTOLOGICAL ASSESSMENT IN THE RATS' SUBSTANTIA NIGRA PARS COMPACTA

As shown in Figure 5, the neuron count in the PQ group was significantly lower than that in the normal group. Conversely, the number of surviving neurons in the NAC+PQ and *Z. zerumbet* (200+PQ and 400+PQ) groups was significantly higher than in the PQ group.

According to Figure 6, the nervous tissue section of the PQ group (Figure 6C), which did not receive any extract or supplement treatment, exhibited an indistinct substantia nigra pars compacta (SNpc) layer. The blue staining intensity was reduced compared to the normal group (Figure 6A). Furthermore, in the untreated PQ group, the SNpc layer was difficult to distinguish from other tissue layers, appearing more homogeneous and blending with the surrounding tissue. In contrast, the SNpc tissue in the treatment group that received *Z. zerumbet* ethyl acetate extract at a dose of 400 mg/kg (Figure 6G) exhibited the most distinct structural differences. Microscopic examination revealed that the SNpc layer in the extract-treated group contained a higher number of neurons, which displayed a more intense bluish colour, making the layer more clearly visible compared to the surrounding tissue.

In the PQ-untreated group, most neurons in the SNpc tissue exhibited signs of degeneration and apoptosis (Figure 6D). The degeneration process was evident through the significant loss of neurons in the SNpc region compared to other groups. This was characterised by the presence of numerous empty spaces between neurons, in contrast to the normal nervous tissue (Figure 6B), which appeared more compact with fewer empty spaces. Additionally, a key morphological feature observed was chromatin clumping in the neuronal nuclei, indicating neuronal degeneration (Figure 6D). PQ induction led to a significant loss of dopaminergic neurons in the SNpc, with most neurons exhibiting nuclear and cytoplasmic shrinkage.

When comparing neuronal morphological features between treatment groups, the SNpc tissue of rats treated with *Z. zerumbet* ethyl acetate extract at a dose of 400 mg/kg (Figure 6H) displayed more intense and uniform cytoplasmic staining than those treated with 200 mg/kg (Figure 6F). Additionally, the neurons appeared more numerous and densely packed, closely resembling the normal SNpc tissue structure. The primary distinguishing feature was the presence of intact, spherical nuclei with well-defined axons and dendrites, along with clearly visible cytoplasm. Moreover, the abundance of glial cells was notably lower in the extract-treated groups compared to the PQ group, which exhibited a high density of glial cells.

DISCUSSION

A previous study using the same model reported that PQ exposure affects behavioural outcomes, particularly tremor severity, and increases oxidative stress markers (Ibrahim et al. 2018). Expanding on these findings, the present study investigates the inflammatory response in the striatum and SNpc, providing deeper insights into PQ-induced neurotoxicity. Our results demonstrate that PQ exposure leads to significant neurodegeneration, as indicated by increased total protein levels, elevated inflammatory biomarkers, and reduced neuronal counts in the SNpc. However, treatment with *Zingiber zerumbet* ethyl acetate extract, particularly at 400 mg/kg, exhibited neuroprotective effects by reducing inflammation and preserving neuronal integrity.

The significant increase in total protein concentration in PQ-treated rats suggests an ongoing inflammatory process and potential protein aggregation due to cellular stress (Dash et al. 2025; González-Barrio et al. 2021). This elevation likely reflects an intensified inflammatory response. In contrast, the NAC+PQ and *Z. zerumbet* treatment groups (200 mg/kg and 400 mg/kg) exhibited a significant reduction in total protein levels, suggesting that *Z. zerumbet* mitigates PQ-induced protein accumulation through its anti-inflammatory properties.

Inflammatory biomarkers, including NF-κB, iNOS, and IL-1β, were markedly increased in the PQ-treated group, highlighting a strong inflammatory

response linked to neurotoxicity. NF-κB activation plays a key role in oxidative stress and inflammation by promoting the production of pro-inflammatory cytokines, chemokines, adhesion molecules, COX-2, and iNOS (Chen et al. 2023; Wu et al. 2022). The upregulation of iNOS, likely due to microglial activation, contributes to dopaminergic neuronal degeneration by producing neurotoxic factors such as IL-1β, TNF-α, and nitric oxide (Chiu et al. 2023; Katsipis et al. 2024). The observed elevation in IL-1β levels in PQ-treated rats suggests a robust glial response to toxin-induced neuronal damage.

