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Antioxidant Role of Zinc Sulfate in the Inhibition of Lead Acetate-Induced Cerebral Toxicity in Wistar Rats

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Abstract

Lead exerts neurotoxic effects in the nervous system via oxidative stress. Zinc sulfate ($ZnSO_4$) is a dietary supplement with antioxidant properties. Accordingly, this study investigated the possible attenuative effects of $ZnSO_4$ against lead-induced cerebral toxicity in Wistar rats. Forty-two Wistar rats were randomly distributed into six groups (n=7) and treated for twenty-eight days as follows: Group A – Control; Group B – 100 mg/kg body weight (bw) of lead acetate (LA); Group C – 50 mg/kg bw of $ZnSO_4$ and LA; Group D – 100 mg/kg bw of $ZnSO_4$ and LA; Group E – 50 mg/kg bw of $ZnSO_4$; Group F – 100 mg/kg bw of $ZnSO_4$. Thereafter, neurobehavioural, antioxidant, lipid peroxidation, and histological assessments were done. The findings revealed a significantly impaired ($p<0.05$) discrimination index and antioxidant enzymes activity as well as a significantly elevated ($p<0.05$) transfer latency and lipid peroxidation in the LA-exposed rats. Also, histological findings revealed cytoplasmic vacuolations and neuronal degeneration in the cerebral cortex of LA-exposed rats. However, these LA-induced effects were significantly attenuated in the cortex of rats pretreated with $ZnSO_4$. Altogether, these findings provide valuable insights into the therapeutic potential of $ZnSO_4$ as a neuroprotective agent, thus offering hope for the development of novel neurotherapeutic strategies against lead and other heavy metals.

Keywords: Lead, Zinc Sulfate; Cerebrum; Lead Acetate; Neuroprotection

INTRODUCTION

Lead is a highly poisonous heavy metal that is bluish gray with an atomic number of 82, molecular weight of 207.2, a density of 11.34 g/cm^3, and a melting point of 621.43 °F (Kumar et al., 2020). It is a significant environmental pollutant with sources both from anthropogenic activities and natural processes, causing extensive contamination and health risks. Anthropogenic activities such as industrial processes, mining, smelting, and combustion of fossil fuels contribute to the release of lead into the environment (Tchounwou et al., 2012). These activities result in the emission of lead-containing particulate matter and the deposition of lead compounds onto soil and water surfaces.

Lead toxicity remains a significant public health concern globally, with adverse effects on multiple organ systems and widespread environmental contamination (Sridevi and Umamaheswari, 2020). Lead toxicity is primarily mediated through oxidative stress, disruption of enzymatic function, interference with neurotransmitter signaling, and induction

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of inflammation (Enogieru and Idemudia, 2025; Virgolini and Aschner, 2021). The central nervous system is particularly vulnerable to lead toxicity, with adverse effects on neurodevelopment, cognitive function, and neurobehaviour (Enogieru and Idemudia, 2024; Gundacker et al., 2021). Additionally, lead exposure impairs the functioning of several systems of the body, including the renal, neurological, cardiovascular, reproductive, hematological, developmental, and immunological systems, contributing to increased morbidity and mortality in affected populations (Harshitha et al., 2024). The detrimental effects of lead on human health, particularly in vulnerable populations such as children and pregnant women, underscore the urgent need for effective interventions to mitigate lead toxicity.

Zinc, an essential trace element, plays a pivotal role in various physiological processes, including growth, development, immunity, and antioxidant defense (Kiouri et al., 2023). Emerging research suggests that zinc supplementation may offer significant protective effects against the adverse effects of toxins in the body (Kiouri et al., 2023). Zinc serves as a crucial cofactor for antioxidant enzymes, which play a pivotal role in neutralizing reactive oxygen species and preventing oxidative damage to cells and tissues (Marreiro et al., 2017). Furthermore, zinc supplementation has been shown to upregulate the expression of metallothionein, a metal-binding protein that sequesters toxic metals such as cadmium, mercury, and lead, thereby reducing their availability for inducing oxidative stress (Marreiro et al., 2017). Zinc supplementation has been investigated as a potential protective measure against the adverse effects of environmental toxins. For example, studies have shown that zinc supplementation can mitigate pesticide-induced oxidative stress, neurotoxicity, and reproductive toxicity in animal models (Saad-Hussein et al., 2020). Furthermore, zinc has been shown to modulate the expression of detoxification enzymes such as cytochrome P450, glutathione S-transferase, and metallothionein, thereby enhancing the body's ability to metabolize and eliminate environmental toxins (Wu et al., 2011).

