



## OPEN Preliminary study of polyethylene microplastics disrupting energy Metabolism, redox Balance, and prefrontal cortex structure in Wistar rats

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Plastic pollution has become a major environmental and health concern, with microplastics (MPs) increasingly implicated in biological toxicity. This study examined the neurotoxic effects of polyethylene microplastics (PE-MPs) on prefrontal cortex (PFC) bioenergetics, mitochondrial redox balance, and inflammatory responses. Fifteen male Wistar rats were divided into control and treatment groups, receiving oral PE-MP doses (15 or 60 mg/kg body weight) for 28 days. Biochemical assessments revealed significant disruption of PFC bioenergetic enzymes critical for energy metabolism. Oxidative stress and inflammation were evident, characterized by antioxidant depletion, enhanced oxidation, and impaired redox homeostasis. Histological analysis further demonstrated neuronal degeneration, vacuolation, and vascular congestion within the PFC. These findings indicate that PE-MP exposure compromises cortical bioenergetics, disturbs mitochondrial redox equilibrium, triggers inflammatory modulation, and induces structural damage in the rat prefrontal cortex.

**Keywords** Metabolism, Mitochondrial, Inflammation, Oxidative phosphorylation, Brain, Oxidative stress, Neurotoxicity, Pre-frontal cortex

The widespread of microplastics (MPs) in the environment has raised increasing concern regarding their potential adverse impacts on both human health and ecological systems. MPs are known to originate either from the fragmentation of larger plastic debris or are intentionally manufactured for various industrial and commercial applications<sup>1</sup>. Human exposure to MPs occurs through multiple pathways, including drinking water, salt, plant-derived products, animal-derived products, particularly seafood, as well as alcoholic beverages and processed foods. Among the various types of MPs, polyethylene microplastics (PE-MPs) are among the most commonly produced and detected, owing to their extensive use in packaging, textiles, and consumer goods<sup>2</sup>. PE-MPs have been shown to accumulate in both terrestrial and aquatic organisms, leading to physiological and behavioural disruptions<sup>3,4</sup>. Emerging evidence suggests that these particles can translocate across biological barriers, accumulate in tissues, and exert toxic effects in multiple organs, including the central nervous system (CNS)<sup>5,6</sup>.

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Notably, several studies have demonstrated that microplastics can access the brain via systemic circulation or olfactory transport, particularly when particle size permits crossing of the blood–brain barrier<sup>7–9</sup>. Once in the brain, MPs may interfere with key cellular and molecular pathways, ultimately impairing cognitive and behavioural functions. Despite increasing interest in the neurotoxic potential of microplastics, the underlying mechanisms, especially those involving cellular energy metabolism, mitochondrial function, oxidative stress, neuroinflammation, and brain histoarchitecture, remain poorly understood.

The brain is a highly metabolically active organ that relies on tightly regulated energy dynamics to support neuronal function and cognitive processes. Within the brain, the prefrontal cortex (PFC) plays a central role in higher-order functions such as working memory, decision-making, attention, and emotional regulation<sup>10</sup>. Its structural and functional integrity is essential for neurobehavioral competence and is particularly vulnerable to disruptions in metabolic and oxidative homeostasis<sup>11,12</sup>. Given its sensitivity to environmental insults, including nanoparticles and pollutants, the PFC represents a critical region for evaluating the neurotoxic effects of microplastics. Neurons, the fundamental building blocks of the PFC, are heavily dependent on mitochondrial oxidative phosphorylation for ATP production, and disturbances in this energy-generating process can compromise synaptic transmission, plasticity, and overall neuronal viability<sup>13,14</sup>. Exposure to PE-MPs has been implicated in the disruption of essential biochemical pathways, including impaired glucose metabolism, hormonal imbalance, mitochondrial dysfunction, altered antioxidant status<sup>15–17</sup>, penetrates the blood–brain barrier, accumulates in brain tissue, and triggers deleterious effects<sup>18,19</sup>.

Since accumulating evidence indicates that PE-MPs may exert significant neurotoxic effects by targeting fundamental biological processes, the present study was designed to address a critical knowledge gap. We hypothesized that oral exposure to PE-MPs would induce dose-dependent impairments in PFC bioenergetics, oxidative balance, inflammatory status, and histoarchitecture. Notably, this work is among the first to integrate mitochondrial energy metabolism, redox regulation, inflammatory markers, and structural analysis specifically in the PFC, a region central to cognition and executive function and highly vulnerable to metabolic stress. Whereas most prior studies have focused on the hippocampus and other brain regions<sup>20–24</sup>, our work uniquely emphasizes the PFC, thereby advancing understanding of microplastic-induced neurotoxicity in a domain essential for higher-order cognitive processes.

## Materials and methods

### Chemicals and reagents

PE-MPs (34–50 µm particle size, Cat No. 434272, CAS-No. 9002-88-4, Melting point 144 °C, Density 0.94 g/mL at 25 °C, Ultra-high molecular weight) were sourced from Sigma-Aldrich, Missouri, USA. Lactate dehydrogenase assay kits were obtained from CYPRESS Diagnostics, Langdrop, Belgium. All other reagents used were of analytical grade, supplied by Carl Roth GmbH, Karlsruhe, Germany.

### Animals and treatment

A detailed methodological approach is provided as a supplementary file. Fifteen (15) male Wistar rats (180–200 g) of 8 weeks old were kept in the animal facility at Ajayi Crowther University for a 7-day acclimatization. Housed in standard cages, the animals were given clean drinking water and a regular rat pellet diet manufactured by Vita Feeds Nigeria Limited. Throughout the course of the study, environmental conditions, temperature of 22 ± 2 °C, a 12-hour light and dark cycle of 06:00–18:00 h were kept. All Animal experimental procedures were approved by the Institutional Ethical Review Committee on Animal Care and Use of the Faculty of Natural Sciences of Ajayi Crowther University, Nigeria (under reference number FNS/ERC/2024/017NH), in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council<sup>25</sup>. All animal experimental procedures and methods are reported in accordance with ARRIVE guidelines.

### Experimental groups

The rats were assigned into three (3) groups, five (5) each, and received the following treatment: group 1 (control) rats received normal saline orally for 28 days; group 2 and 3 received 15 and 60 mg/kg PE-MPs orally (gavage), respectively. The PE-MPs dose was chosen based on a previous study by Farag et al.<sup>26</sup>. A stainless-steel gavage needle was used to minimize particle adherence. The gavage needle was weighed before and after administration to ensure accurate dose delivery, and rinsed between animals to prevent dose loss or cross-contamination. This study was designed as a preliminary investigation with the primary aim of generating initial data on dose-dependent effects rather than establishing definitive conclusions, and the sample size of 15 rats (5 per dose group) was determined based on ethical committee approval, which strictly limited the number of animals permitted for this exploratory phase.