Treatment with *Z. zerumbet* extract at both doses (200 mg/kg and 400 mg/kg) significantly reduced NF-κB, iNOS, and IL-1β levels, reinforcing its anti-inflammatory and neuroprotective properties. The inhibition of NF-κB suggests that *Z. zerumbet* may prevent IκB protein degradation, thereby suppressing pro-inflammatory signalling pathways (Paladino et al. 2023). The reduction in iNOS activity further indicates its role in limiting excessive microglial activation and inflammation-related neuronal damage. Notably, IL-1β levels were significantly lower in the 400 mg/kg group, suggesting a stronger anti-inflammatory effect at this dose. These findings align with studies demonstrating the ability of *Z. zerumbet* and its bioactive compound, zerumbone, to suppress NF-κB activation and iNOS expression (Su et al. 2021; Yeh et al. 2022). The superior anti-inflammatory effects of the 400 mg/kg dose compared to the 200 mg/kg and NAC treatments suggest a dose-dependent protective mechanism against PQ-induced neurotoxicity.

Although α-synuclein is often associated with Parkinsonism, the low levels observed in the PQ-treated group may indicate increased microglial activation. Microglia play a crucial role in clearing extracellular α-synuclein, but excessive activation can contribute to neuroinflammation and neuronal loss. Research suggests that while microglia efficiently phagocytose α-synuclein, their capacity diminishes when overwhelmed, exacerbating neurodegeneration (Sirerol-Piquer et al. 2025).

The consistently low α-synuclein levels in the NAC+PQ and low-dose *Z. zerumbet* (200 mg/kg) groups suggest that these treatments were less effective in suppressing excessive microglial activation, which could contribute to neuronal damage. Conversely, the high-dose *Z. zerumbet* (400 mg/kg) group exhibited α-synuclein levels comparable to the normal control group, suggesting that this dose modulates microglial activity and preserves α-synuclein homeostasis. Given α-synuclein's role in dopamine regulation (Barba et al. 2022), its preservation at 400 mg/kg may help prevent dopamine-related neurotoxicity.

These findings highlight the potential of enhancing microglial phagocytosis while preventing excessive inflammation as a strategy for neuroprotection. The ability of *Z. zerumbet* at a higher dose to regulate α-synuclein levels suggests its neuroprotective effects, likely through microglial modulation and dopaminergic neuronal preservation.

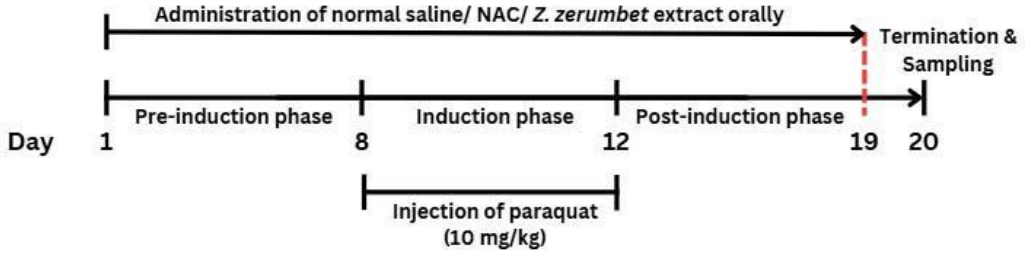

FIGURE 1 The Experimental Timeline. *Z. zerumbet* extract was administered orally via gavage for 19 consecutive days. From Day 8 to Day 12, paraquat was injected daily via intraperitoneal (i.p.) administration. On Day 20, all rats were sacrificed for sample collection. The striatum and substantia nigra pars compacta (SNpc) were collected for biochemical and histological analyses.