Zinc sulfate, containing the essential mineral zinc, has been suggested to protect against diseases linked to oxidative stress. Despite these potential benefits, there is limited research evidence regarding the neuroprotective effects of zinc sulfate against heavy metal toxicity, specifically lead. Therefore, this investigated the potential protective activity of zinc sulfate against the neurotoxic effects of lead in order to provide novel insights into its therapeutic potential.

MATERIALS AND METHODS

Chemicals and Reagents

Zinc Sulfate ($ZnSO_4$; 99% purity) and Lead acetate ($Pb(C_2H_3O_2)_2$; 99% purity) obtained from Loba Chemie, India, were used for this study.

Animals and Treatment Regimen

Forty-two Wistar rats, between 130 g-145 g were acquired and reserved in the Department of Anatomy animal house for a fourteen-day acclimatization period. The experiment was approved by the Research Ethics Committee, University of Benin, Nigeria (CMS/REC/2024/575). The experimental rats were treated as shown below (Table 1).

Table 1: Treatment protocol

Groups	Treatment
Group A (Control)	1 ml normal saline
Group B (Pb)	100 mg/kg Pb
Group C (ZS1 + Pb)	50 mg/kg $ZnSO_4$ + 100 mg/kg Pb
Group D (ZS2 + Pb)	100 mg/kg $ZnSO_4$ + 100 mg/kg Pb
Group E (ZS1)	50 mg/kg $ZnSO_4$
Group F (ZS2)	100 mg/kg $ZnSO_4$

Pretreatment of $ZnSO_4$ was done one hour before the administration of Pb, daily via an oral gavage, for twenty-eight days.

Neurobehavioural assessments

The novel object recognition test was carried out as previously reported (Enogieru and Egbon, 2022; Okhah and Enogieru, 2023). The discrimination between the familiar and novel objects served as the discrimination index and was calculated as follows: Discrimination Index = (Novel Object – Familiar Object 1) / (Novel Object + Familiar Object 1). Also, the elevated plus maze test was carried out as previously reported by our laboratory (Enogieru and Williams, 2024). The Transfer Latency (TL) was defined as the duration taken to transition from the open arm into any of the closed arms, as previously reported (Khan et al., 2023).

Biochemical Assessments

The cerebra were homogenized in ice-cold 20 Mm Tris-HCl buffer (pH 7.4), and the homogenate was then centrifuged at 10,000 g for 10 min at 4°C (Enogieru and Iyoha, 2023). The supernatant was obtained and evaluated for Superoxide dismutase – SOD (Misra and Fridovich, 1972), Catalase – CAT (Cohen et al., 1970), Glutathione peroxidase – GPx (Nyman, 1959), Malondialdehyde – MDA (Buege and Aust, 1978), and Glutathione - GSH (Ellman, 1959) activities.

Histological evaluation

The cerebra were fixed in 10% buffered formal saline for seventy-two hours, and the Hematoxylin and Eosin staining method was utilized for further processing (Drury and Wallington, 1980).

Statistical analysis

Data analysis was done using the GraphPad Prism Software V9. One-way analysis of variance (ANOVA) and Tukey's multiple comparisons post hoc test were employed to ascertain significance ($p < 0.05$). Values are presented as Mean ± Standard Error of Mean (SEM).

RESULTS

Neurobehavioural Findings

For the discrimination index, a significant decrease ($p<0.05$) was observed in the Pb group following comparison to control. On the contrary, a significant increase ($p<0.05$) was observed in the pretreated groups (ZS1 + Pb and ZS2 + Pb) when compared to the Pb group. For transfer latency, a significant increase ($p<0.05$) was noticed in the Pb group following comparison to control. However, a significant decrease ($p<0.05$) was noted in the pretreated groups (ZS1 + Pb and ZS2 + Pb) when compared to the Pb group.

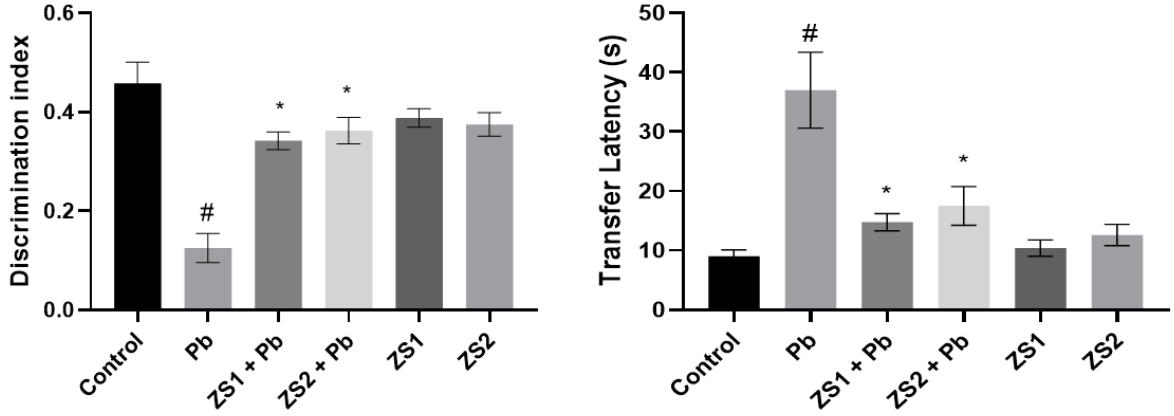

Figure 1: Neurobehavioural parameters across experimental groups.
# p<0.05 compared with the control group; * p<0.05 compared with Pb-group.