### Organ sampling and biochemical techniques

Following an overnight fast after 28 days of exposure to PE-MPs, the animals were sacrificed under ketamine-xylazine anaesthesia (Dosage: 75 mg Ketamine/kg, 10 mg Xylazine/kg; Concentration: 300 mg/ml ketamine, 30 mg/ml Xylazine, intraperitoneal injection). For biochemical assessments, the animals' brains were excised, and the PFC was sectioned. The PFC was rinsed in 1.15% ice-cold KCl and then homogenized in 5 volumes/weight of ice-cold 0.1 M phosphate buffer (pH 7.4)<sup>27</sup>. The post-mitochondrial fraction was obtained by centrifuging the homogenate at 10,000 g for 15 min at 4 °C. According to Fernández-Vizarra et al.'s, mitochondria were isolated from the rats' PFC<sup>28</sup> and stored at –40 °C. Total protein concentration in PFC was assessed using the Biuret method described by Gornall et al.<sup>29</sup>.

#### *Glycolytic enzyme activities in PFC*

The activities of key metabolic enzymes, hexokinase (HK) was assessed following the method by Hass and Hurley<sup>30</sup>, aldolase (ALD) activity was evaluated using the procedure of Jagannathan et al.<sup>31</sup>, lactate dehydrogenase (LDH) activity was determined using the instructions provided by the LDH assay kit manufacturer (LABKIT), and NADase activity was assessed based on the method outlined by Tatsuno et al.<sup>32</sup>.

#### *Oxidative phosphorylation enzyme activities in PFC*

Enzymatic activities of key PFC mitochondrial markers were quantified using established spectrophotometric protocols. Citrate synthase (CS) activity was measured according to the method of Nulton-Persson and Szweda<sup>33</sup>, serving as a reference marker for mitochondrial content. Isocitrate dehydrogenase (IDH) activity was assessed at 340 nm by monitoring NAD<sup>+</sup> reduction, following the protocol of Romkina and Kiriukhin<sup>34</sup>. Malate dehydrogenase (MDH) activity was determined using the approach described by López-Calcano et al.<sup>35</sup>. Succinate dehydrogenase (SDH) activity was evaluated using the method developed by Jones and Hirst<sup>36</sup>. Activities of electron transport chain (ETC) complexes I–IV were quantified to assess the PFC mitochondrial functional capacity: NADH ubiquinone oxidoreductase (Complex I), succinate ubiquinone oxidoreductase (Complex II), cytochrome c oxidoreductase (Complex III), and cytochrome c oxidase (Complex IV), following the protocol outlined by Medja et al.<sup>37</sup>.

#### *Mitochondrial redox and inflammatory status of PFC*

Mitochondrial oxidative and antioxidant status were assessed in the PFC tissue using different biochemical assays. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using the method of Olajide et al.<sup>38</sup>. Nitric oxide (NO) concentration was determined via the Griess reagent, following Kehinde et al.<sup>39</sup>. Myeloperoxidase (MPO) activity was quantified spectrophotometrically as per Hanning et al.<sup>40</sup>. Reduced glutathione (GSH) levels were measured using the protocol by Moron et al.<sup>41</sup>, ascorbic acid (AA) concentration determined by Olajide et al.<sup>38</sup> method, and activities of glutathione S-transferase (GST) by Skopelitou & Labrou<sup>42</sup>, superoxide dismutase (SOD) by Misra & Fridovich<sup>43</sup>, and catalase (CAT) by Hadwan<sup>44</sup>.

#### **Histopathological analysis of PFC section of the brain tissue**

At termination of study, rats were euthanized under deep anaesthesia in accordance with institutional ethical approval and relevant animal welfare guidelines. The brain was rapidly excised, and the pre-frontal cortex was anatomically identified using standard rat brain landmarks and dissected bilaterally on an ice-cold surface. Tissues were briefly rinsed in cold phosphate-buffered saline to remove blood contaminants. For histological assessment, samples were fixed in 10% neutral buffered formalin for 24–48 h, processed through graded ethanol, cleared in xylene, and embedded in paraffin. Coronal Sects. (4–5 µm) were prepared using a rotary microtome and mounted on glass slides. Sections were stained with hematoxylin and eosin and examined using a camera-equipped Leica DM750 microscope for evaluation of cortical histoarchitecture and pathological alterations.

#### **Statistical analysis**

All the results are presented in form of mean ± standard deviation (SD). To make comparisons within the groups and check for overall differences, we used analysis of variance (ANOVA). When the ANOVA indicated a significant effect, we followed up with the use of Tukey's post hoc test to pinpoint where those differences lay. Statistical significance was assumed at a p-value < 0.05.