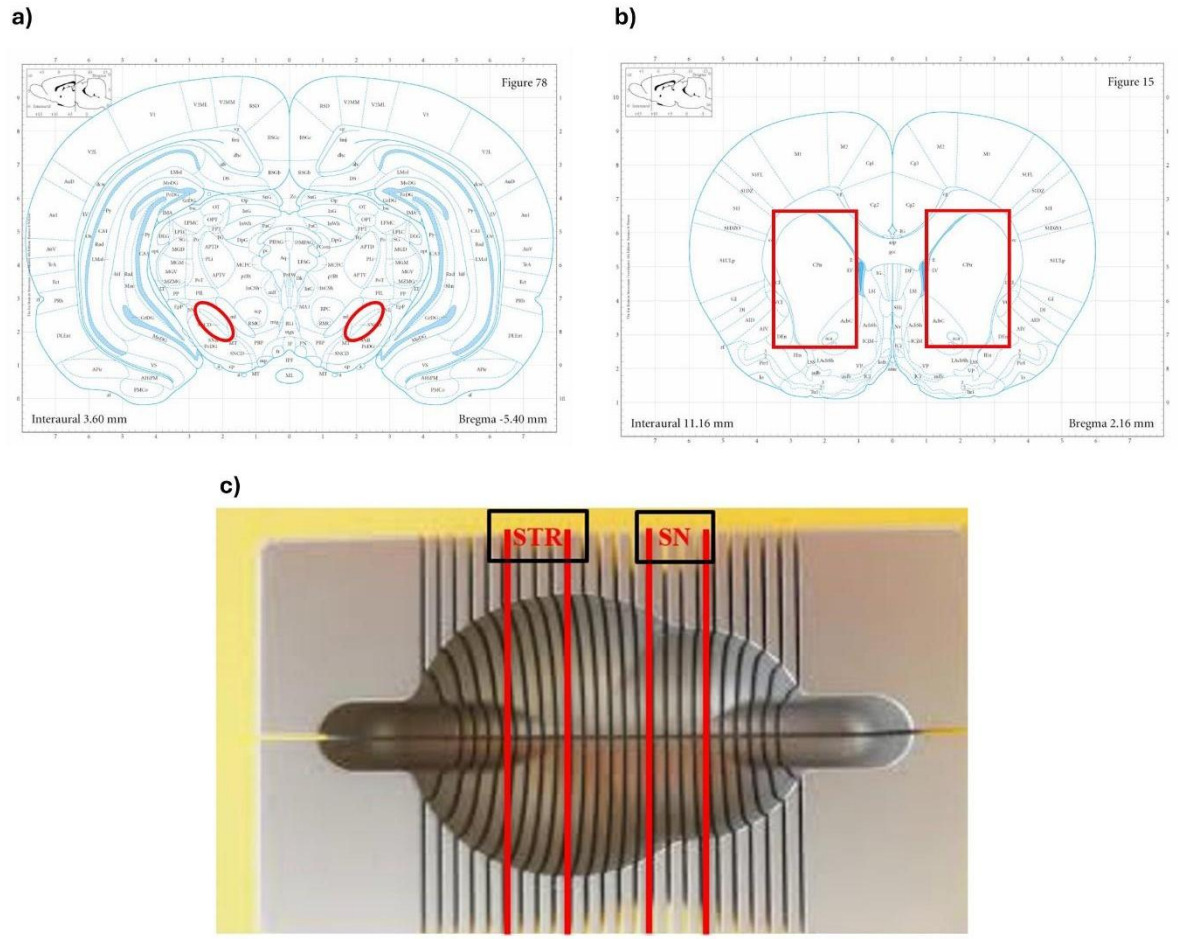

FIGURE 2 Brain Regions of Interest for Biochemical and Histological Analysis. (a) Coronal section from the Paxinos and Watson rat brain atlas highlighting the substantia nigra pars compacta (SNpc) (red ovals) at Bregma -5.40 mm. (b) Coronal section from the Paxinos and Watson atlas indicating the striatum (red rectangles) at Bregma 2.16 mm. (c) Brain matrices showing sectioning of the striatum and substantia nigra (SN) for analysis.