Biochemical Findings

For SOD, CAT, GSH. and GPx, a significant decrease (p<0.05) was observed in the Pb group following comparison to control. On the contrary, for SOD and GSH, a significant increase (p<0.05) was observed in the pretreated groups (ZS1 + Pb and ZS2 + Pb) when compared to the Pb group. For CAT and GPx, only pretreated group D (ZS2 + Pb) was significantly higher (p<0.05) when compared to the Pb group. For MDA, a significant increase (p<0.05) was noticed in the Pb group following comparison to control. However, a significant decrease (p<0.05) was noted in the pretreated groups (ZS1 + Pb and ZS2 + Pb) when compared to the Pb group.

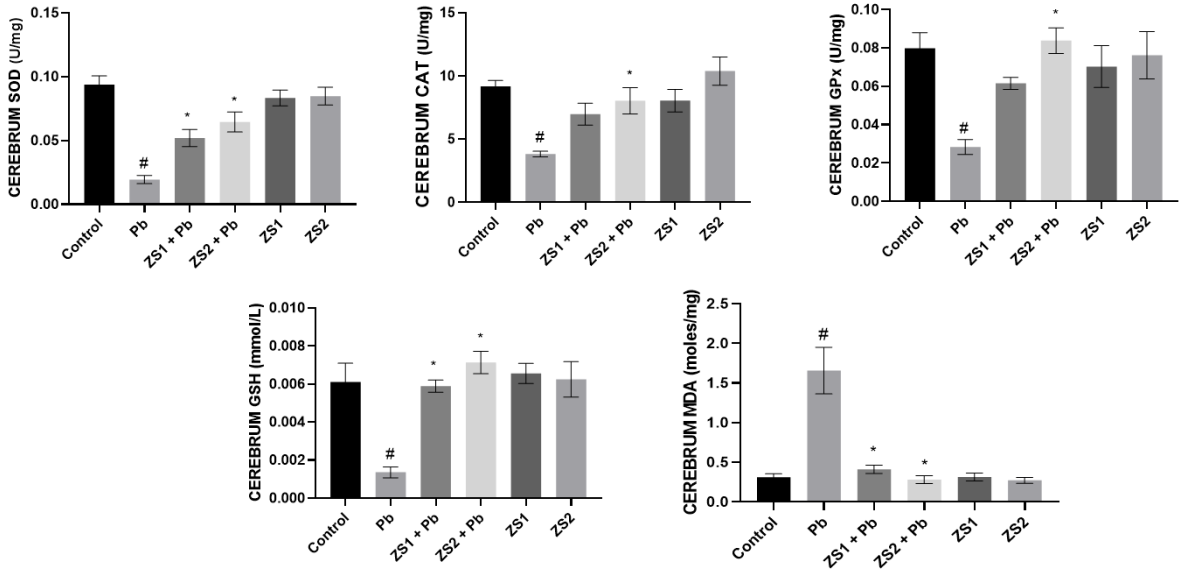

Figure 2: Biochemical Parameters across experimental rats.
# p<0.05 compared with the control group; * p<0.05 compared with Pb-group.

Histological Findings

Plate 1A shows the normal histology of the cerebrum (internal granular layer) of control displaying normal granular cells. Plate 1B shows the cerebrum of Pb-treated rats displaying cytoplasmic vacuolization in neuronal cell bodies, and normal granular cells are hardly seen. Plates 1C-D show the cerebrum of $ZnSO_4$-pretreated rats displaying relatively normal features of granular cells following comparison to the Pb group. Plates 1E- F show the cerebrum of $ZnSO_4$-treated rats displaying normal histological features.