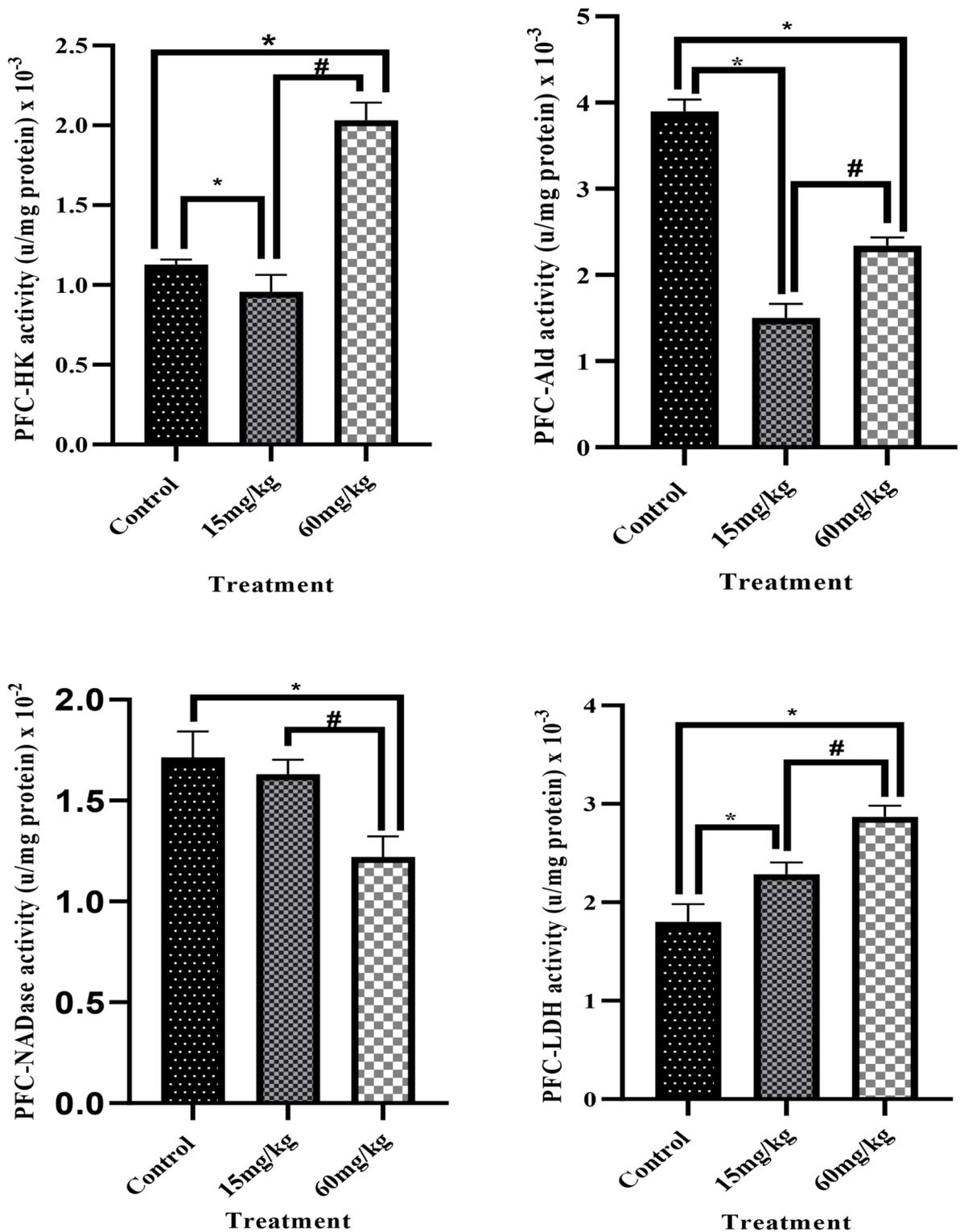
### **Results**

#### **PE-MPs perturb glycolytic enzyme activities in PFC in rats**

Figure 1 illustrates the effects of PE-MPs exposure on PFC glycolytic enzyme activities, including HK, ALD, NADase, and LDH. HK activity exhibited a biphasic response to PE-MP exposure. Relative to control, the 15 mg/kg group showed a modest reduction (13.1%), whereas the 60 mg/kg group demonstrated a marked upregulation (66.7%;  $P < 0.05$ ), representing a significant 42.9% increase compared with the 15 mg/kg group ( $P < 0.05$ ). This pattern indicates both dose dependency. In contrast, ALD activity was significantly suppressed following PE-MP exposure. The 15 mg/kg group exhibited a twofold reduction, while the 60 mg/kg group showed a 32.6% decrease relative to control ( $P < 0.05$ ). Although both treated groups differed significantly from the control, ALD activity partially recovered at the higher dose, as evidenced by a significant 40.8% increase at the 60 mg/kg group ( $P < 0.05$ ). NADase activity remained comparable to control at 15 mg/kg but was significantly reduced at 60 mg/kg, showing a 38.9% decrease ( $P < 0.05$ ), which was also significant relative to the 15 mg/kg group. LDH activity increased in a clear dose-dependent manner, rising by 34.8% at 15 mg/kg ( $P < 0.05$ ) and by 56.5% at 60 mg/kg ( $P < 0.05$ ) compared with control, with a significant difference between treatment groups ( $P < 0.05$ ). Collectively, the elevation of HK and LDH activities suggests a compensatory upregulation of glycolysis, whereas the suppression of ALD and NADase indicates disrupted glycolytic flux and impaired energy metabolism, potentially reflecting mitochondrial dysfunction at higher PE-MP exposure levels.

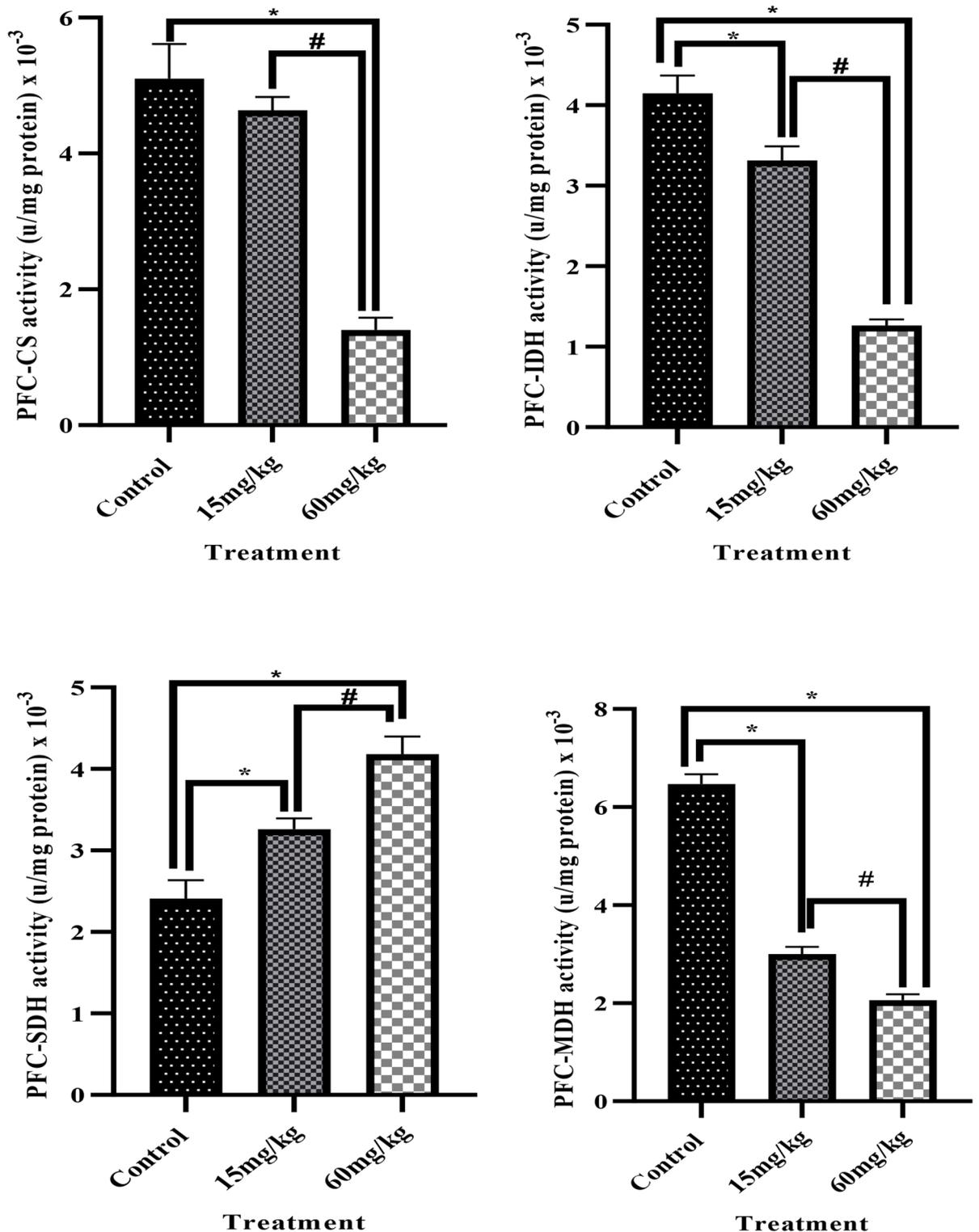
#### **PE-MPs alter tricyclic acid cycle enzyme activity in the PFC of rats**