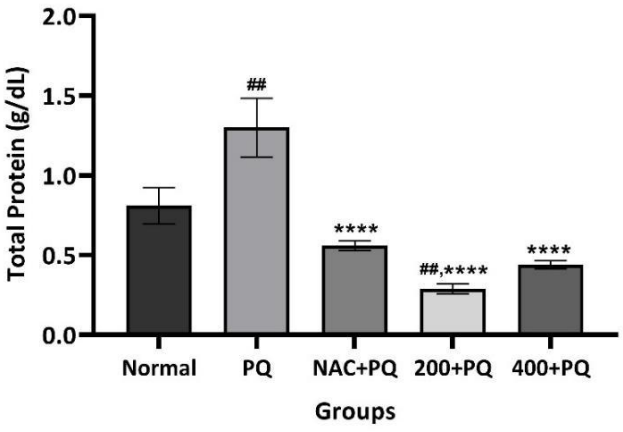

FIGURE 3 Effects of *Z. zerumbet* on Total Protein Levels in the Rats' Striatum. Statistical analysis was performed using one-way ANOVA. Data are presented as mean ± SEM (n=10). Post hoc: $^{\#\#}p<0.01$ compared to the normal group, ****$p < 0.0001$ compared to the PQ-treated group

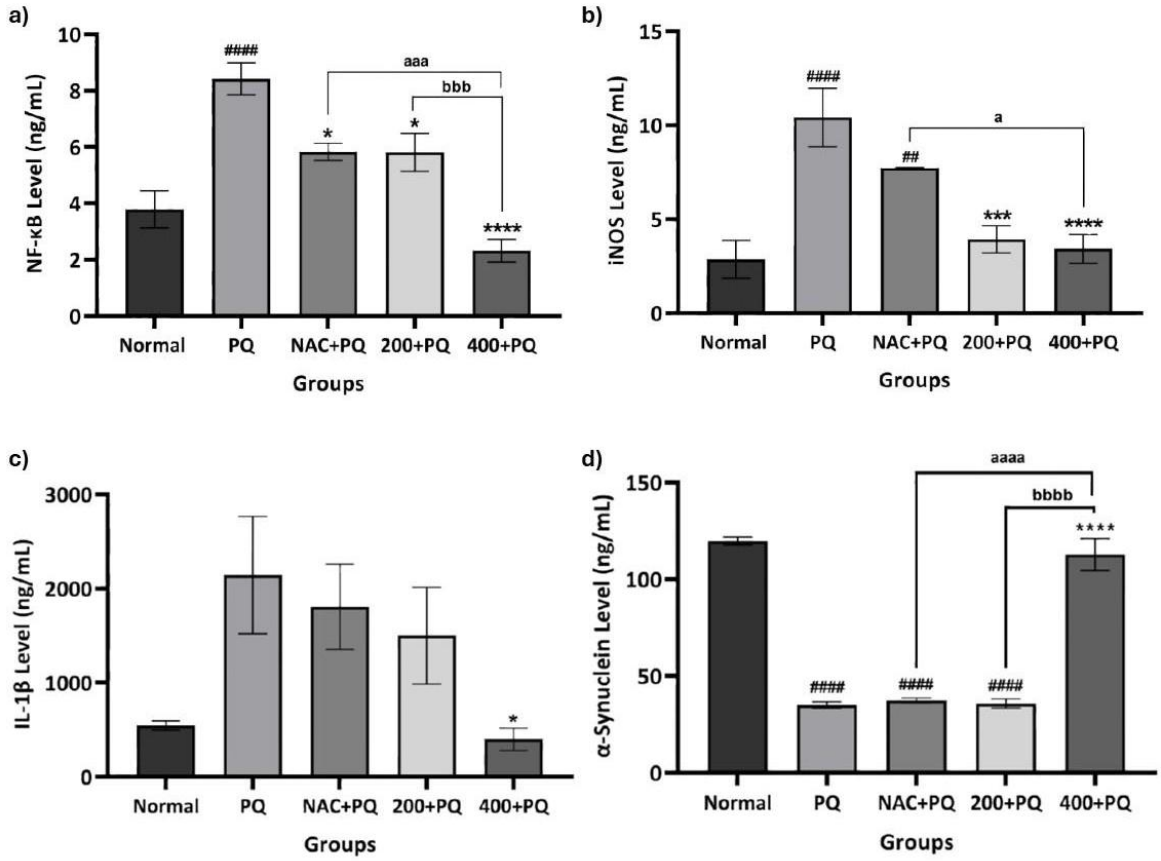

FIGURE 4 Effects of *Z. zerumbet* on Inflammatory and Disease-Related Biomarkers in the Rats' Striatum. (a) NF-κB, (b) iNOS level, (c) IL-1β and (d) α-Synuclein levels. Statistical analysis was performed using one-way ANOVA. Data are presented as mean ± SEM (n=10). Post hoc: $^{\#\#}p<0.01$, $^{\#\#\#\#}p< 0.0001$ compared to normal group, *p< 0.05, ***p< 0.001, ****p < 0.0001 compared to PQ-treated group, $^{a}p< 0.05$, $^{aa}p< 0.01$, $^{aaaa}p< 0.0001$ compared to NAC+PQ group, $^{bbb}p< 0.001$, $^{bbbb}p< 0.0001$ compared to 200+PQ group.

