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Mercury-Induced Oxidative Stress and Neuropathologic Changes in the Prefrontal Cortex of Wistar Rats

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ABSTRACT

Access to clean water is necessary for human, animal, and environmental health. Anthropogenic human activities such as mining, agriculture, and continuous pollution from industrial waste, as well as population growth, continue to compromise the quality of water. The neurodegenerative impact of mercury with a similar concentration as obtained from waterways on the prefrontal cortex of Wistar rats was studied. Twenty adult rats were randomised into two groups: a control group and a 0.001 mg/L mercury (II) thiocyanate (Hg(SCN)₂) group, *ad libitum* for 65 days, after which the animals were euthanised via cervical dislocation. Blood and tissue samples were obtained for analyses of oxidative stress biomarkers: superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA), hydrogen peroxide (H₂O₂), histological, and glial fibrillary acidic protein (GFAP) and inducible nitric oxide synthase (iNOS) immunohistochemical evaluation. Statistical data were analysed using a t-test, set at $p < 0.05$. When comparing the mercury-treated groups to the control, a significant difference was observed in the SOD, GPx, MDA, and H₂O₂ values, with cellular alterations evident in the mercury-treated rats. Additionally, mercury treatment resulted in the distortion of the cortical histoarchitecture with intense immunoreactivity of GFAP and iNOS suggesting neuroinflammation. This study highlighted that mercury exposure induced oxidative stress and led to distortion in the histoarchitecture of the prefrontal cortex of adult Wistar rats.

Keywords

Mercury, Neurodegeneration, Neuroinflammation, Oxidative stress, Pollution

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INTRODUCTION

Human populations face constant exposure to environmental toxicants, intensifying global concerns over public health and contamination (Adeniyi *et al.*, 2023; Goel *et al.*, 2023; Moronkeji and Akinbo, 2024). Availability and accessibility to clean water remain necessary for human, animal, and environmental health. However, recently, water quality has been compromised by a continuous increase in industrial, agricultural, and mining activities and population

growth (Carolin *et al.*, 2017; Vardhan *et al.*, 2019; Sonone *et al.*, 2020; Dehkordi *et al.*, 2024). Major pollutants in the water bodies include heavy metals, plastics, dyes, pharmaceutical products, organic matter, pathogens, and several others (Villarin and Merel, 2020). One of the most abundant pollutants in the ecosystem is heavy metal ions, making it of great public health concern (Azimi *et al.*, 2017).

Heavy metals are important environmental toxicants of public health importance. They are considered toxic and

harmful to human and animal health. Sources of heavy metals in the environment include natural sources such as volcanic eruptions, disintegration of rocks containing heavy metals, and human activities such as mining, extraction of minerals, agricultural practices, and other industrial processes (Kapoor and Singh, 2021; Wu *et al.*, 2024). All these activities have increased the concentration of heavy metals within the ecosystem (Tchounwou *et al.*, 2012; Bharti and Sharma, 2022). A study by Adeniyi *et al.* (2017) found that heavy metals polluted waterways in three geopolitical zones of Kwara State, Nigeria, with the average concentrations of mercury in the water being between 0.001 mg/L and 0.006 mg/L. Heavy metals in a prepared solution sample were compared to those found in waterways in Kwara State by Adeniyi *et al.* (2019; 2023), who reported that both had similar negative effects on the structure and function of the prefrontal cortex and visceral organs in Wistar rats. Furthermore, the impact of mercury alone on the prefrontal cortex at concentrations earlier reported on the waterways is currently being studied to determine the singular effect of mercury toxicity on the brain. There are several compounds of mercury among them; methylmercury causes neurological alterations that occur in living tissues (Novo *et al.*, 2021; Wu *et al.*, 2024). Studies have reported that the mechanisms of mercury-induced toxicity are related to the oxidative damage caused by increased reactive oxygen species (ROS) in the tissue (Kang *et al.*, 2024). Oxidative stress has been connected to the pathogenesis of neurodegenerative diseases, which include Alzheimer's disease and Parkinson's disease (Bridges and Zalups, 2010; Fernandes Azevedo *et al.*, 2012; Zafar *et al.*, 2024). This study aimed to evaluate mercury-induced toxicity in the same concentration as obtained from the waterways in Kwara State, Nigeria.

MATERIALS AND METHODS

Procurement of Animal

Twenty adult male Wistar rats (150–180 g) were obtained from the animal facility of the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan. The rats were kept in a plastic cage and allowed to acclimatise for two weeks before the commencement of the experiment.