Figure 2 shows the impact of PE-MPs exposure on TCA-cycle enzymes in the PFC. PE-MPs induced dose-dependent mitochondrial dysfunction, with significant reductions in CS, IDH, and MDH activities, alongside a paradoxical increase in SDH. CS activity showed a progressive decline, with a non-significant decrease at 15 mg/kg but a drastic 81.8% reduction at 60 mg/kg ( $P < 0.05$ ), indicating disruption of TCA cycle initiation. IDH activity followed a similar pattern, decreasing by 15.1% at 15 mg/kg and 77.4% at 60 mg/kg ( $P < 0.05$ ), suggesting impaired oxidative metabolism. In contrast, SDH activity increased significantly, rising by 44.0% at 15 mg/kg ( $P < 0.05$ ) and further by 19.4% at 60 mg/kg compared to the 15 mg/kg group, pointing to compensatory



**Fig. 1.** Neural glycolytic enzyme activities in PE-MPs exposed rats. The values are presented as mean  $\pm$  SD. ( $n=5$ ) per group. \* Significantly ( $P<0.05$ ) different when compared with control; # significantly ( $P<0.05$ ) different relative to 15 mg/kg PE-MPs. PFC-Pre-frontal cortex, HK-Hexokinase, ALD-Aldolase, LDH-Lactate dehydrogenase, NADase-  $\text{NAD}^+$  Nucleosidase.

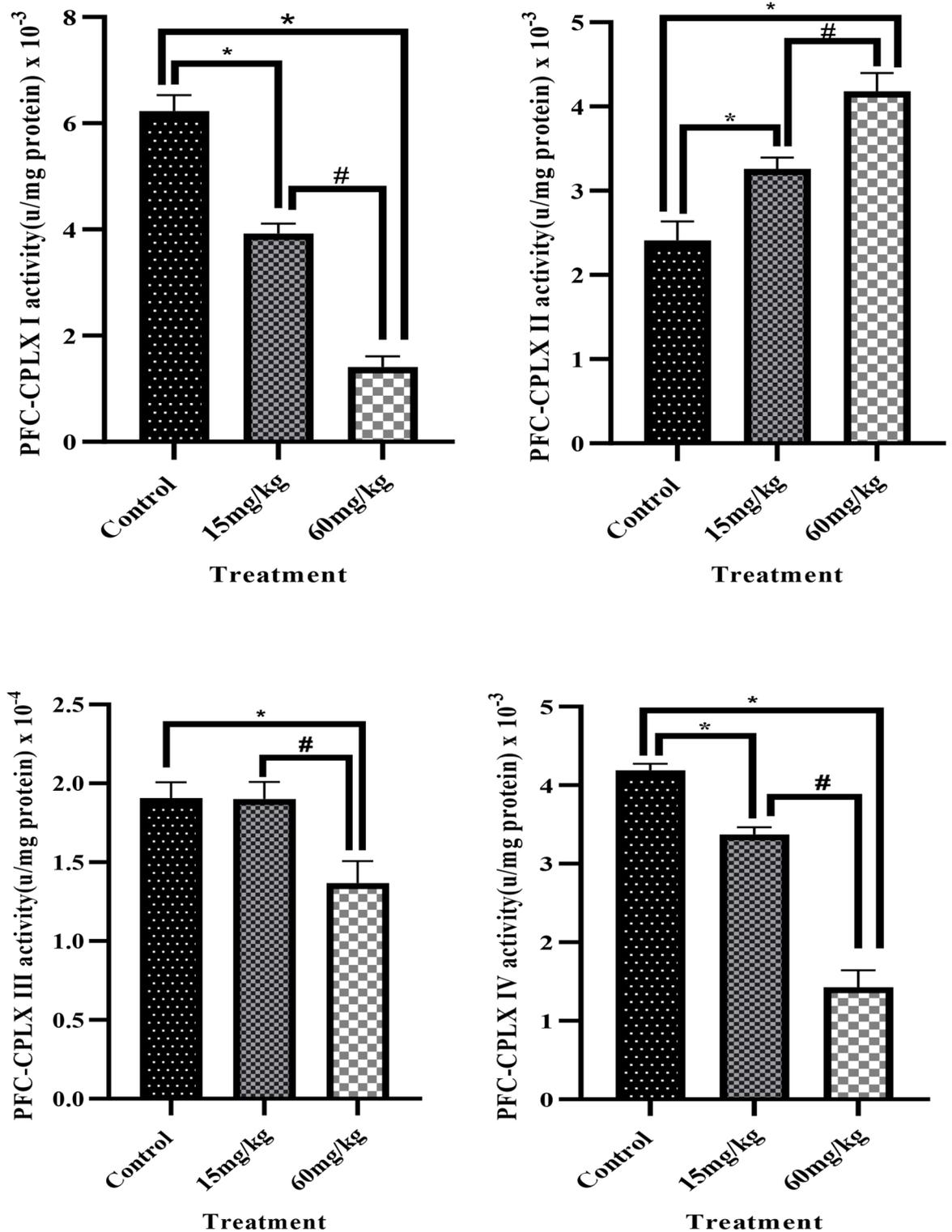
adaptation or altered redox regulation. MDH activity was markedly suppressed, with a 44.1% reduction at 15 mg/kg ( $P<0.05$ ) and a 70.6% reduction at 60 mg/kg ( $P<0.05$ ), reflecting impaired oxaloacetate regeneration and compromised cycle continuation. Collectively, these enzyme alterations highlight profound mitochondrial imbalance, likely driven by oxidative stress and disrupted redox signalling.