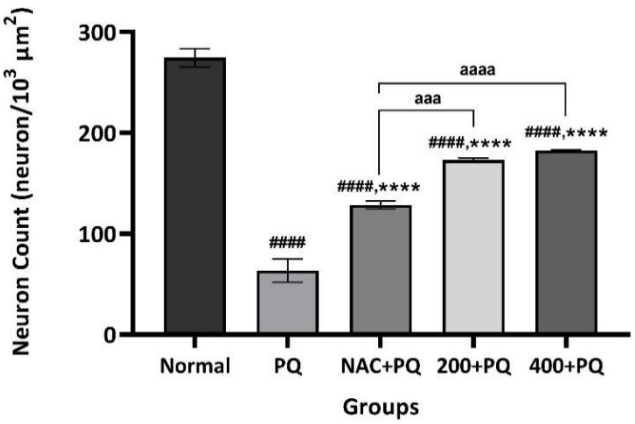

FIGURE 5 Effects of *Z. zerumbet* on Neuron Count in The Rats' Substantia Nigra Pars Compacta (SNpc). Statistical analysis was performed using one-way ANOVA. Data are presented as mean ± SEM (n=10). Post hoc: $^{\#\#\#\#}p<0.0001$ compared to normal group, ****p < 0.0001 compared to PQ-treated group, aaap< 0.001, aaaap< 0.0001 compared to NAC+PQ group.