Preparation of Solutions

Mercury (II) thiocyanate [$\text{Hg}(\text{SCN})_2$, 0.001 g, Sigma-Aldrich Chemical Co., USA] was dissolved in a litre of double-distilled water to form a 0.001 mg of $\text{Hg}(\text{SCN})_2$ per litre solution. This was determined by the empirical measurement of heavy metals collected from the waterways of Kwara State, Nigeria (Adeniyi *et al.*, 2017).

Experimental Design

The twenty adult male rats were randomly divided into two groups of ten animals each selected by a simple randomisation method as described by Svendsen and Hau (1994). The study was for 65 days, and the duration was selected Adeniyi *et al.*

based on a previous report (Adeniyi *et al.*, 2019). The rats were maintained under standard laboratory conditions: light and dark cycles of 12 h were provided at room temperature. They were fed with pelletised rat feed and water *ad libitum* as depicted in Table 1.

Table 1: Grouping and Treatment of Animals.

Group	Treatment
1	Control group was fed with pelletized feed and double distilled water <i>ad libitum</i>
2	Test group had access to pelletized feed and dissolved 0.001 mg/L of $\text{Hg}(\text{SCN})_2$ water <i>ad libitum</i>

Research Ethical Approval

The protocol and procedures for this study were approved by the College of Medicine Research Ethics Committee, University of Nigeria, Enugu campus, Enugu State, with protocol number 025/02/2017, which is consistent with the set down guide by the National Institute of Health (NIH) in the "Guide to the Care and Use of Animals in Research and Teaching" (National Academy of Science, 2011).

Measurement of Body Weight

The weight of the rats was obtained weekly using a sensitive digital weighing balance (SP 401, OHAUS, Parsippany, USA) to account for possible resulting physical changes in the rats for the 65-day treatment duration (Adewole and Ayoka 2009; Ibrahim *et al.*, 2012).

Collection of Blood Samples and Tissue from Laboratory Animals

The animals were euthanised by cervical dislocation twelve hours after administration of the final dose. Blood samples were collected via cardiac puncture under aseptic conditions, transferred into plain bottles, allowed to clot, and centrifuged at 4,000 rpm for 5 min to obtain serum for oxidative stress biomarker analysis. Bone forceps were used to open the skull. The brain was gently removed, and the prefrontal cortex was obtained from the anterior cerebral cortex. The harvested tissues were fixed in 10% buffered formal saline, weighed, and processed for paraffin tissue embedding using established protocols (Bancroft and Gamble, 2008) and stained for histological and immunohistochemical evaluation, respectively (Drury and Wellington, 1980; Adeniyi *et al.*, 2019).

Biochemical Analyses

The clotted blood samples were spun for 5 min at 4,000 revolutions per minute to get the serum on a bench centrifuge. The sera were pipetted into vials that were stored at -20°C until biochemical analyses were carried out. The preserved sera were assayed for superoxide dismutase (SOD) using the method outlined by Ijomone *et al.* (2018), glutathione peroxidase (GPx) using the method of Paglia and Valentine (Gallo and Martino, 2009; Mikulková *et al.*, 2020), hydrogen peroxide using the method of Bopitiya *et al.* (2021), and malondialdehyde (MDA) using the method of Deger *et al.* (2023).

Histological Evaluation

Histological analysis was carried out using haematoxylin and eosin (H&E) stain to demonstrate the general architectural profile of the prefrontal brain neurons (Bancroft and Gamble, 2008; Adeniyi *et al.*, 2023).

Immunohistochemical Evaluation

Glial fibrillary acidic protein (GFAP) immunohistochemistry was done to demonstrate the distribution of glial cells during oxidative stress. Glial activation is a primary indicator of stress-induced cell death in neuronal cell layers (Lyck *et al.*, 2008; Delcambre *et al.*, 2016). Inducible nitric oxide synthase (iNOS) was used to demonstrate oxidative stress in the prefrontal cortex tissue (Hoehn *et al.*, 2003). Briefly, the antigen retrieval process was carried out on the paraffin sections using a citrate-based antigen unmasking buffer solution (pH: 6.0). Endogenous peroxidase activity was then blocked in 0.3% hydrogen peroxide. Sections were incubated at room temperature in primary rabbit antibodies GFAP and iNOS, respectively (ThermoFisher, USA; #16825-1-1AP) at 1:7,500. A biotinylated secondary antibody (ThermoFisher, USA) at 1:7,500 was applied, and colour was developed with 3,3'-diaminobenzidine peroxidase. Sections were finally counterstained in Meyer's haematoxylin.