**Fig. 2.** Neural tricarboxylic acid cycle (TCA) enzyme activities in PE-MPs exposed rats. The values are presented as mean + SD. ( $n=5$ ) per group. \* Significantly ( $P<0.05$ ) different when compared with control; # significantly ( $P<0.05$ ) different relative to 15 mg/kg PE-MPs. PFC- Pre-frontal cortex, Citrate synthase-CS, Isocitrate Dehydrogenase-IDH, SDH-Succinate dehydrogenase, MDH-Malate dehydrogenase.

### PE-MPs perturb the PFC electron transport chain enzyme activity in rats

Figure 3 depicts the effects of PE-MPs on PFC electron transport chain enzymes. CPLX I activity declined significantly, with a 33.9% reduction at 15 mg/kg ( $p<0.05$ ) and a 72.4% decrease at 60 mg/kg ( $p<0.05$ ), the latter also 58.3% lower than the 15 mg/kg group, indicating dose-dependent inhibition. In contrast, CPLX II activity



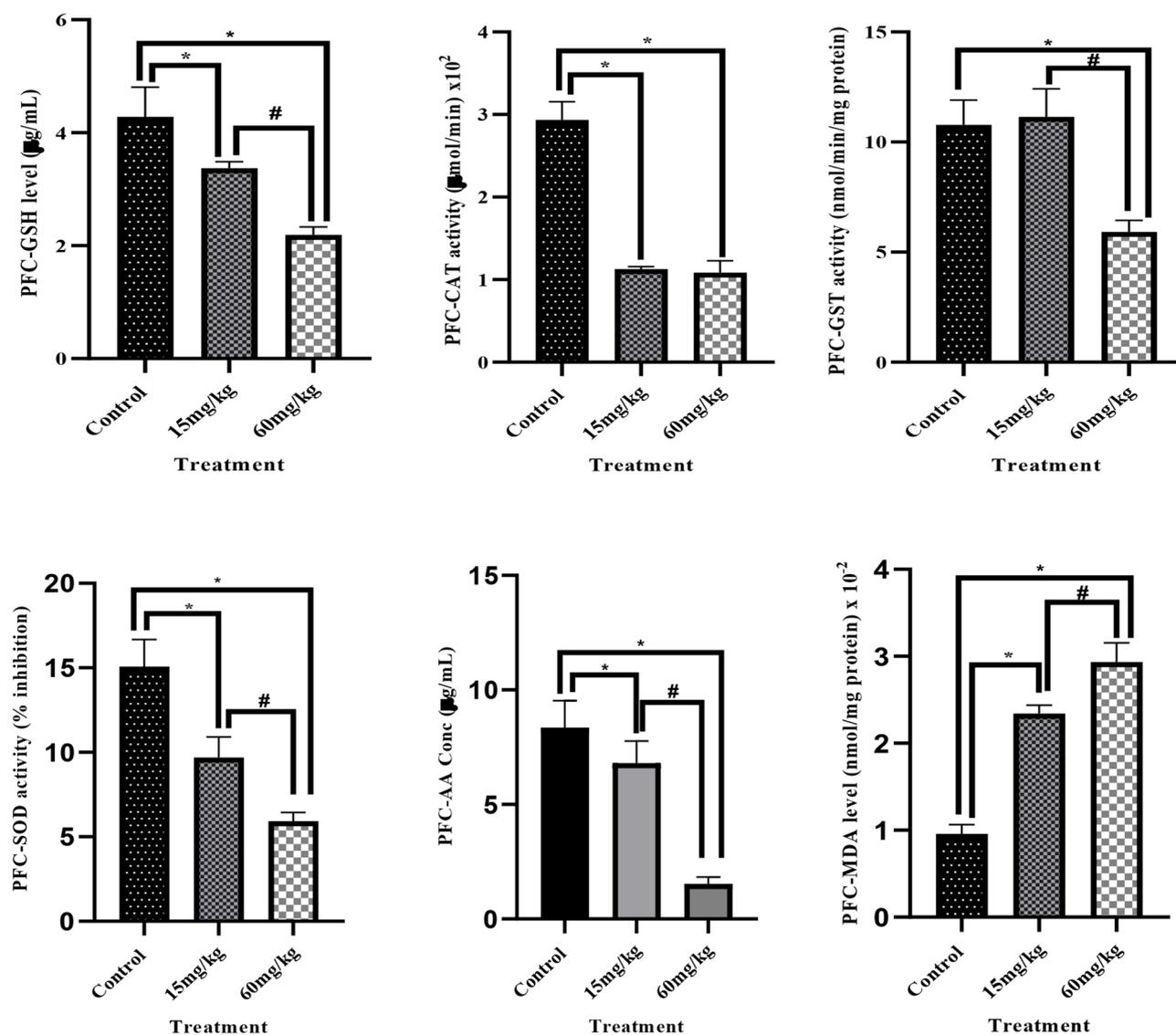
**Fig. 3.** Neural respiratory chain enzyme activities in rats exposed to PE-MPs. The values are presented as mean  $\pm$  SD. ( $n=5$ ) per group. \* Significantly ( $P<0.05$ ) different when compared with control; # significantly ( $P<0.05$ ) different relative to 15 mg/kg PE-MPs. PFC-Pre-frontal cortex, Complex I- CPLX I, Complex II- CPLX II, Complex III-CPLX III, Complex IV-CPLX IV.

was significantly increased at both 15 and 60 mg/kg of PE-MPs doses, suggesting compensatory mitochondrial adaptation. CPLX III activity remained unchanged at 15 mg/kg but decreased by 20.8% at 60 mg/kg ( $p<0.05$  vs. control), consistent with a threshold effect. CPLX IV activity was suppressed at both doses, with a 30.1% reduction at 15 mg/kg ( $p<0.05$ ) and 74.4% decline at 60 mg/kg ( $p<0.05$ ). Overall, PE-MPs exert dose-dependent

inhibitory effects on CPLX I, III, and IV, while paradoxically stimulating CPLX II, reflecting compensatory responses and differential vulnerability of respiratory chain complexes to oxidative or structural stress.