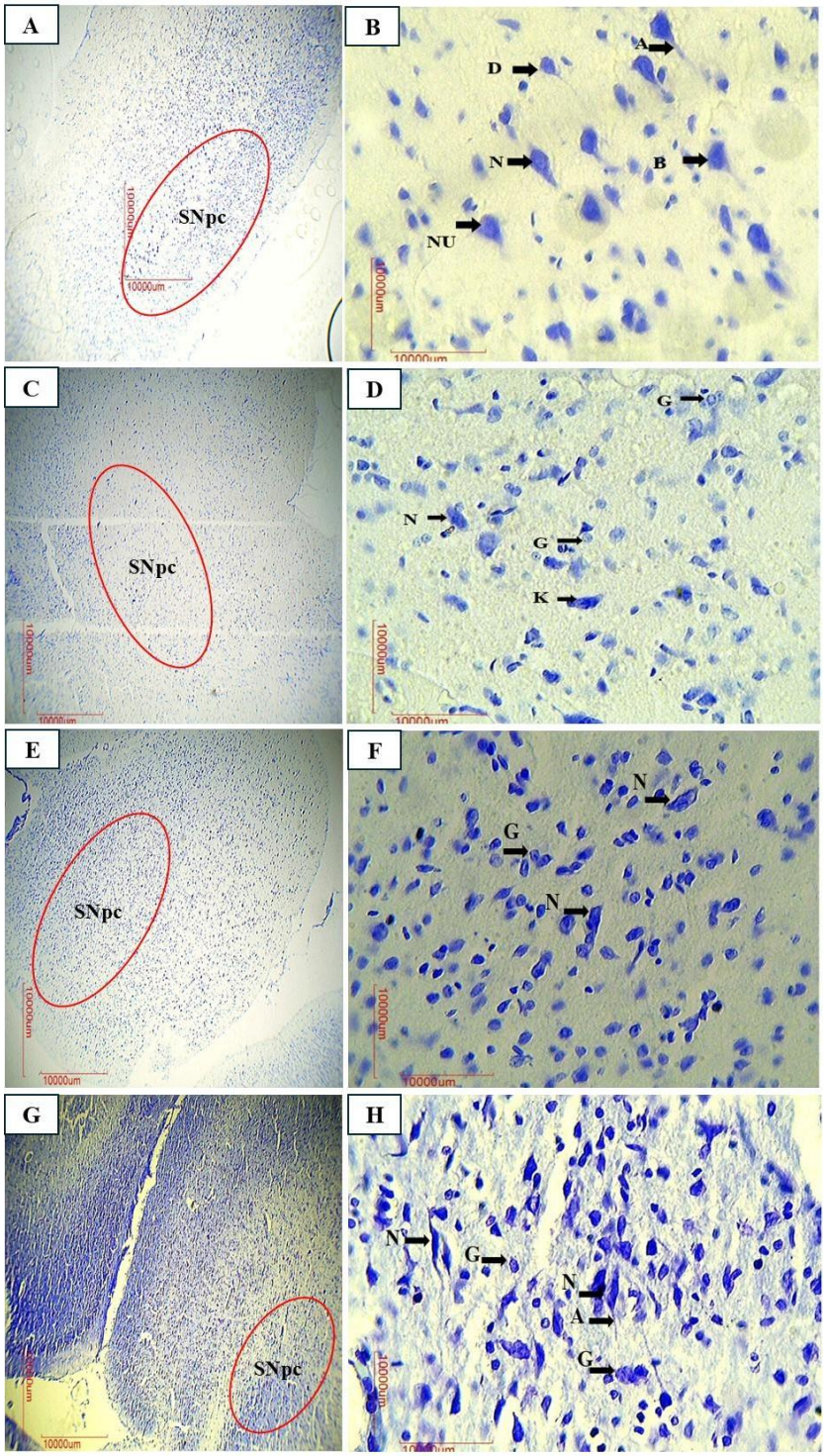

FIGURE 6 Histology Observation of Nissl Staining in Substantia Nigra Pars Compacta (SNpc). Nissl-stained images of substantia nigra pars compacta (SNpc) at 40× magnification (A, C, E, G) and 400× magnification (B, D, F, H). (A, B) Normal group. (C, D) Paraquat group. (E, F) 200 mg/kg *Zingiber zerumbet* ethyl acetate extract. (G, H) 400 mg/kg *Zingiber zerumbet* ethyl acetate extract. Arrow: A = axon, B = body cells, D = dendrite, N = neuron, NU = nucleoplasm, G = glial cells, K = chromatin clumping. Scale bars: 10,000 µm (40×), 1,000 µm (400×). The NAC+PQ group served as the positive control; however, representative images for this group are not shown due to insufficient image quality. All related data were analyzed and included.

Histological analysis further supports the neuroprotective effects of *Z. zerumbet* in the SNpc. Both treatment groups (200 mg/kg and 400 mg/kg) exhibited a significant increase in neuronal count compared to the PQ group, with preserved neuronal morphology and minimal apoptosis-related features (nuclear shrinkage, chromatin clumping). The low abundance of glial cells suggests that *Z. zerumbet* reduces inflammation by limiting excessive microglial activation. Notably, neuronal counts in both treatment groups were comparable to those in the normal control, indicating strong neuroprotection. However, the 400 mg/kg dose appeared more effective in maintaining SNpc structure, reducing glial proliferation, and preventing neurodegeneration. Overall, these findings support the therapeutic potential of *Z. zerumbet*, particularly at 400 mg/kg, in protecting dopaminergic neurons from PQ-induced toxicity by modulating inflammation and microglial activation.

CONCLUSION

This study demonstrates the anti-inflammatory and neuroprotective effects of *Z. zerumbet* ethyl acetate extract in a PQ-induced Parkinsonian rat model. PQ exposure resulted in increased neuroinflammation, as indicated by elevated NF-κB, iNOS, and IL-1β levels, alongside significant neuronal loss in the SNpc. Treatment with *Z. zerumbet*, particularly at 400 mg/kg, effectively reduced inflammation, preserved α-synuclein homeostasis, and maintained neuronal integrity. These findings suggest that *Z. zerumbet* exerts neuroprotection by modulating microglial activity and suppressing inflammatory pathways. Given its dose-dependent effects, *Z. zerumbet* could serve as a promising natural therapeutic candidate for neurodegenerative diseases associated with chronic neuroinflammation, such as PD. Future studies should further investigate its mechanisms, particularly its role in microglial regulation and neuroprotection.

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Received: 17 March 2025
Revised: 16 May 2025
Accepted for Publication: 26 May 2025