Statistical Analyses

Statistical analyses were conducted using GraphPad Prism version 8 (GraphPad Inc, San Diego, US) statistical software. The statistical tests included the student's t-test and the Mann-Whitney U test, with P values less than 0.05 ($p < 0.05$) considered statistically significant. The data are presented as means plus or minus the standard deviation (SD) with appropriate symbols to indicate significant differences on the graphs.

Microscopy and Photomicrography

The Olympus U-D03 microscope was used to view the stained sections, while the Olympus DP21 microscope was used to snap. Photomicrographs of stained sections were obtained and reported.

RESULTS

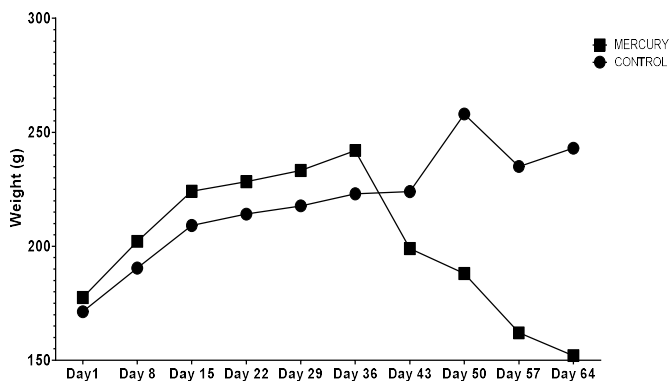
Weight Changes and Mortality

All animals were observed to steadily gain weight during the period of acclimatisation and the early half of the period of the study. Rats in the control group continued to gain weight steadily after acclimatisation while their counterparts in the mercury-treated group steadily lost weight from the end of week 6. This steady weight loss was observed to continue till the end of the period of administration in rats from the mercury-treated group, which resulted in a 10% mortality rate (Fig. 1).

Biochemical Analyses

The effect of mercury on SOD, GPx, lipid peroxidation, and hydrogen peroxide. The activities of SOD and GPx were determined in this study to understand the possible toxicity Adeniyi *et al.*

effects of mercury administration. It was observed that mercury exposure significantly decreased the activity of enzymes of oxidative stress. The SOD value was significantly reduced in the group 2 animals relative to the unexposed control group 1 ($P < 0.05$) as shown in Figure 2.



posed control group 1 ($P < 0.05$) as shown in Figure 2.
Fig. 1: Graph representing the pattern of weight change

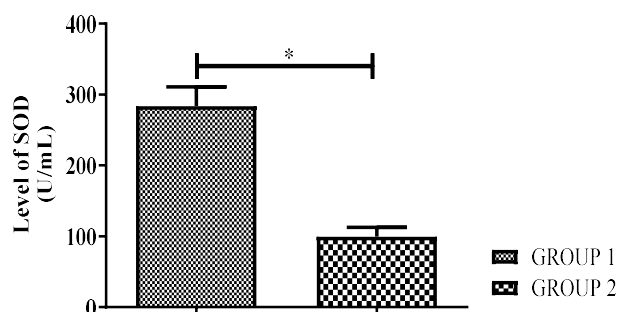


Fig. 2: Depicts the effect of mercury on SOD. Values are expressed as mean + SD. Student's t-test followed by the Mann-Whitney U test. * $p < 0.05$; SOD - superoxide dismutase

As shown in Figure 3, analysed data showed a statistically significant reduction in the GPx level in the group 2 rats when compared to the unexposed control group ($p < 0.05$).

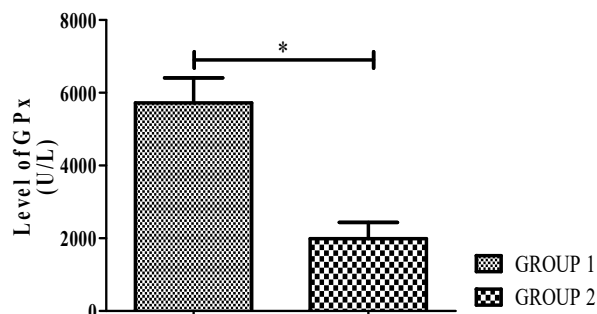


Fig. 3: Depicts the effect of mercury on GPx. Values are expressed as mean + SD. Student's t-test followed by the Mann-Whitney U test. * $p < 0.05$; GPx - Glutathione peroxidase

MDA is one of the secondary products of lipid peroxidation. The MDA finding in this study greatly enhanced lipid peroxidation in rats in the test group compared to the control.