#### PE-MPs perturb PFC mitochondria redox status in rats

Figure 4 shows the impact of PE-MPs on PFC redox status. GSH levels declined significantly in both treatment groups, with reductions of 28% at 15 mg/kg and 35% at 60 mg/kg ( $p < 0.05$ ), indicating a dose-dependent effect. CAT activity was markedly suppressed, decreasing by 77% at 15 mg/kg and 64% at 60 mg/kg compared to controls, though no difference was observed between the two doses. GST activity exhibited a biphasic response: a 22% increase at 15 mg/kg ( $p < 0.05$ ) followed by a 50% decrease at 60 mg/kg ( $p < 0.05$ ). SOD activity was impaired in a dose-dependent manner, falling by 33.3% at 15 mg/kg and 66.7% at 60 mg/kg ( $p < 0.05$ ), reflecting compromised superoxide neutralization. Similarly, AA levels dropped by 18% and 73% at 15 and 60 mg/kg, respectively ( $p < 0.05$ ), with a significant difference between the two groups. Collectively, these findings demonstrate that PE-MPs exposure disrupts redox balance, leading to oxidative distress in the PFC. MDA levels, an indicator of lipid peroxidation, were significantly elevated following PE-MP exposure. The 15 mg/kg and 60 mg/kg groups showed moderate but statistically significant increases compared to the control ( $p < 0.05$ ), consistent with enhanced oxidative stress.



**Fig. 4.** Neural antioxidant enzymes activities in PE-MPs exposed rats. The values are presented as mean  $\pm$  SD. ( $n=5$ ) per group. \* Significantly ( $P < 0.05$ ) different when compared with control; # significantly ( $P < 0.05$ ) different relative to 15 mg/kg PE-MPs. PFC-Pre-frontal cortex, GSH- Glutathione, CAT-Catalase, GST- Glutathione-s-transferase, SOD-Superoxide dismutase, AA-Ascorbic acid, MDA-Malondialdehyde.

### PE-MPs alter PFC inflammatory markers in rats

Figure 5 shows the effects of PE-MPs on PFC NO concentration and MPO activity. NO concentration increased significantly in a dose-dependent manner, rising by 42.9% at 15 mg/kg ( $p < 0.05$ ) and reaching a 2-fold elevation at 60 mg/kg compared to controls, with a further 38.8% increase relative to the 15 mg/kg group ( $p < 0.05$ ). These findings indicate that PE-MPs exposure enhances NO production even at low doses, with greater elevations at higher doses. In contrast, MPO activity decreased following PE-MPs administration. The 15 mg/kg group showed a 16.7% reduction compared to control ( $p < 0.05$ ), while the 60 mg/kg group exhibited a more pronounced 44.4% decline relative to control and a 33.3% decrease compared to the 15 mg/kg group ( $p < 0.05$ ). Increased NO concentration in the PFC indicates activation of inflammatory signalling and enhanced nitrosative responses. In contrast, MPO activity reduction suggests a suppression of neutrophil-associated oxidative activity. This pattern reflects an imbalance in inflammatory response, which may influence the overall inflammatory status and redox balance of the tissue, and the underlying mechanisms require further investigation.

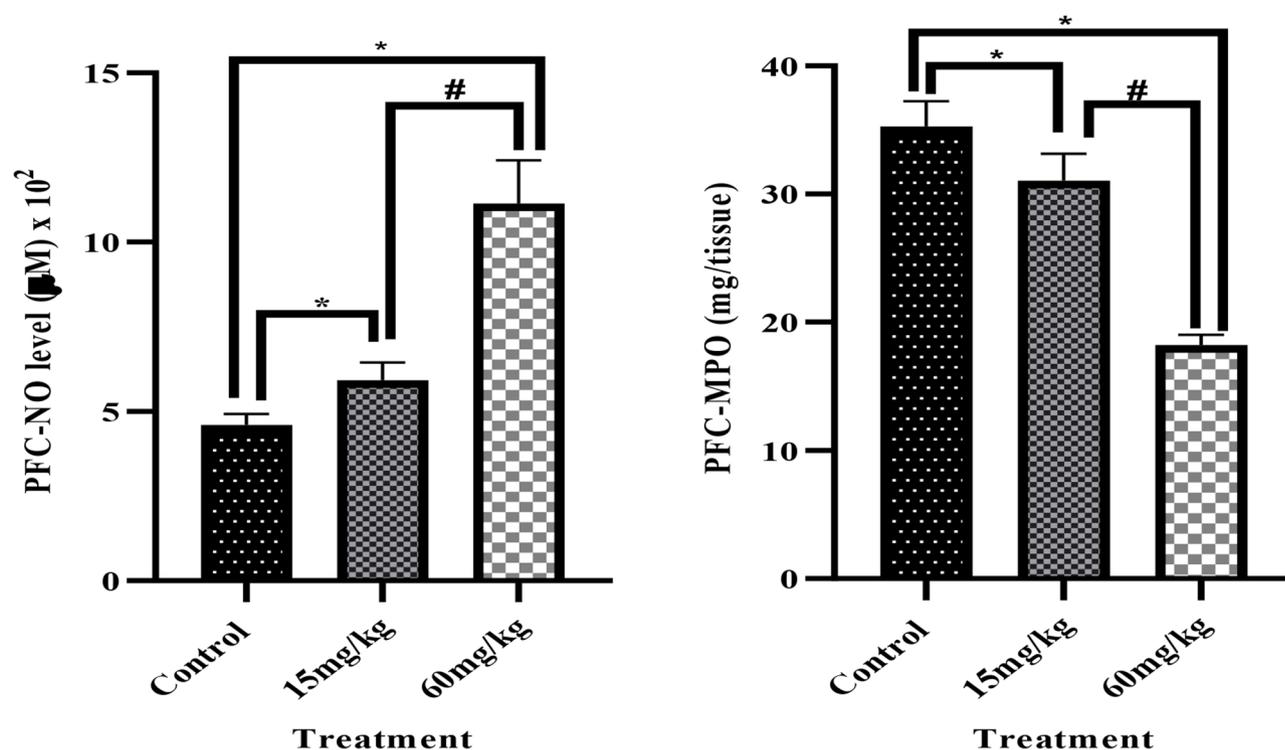
### PE-MPs distorts PFC histoarchitecture of the PFC in rats

Figure 6 depicts histoarchitectural analysis of PFC from PE-MPs-exposed rats which revealed significant structural changes compared to the control group. Haematoxylin and eosin (H&E) stained sections of the PFC from (A) control (CNT), (B) 15 mg/kg PE MPs, and (C) 60 mg/kg PE MPs groups (magnification  $\times 400$ ). Histological analysis of the PFC revealed dose-dependent neurotoxic effects of PE-MPs. The control group exhibited normal cortical architecture with no observable pathological changes. In the 15 mg/kg PE-MP group, mild neuronal degeneration, vacuolation, and gliosis were observed ( $5.00 \pm 0.82$ ), which was significantly higher than control ( $p < 0.01$ ). The 60 mg/kg group showed extensive neuronal loss, pronounced vacuolation, significant perivascular edema, glial proliferation, and marked disruption of cortical organization ( $13.00 \pm 1.41$ ), significantly higher than both the control ( $p < 0.001$ ) and 15 mg/kg group ( $p < 0.01$ ). These results indicate that PE-MP exposure induces a dose-dependent increase in neurodegenerative damage in the PFC.