MDA was significantly elevated ($p < 0.05$) in animals exposed to mercury compared with animals from the control. The alteration in the level of MDA in Group 1 and Group 2 is presented in Figure 4.

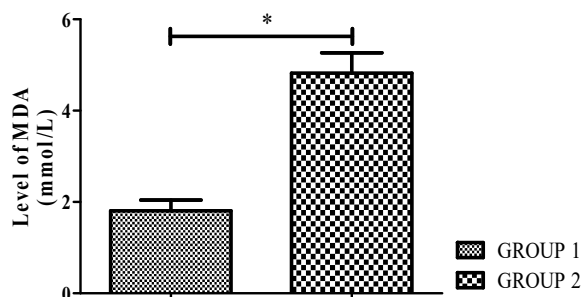


Fig. 4: Depicts the effect of mercury on MDA. Values are expressed as mean + SD. Student's t-test followed by the Mann–Whitney U test. * $p < 0.05$; MDA - malondialdehyde

Hydrogen peroxide is one of the free radicals or ROS. In this study, it was observed that the level of hydrogen peroxide significantly increased in the mercury-exposed group when compared to the control group (Fig. 5).

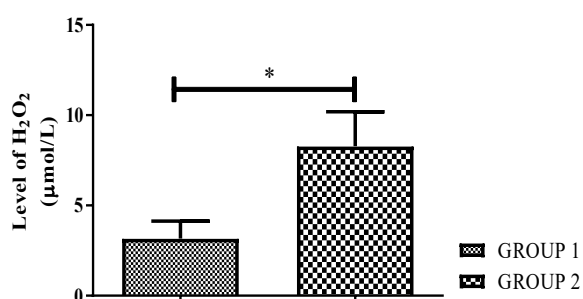


Fig. 5: Depicts the effect of mercury on the level of hydrogen peroxide. Values are expressed as mean + SD. Student's t-test followed by the Mann–Whitney U test. * $p < 0.05$

Effect of Mercury on the Histological and Immunohistochemical Evaluation

Histological Evaluation

The histological evaluation shows that the control group had normal prefrontal cortex histoarchitecture with intact neurons while the mercury-exposed group showed features of neurodegeneration (Fig. 6). The control rats had intact neurons and normal neurophils with normal glial cells present within the neurophils. The blood vessels also appeared normal (Fig. 6a). The prefrontal cortex of the mercury-exposed rats showed features of neurodegeneration typified by the swelling of the soma, reactive astrocytic gliosis, and a marked-up neurophil vacuolation degeneration (Fig. 6b).

Immunohistochemical Evaluation

Immunohistochemical evaluation of sections from the control group shows mild immunohistochemical reactivity to GFAP and iNOS, respectively, while the treated group showed marked reactivity (Fig. 7 and 8).

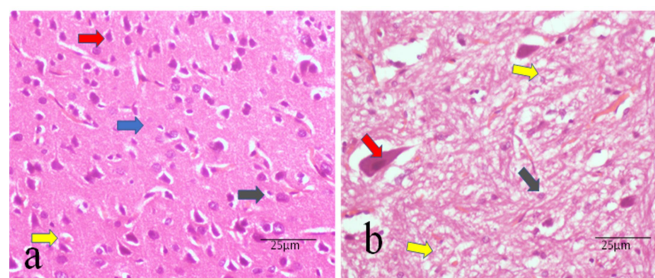


Fig. 6: Photomicrographs of the prefrontal cortex. (a) The control group shows intact neurons (red arrow), normal blood vessels (yellow arrow) in neuropil (blue arrow), and normal glial cells scattered within the neuropil (green arrow) (H&E ×400). (b) Mercury-exposed group: swollen soma (red arrow), glia (green arrow) with marked neuropil vacuolation degeneration (yellow arrow) (H&E, ×400).