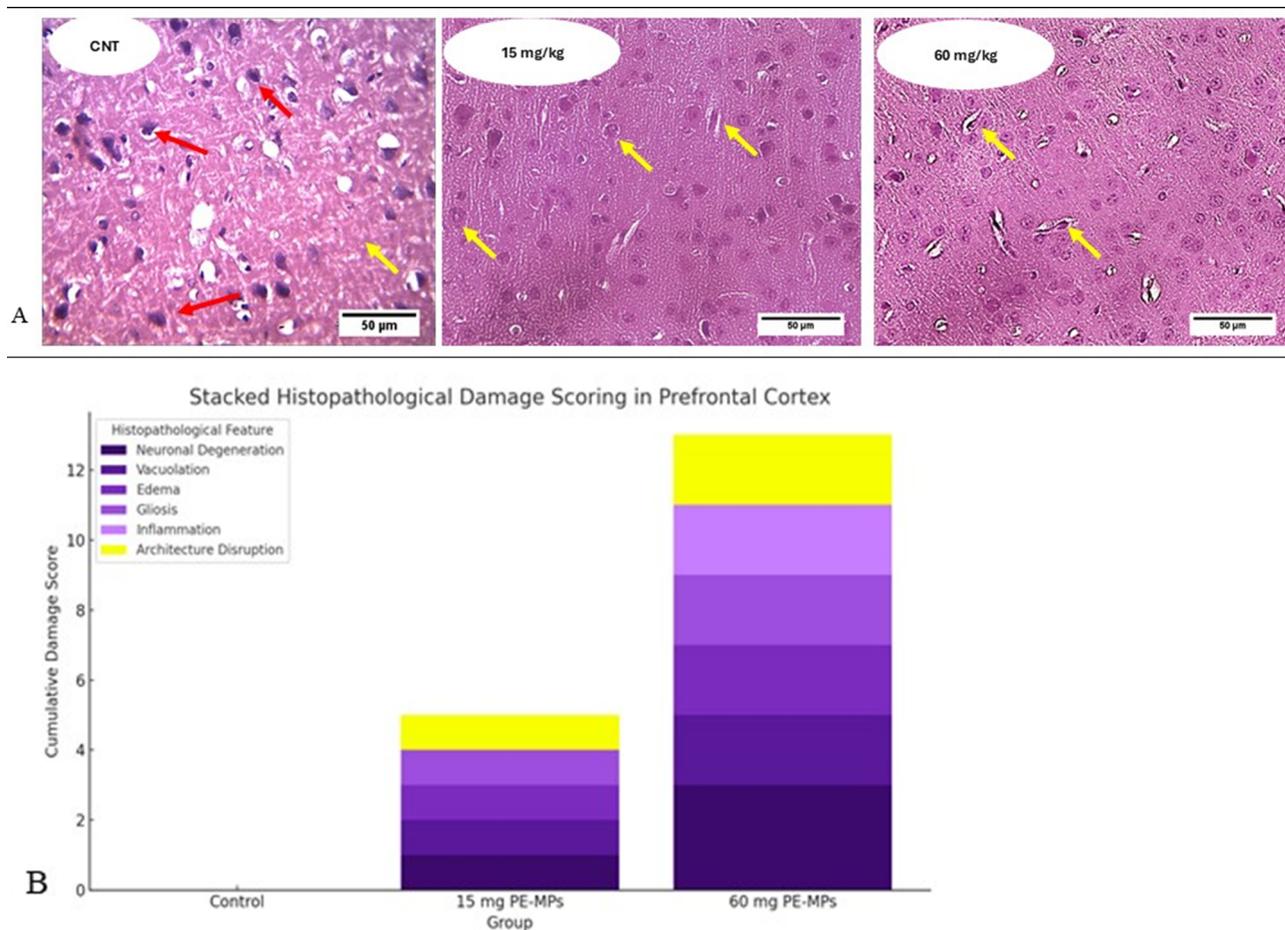
### Discussion

This study offers novel insights into the neurotoxic potential of PE-MPs, specifically examining their impact on bioenergetics, mitochondrial redox, inflammation, and histoarchitecture in the PFC section of the brain. The findings strongly support the emerging hypothesis that chronic microplastic exposure is not merely an ecological hazard but a plausible contributor to neurodegenerative processes in mammals, including humans<sup>45</sup>.

Our findings showed that exposure to PE-MPs disrupts key enzymes involved in PFC glycolysis in a dose-dependent manner, indicating both metabolic impairment and compensatory adaptations. A striking aspect of this study is the biphasic modulation of key glycolytic enzymes, particularly HK and ALD, highlighting an initial suppression of energy entry points, followed by a compensatory but maladaptive glycolytic upregulation. This



**Fig. 5.** Neural inflammatory biomarkers in PE-MPs exposed rats. The values are presented as mean  $\pm$  SD. ( $n=5$ ) per group. \* Significantly ( $P < 0.05$ ) different when compared with control; # significantly ( $P < 0.05$ ) different relative to 15 mg/kg PE-MPs. PFC-Pre-frontal cortex, NO- Nitric oxide, MPO-Myeloperoxidase.