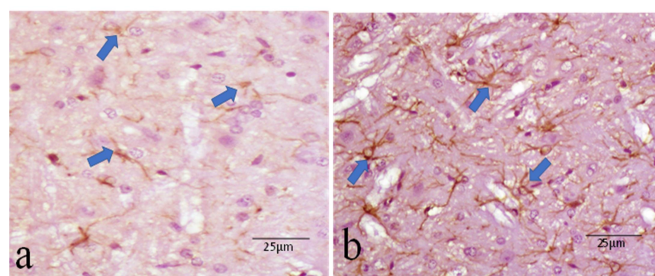


Fig. 7: Photomicrographs of an immunohistochemical assessment of the prefrontal cortex. (a) The control group shows mild GFAP immunoreactivity with specific and uniform expression (blue arrows). (b) The mercury-treated group shows strong immunoreactivity and several deeply expressed GFAP (blue arrows) (GFAP, ×400).

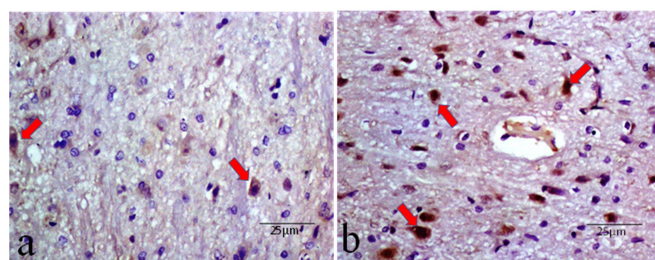


Fig. 8: Photomicrographs of an immunohistochemical assessment of the prefrontal cortex. (a) The control group shows low immunoreactivity to iNOS. (b) The mercury-treated group shows intensely expressed immunoreactivity (red arrows) with iNOS (iNOS, ×400).

DISCUSSION

There is no known biological need for mercury in the human body. Mercury is an extremely toxic heavy metal that exists in three forms: elemental, organic, and inorganic, which all have adverse effects on the ecosystem (Zafar *et al.*, 2024). In this study, our results show that treatment with mercury in rats induced body weight loss. Reduction in body weight is a vital indicator of the disruption in the overall health of the animals (Uzunhisarcikli *et al.*, 2016).

Mahboob *et al.* (2001) identified weight loss as the principal symptom of mercuric chloride toxicity, linking it to animals' low food consumption. Other possible causes could include anorexia, abnormalities in the functional integrity of the gastrointestinal tract, systemic toxemia, and muscle weakness associated with exposure to toxic metals (Jadhav *et al.*, 2007; Olatunji *et al.*, 2015). Our result agrees with the works of Shalan (2022), who reported a significant decrease in the body weight of rats orally administered with mercury. The rats continued to lose weight steadily until the end of the administration period, which led to a 10% mortality rate at 150 g. The current study observed a significant increase in the hydrogen peroxide level in the mercury-exposed group compared to the control group. This suggests the induction of oxidative stress, which leads to the production of ROS. Hydrogen peroxide increases in cells due to excessive free radicals, which cause tissue damage (Foyer and Noctor, 2005). Mercury alters the inner membrane of mitochondria, leading to an increase in hydrogen peroxide production in the mitochondrial electron transport chain, which in turn decreases glutathione levels in the mitochondria. These alterations in mitochondrial function can cause neuronal cell death via necrosis or apoptosis (Hazelhoff *et al.*, 2021). Researchers have identified two pathways through which mercury initiates oxidative stress and tissue damage: an increase in ROS production and a direct depletion of antioxidant reserves (Shalan, 2022). Our results show that the administration of mercury significantly elevated MDA levels, which is an indicator of enhanced lipid peroxidation (Xia *et al.*, 2010; Demir *et al.*, 2011; Uzun and Kalender, 2013). MDA is a secondary product of lipid peroxidation, which serves as a biomarker of cell membrane injury. The increase in MDA levels in neurotoxicity suggests intense lipid peroxidation leading to tissue damage. The brain is very sensitive to ROS because of its huge concentration of unsaturated lipids and high oxidative metabolism. Exposure to mercury can result in the production of ROS, which can lead to oxidative stress. These species produce peroxides and superoxide anion radicals, which damage deoxyribonucleic acid and break down proteins (Sudjarwo *et al.*, 2017; Shalan, 2022). Furthermore, studies have shown that mercury inhibits lipid metabolism in animal brains, disrupting total lipid metabolism, cholesterol, and triglycerides, leading to a discrepancy between lipid synthesis and degradation. This result agrees with previous studies that have reported similar findings (Adeniyi *et al.*, 2019; Oliveira *et al.*, 2020; Shalan, 2022). Additionally, studies by Wu *et al.* (2024) have also documented the mechanisms and environmental health impact of mercury. Endogenous antioxidant enzymes are important because they defend the body against oxidative stress injuries by scavenging free radicals in the cell. The body uses SOD and GPx, two potent endogenous enzymes, as indicators to measure oxidative stress. SOD catalyses the dismutation of superoxide radicals to molecular oxygen and hydrogen peroxide (Boujbiha *et al.*, 2009). Similarly, GPx catalyses the reduction of hydrogen peroxide to water and oxygen, which also protects the cell from oxidative injury. In this present study, mercury exposure significantly decreased the activity of

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SOD and GPx in the exposed animals when compared to the unexposed control group. This suggests that mercury causes the inactivation of antioxidant enzymes and the suppression of antioxidant defence. Adeniyi *et al.* (2019) reported a similar result.

Histological evaluation of the prefrontal cortex of the mercury-exposed group showed gross disruption of general cortical histoarchitecture, blood vessel congestion, a large glia population, and extensive necrosis of cortical neurons and vacuolations. As stated earlier, the brain is very sensitive to ROS because of its huge concentration of unsaturated lipids and high oxidative metabolism, which makes the neuronal membrane vulnerable to lipid peroxidation, as evident in the MDA level. Researchers have reported that mercury increases the expression of vascular endothelial growth factor in brain tissue, leading to damage to neurons and the blood-brain barrier. This allows inflammatory and cytotoxic cells from the blood to enter the brain, leading to neuronal cell death (Takahashi *et al.*, 2017; Takahashi and Shimohata, 2019). These findings are in line with previous studies in which heavy metal toxicity resulted in neuronal damage and cell death (Owolabi *et al.*, 2014; Augustine *et al.*, 2021; Said *et al.*, 2021).

GFAP immunostaining is an indicator for mature astrocytes. Astrocytes are important supportive cells of the central nervous system. Oxidative damage triggers them in response to excitotoxicity (Liu *et al.*, 2011). Astrocytes' processes and soma express GFAP. The prefrontal cortex of the mercury-exposed rat showed strong astrocytic immunoreactivity for the GFAP immunostain. The immunoreaction of GFAP indicates the activation of astrocytes as an immunological response to the neurotoxic effects of mercury, which is an indicator of induced neuronal damage (Adekomi *et al.*, 2017; Adeniyi *et al.*, 2019). Our study confirmed that mercury toxicity induces neurodegeneration via oxidative neuronal damage. Shalan (2022) reported corresponding findings that mercury chloride induces necrosis, leukocyte infiltration, and blood vessel congestion in the cerebral cortex.

iNOS are enzymes that catalyse the production of nitric oxide. Inflammatory factors activate astrocytes and other neuroglia to express iNOS (Saha and Pahan, 2006). These elicit the production of nitric oxide and superoxide by neuroglia, which results in the formation of peroxynitrite (Pacher *et al.*, 2007; Brown, 2010). Peroxynitrites are powerful oxidants that cause cell death (Huang *et al.*, 2022). The prefrontal cortex of the mercury-exposed rat showed strong immunoreactivity for the iNOS immunostain. It is clear that strong iNOS reactivity causes oxidative damage by making nitric oxide (Brown, 2010; Adeniyi *et al.*, 2019).

Conclusion

This study showed that mercury depleted body weight, caused cell damage due to decreased antioxidant levels, and disrupted brain histoarchitecture, which strongly responded to GFAP and iNOS immunoreactivity. Deliberate efforts should be taken to ensure the safety of human and

animal lives by reducing the concentration of mercury in the environment.

DECLARATION

Acknowledgements

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Grants and Financial Support

None.

Conflict of Interest

None declared.

Ethical Approval

The protocol and procedures for this study were approved by the College of Medicine Research Ethics Committee, University of Nigeria, Enugu campus, Enugu State, with protocol number 025/02/2017.

Consent to Participate and Publish Data

Not applicable.

Authors' Contribution

TA: Conceptualisation, project administration and supervision, writing the original draft, methodology, data acquisition and analysis, visualisation, and validation. AM: Writing, review and editing, methodology and validation. GO: Writing, review and editing, methodology, and validation. AA: Writing, review and editing, methodology and data analysis. GA: Writing, review, methodology, and data analysis.

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