**Fig. 6.** (A) Representative photomicrographs of prefrontal cortex from control (CNT) and (PE MPs)-exposed groups (15 and 60 mg) (Upper). Hematoxylin and eosin (H&E) stained sections of the PFC from control (CNT), 15 mg/kg PE MPs, 60 mg/kg PE MPs groups (magnification  $\times 400$ ). The control group (Red arrow) displays normal neuronal architecture with well-preserved neurons and glial cells. In contrast, the 15 mg/kg group shows (Yellow arrow) mild neuronal shrinkage and scattered vacuolation, while the 60 mg/kg group exhibits pronounced neurodegenerative changes, extensive neuronal loss, cytoplasmic vacuolation, and perivascular edema. (B) Histopathological damage scores in the PFC of control and PE-MP-exposed rats. Scores were assigned based on the severity of neuronal degeneration, vacuolation, edema, gliosis, inflammation, and cortical disorganization, using a semi-quantitative scale (0 = none, 1 = mild, 2 = moderate, 3 = severe). Each parameter was assessed from photomicrographs of H&E-stained brain sections shown (Lower).

response mirrors metabolic reprogramming seen in neurons under chronic stress, where impaired mitochondrial oxidative phosphorylation forces a shift toward anaerobic glycolysis, a less efficient and potentially harmful energy pathway, or disrupted glucose transport<sup>46,47</sup>. The upregulation of LDH and suppression of NADase activities collectively indicate a metabolic pivot toward anaerobic glycolysis. While this shift may transiently sustain ATP production in the PFC, it comes at a steep cost, promoting lactate accumulation, intracellular acidosis, and redox imbalance, all of which are hallmarks of neurodegenerative pathophysiology<sup>48–50</sup>.

Furthermore, PE-MPs induce systemic mitochondrial failure by impairing oxidative phosphorylation, the terminal stage of cellular respiration essential for ATP synthesis. Specifically, the dose-dependent inhibition of key TCA cycle enzymes, CS, IDH, and MDH, combined with reduced activity of ETC complexes I, III, and IV, indicates a collapse of mitochondrial energy metabolism. These impairments hinder the production of NADH and FADH<sub>2</sub>, essential electron donors for the ETC, ultimately compromising ATP output and neuronal viability<sup>51,52</sup>. Paradoxically, the upregulation of SDH, a dual-function TCA/ETC enzyme, may reflect a maladaptive compensatory response. Hyperactivation of SDH is associated with enhanced electron leakage and ROS generation, exacerbating oxidative stress<sup>53,54</sup>. Additionally, disruption at CPLX III, a critical electron relay hub, can further promote ROS accumulation, while inhibition of CPLX IV in the PFC impairs oxygen reduction and compromises mitochondrial membrane potential<sup>54,55</sup>.

Additionally, this study advances the understanding of oxidative stress responses to PE-MPs exposure. Observed alterations in redox markers suggest impaired oxidative balance; however, specific signalling pathways (e.g., Nrf2, microglial activation) were not directly measured and are therefore presented as potential, rather than definitive, mechanisms. The increase in NO concentration and reduced MPO activity highlights a disrupted inflammatory and redox status, consistent with the findings of Kehinde et al.<sup>39</sup>, and warrants further investigation into the mechanisms underlying these divergent pathways. Furthermore, histological evidence provides critical validation for the biochemical disruptions observed in this study. Although PE-MP accumulation within the PFC was not directly quantified, recent studies demonstrate that small polyethylene particles can cross the intestinal barrier and reach the frontal cortex, while indirect mechanisms such as systemic inflammation and gut–brain axis signalling may also mediate neurotoxicity<sup>20–24</sup>.

Thus, it is hypothesized that the observed PFC alterations likely reflect a combination of direct and indirect effects. Emerging evidence further supports the plausibility of indirect neurotoxicity following microplastic exposure via disruption of the gut microbiota and its downstream metabolic and immunological signalling. Recent studies have demonstrated that polyethylene and other polymeric microplastics induce gut dysbiosis, compromise intestinal barrier integrity, and alter microbial-derived metabolites, thereby promoting systemic inflammation and oxidative stress that can propagate to distal organs, including the brain<sup>56–58</sup>. Such microbiota-mediated mechanisms have been shown to influence mitochondrial function, redox homeostasis, and neuroinflammatory tone, providing a biologically coherent link between peripheral microplastic exposure and central nervous system dysfunction. These findings offer important mechanistic context for the present observations and support the hypothesis that PE-MP-induced prefrontal cortex alterations may arise not only from direct tissue exposure but also from gut–brain axis–driven pathways.

### Study limitations and future perspectives

A key limitation of our study is that we did not directly measure PE-MPs in the rat PFC. Although our findings provide important insights into systemic effects, the absence of direct brain measurements restricts conclusions about central nervous system accumulation. Thus, future investigations incorporating direct quantification of PE-MPs in the brain are warranted to substantiate their potential neurobiological impact. As this study was designed as a preliminary investigation to generate initial data on dose-dependent effects rather than to establish definitive conclusions, and the sample size was limited to 15 rats (5 per dose group) in accordance with ethical committee restrictions, the findings should be interpreted with caution. While the present study demonstrates clear neurotoxic outcomes in terms of bioenergetics, oxidative and inflammatory disruption, it does not address the underlying regulatory mechanisms, such as the Nrf2-Keap1 antioxidant pathway, the NF- $\kappa$ B inflammatory axis, or the MAPK signalling network, which future work should examine using approaches like Western blotting, qPCR, and immunohistochemistry. Moreover, the potential contribution of the gut-brain axis remains unexplored, despite evidence that microplastics can alter gut microbiota and thereby influence brain function through metabolic and immune signalling.

### Conclusion

In sum, this study provides evidence that PE-MP exposure is associated with impaired bioenergetics, oxidative imbalance, inflammatory modulation, and structural alterations in the rat prefrontal cortex. These findings extend the neurotoxicity profile of microplastics while emphasizing the need for cautious interpretation and further studies incorporating biodistribution and mechanistic validation.

### Data availability

All data supporting the findings of this study are available within the article.

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### Author contributions

\*\*Samuel Abiodun Kehinde: \*\* Conceptualization, Methodology, Investigation, Supervision, Writing – original draft. \*\*Abiola Busola Tolulope: \*\* Methodology, Data curation, Investigation. \*\*Abosede Temitope Olajide: \*\* Formal analysis, Validation. \*\*Ayokanmi Ore: \*\* Investigation, Data curation. \*\*Tolulope Peter Fatokun: \*\* Visualization, Software, Methodology. \*\*Hafsoh Motunrayo Adejumo: \*\* Investigation, Writing – review & editing. \*\*Sarva Mangala Praveena: \*\* Resources, Validation, Writing – review & editing. \*\*Mariana Teles Pereira: \*\* Project administration, Writing – review & editing. \*\*Sasitorn Chusri: \*\* Conceptualization, Project administration, Funding acquisition, Resources, Writing – review & editing.

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### Declarations

### Competing interests

The authors declare no competing interests.

### Institutional review board statement

The experimental protocol was approved by the Ethical Review Committee of the Faculty of Natural Sciences, Ajayi Crowther University, Oyo, Nigeria under reference number FNS/ERC/2024/017NH.

### Additional information

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