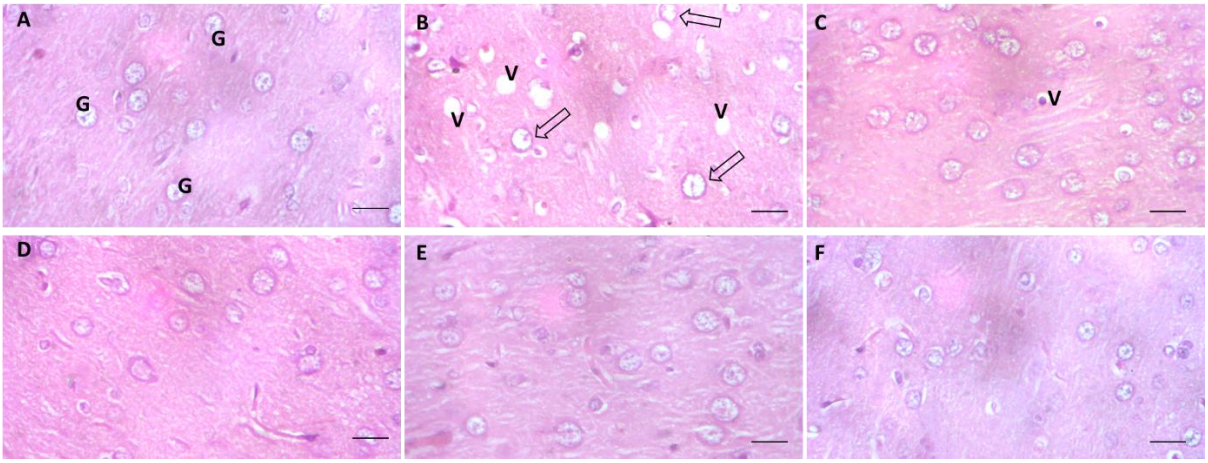

Plate 1: Cerebral cortex across experimental groups. (A) Control - normal granular (G) cells. (B) Pb group - cytoplasmic vacuolization (V); in neuronal cell bodies observed [arrows] (C) ZS1 + Pb - vacuolization (V) (D) ZS2 + Pb (E) ZS1 (F) ZS2 (H&E; 400x; Scale bar: 25µm)

DISCUSSION

Lead exposure has been extensively investigated for its negative effects on the body and brain, especially in experimental models. Reports indicate that lead acetate causes oxidative stress, neurotoxicity, and neurobehavioral abnormalities in rats (Enogieru and Momodu, 2022; Fan et al., 2020). The elevated plus test is a simple method to assess cognition and memory-related behaviour in rats (Danduga and Kola, 2024). In this test, poor cognition or memory retention is demonstrated by an increased duration in movement from an open arm to a closed arm. This parameter, known as transfer latency, is used to assess cognitive function, particularly learning and memory in animal studies. Longer transfer latencies suggest deficits in learning and memory (Haider et al., 2014; Morales-Delgado et al., 2018). In this study, a significant increase in transfer latency was observed in the lead acetate group when compared to the control group, and this is consistent with previous studies demonstrating increased transfer latencies following exposure to lead acetate (Enogieru and Williams, 2024). However, $ZnSO_4$-pretreated rats had a significantly shorter transfer latency, thus indicating that $ZnSO_4$ enhanced learning, memory, and cognitive functions. Similarly, the discrimination index is used to assess an animal's ability to differentiate between a familiar object and a novel one, and a reduction in the discrimination index often suggests a deficit in recognition memory and cognitive impairment (Sivakumaran et al., 2018). In this study, a significant reduction in the discrimination index was noted in rats exposed to lead acetate following comparison to the control group, thus indicating cognitive impairment. This finding is consistent with previous studies demonstrating that lead causes a reduction in the discrimination index in rodents (Enogieru and Egbon, 2022; Enogieru and Idemudia, 2025; Inwang et al., 2024). However, ZnSO4-pretreated rats displayed a higher discrimination index when compared to rats exposed to $ZnSO_4$, thus indicating that $ZnSO_4$ enhances cognition and memory.

Oxidative stress occurs when there is an imbalance between the production of free radicals and the body's inability to neutralize them. This imbalance between ROS production and antioxidant defenses contributes to the pathophysiology of lead-induced damage (Virgolini and Aschner, 2021). To mitigate free radical-induced oxidative damage, cells utilize enzymatic and non-enzymatic antioxidant defence systems. For instance, Superoxide radicals are catalyzed by SOD to become hydrogen peroxide (H_2O_2) and molecular oxygen (O_2); CAT converts H_2O_2 into water (H_2O) and O_2; GPx catalyzes the reduction of H_2O_2 and organic hydroperoxides by utilizing GSH as a cofactor; GSH serves as a coenzyme for a variety of antioxidant enzymes and helps to maintain cellular redox homeostasis (Enogieru and

Momodu, 2021; Irato and Santovito, 2021). Lead exposure has been demonstrated to inhibit the activities of SOD, CAT, GSH, and GPx, thereby weakening the antioxidant defense system and increasing vulnerability to oxidative damage. In this study, a significant decrease was observed in rats treated with lead acetate following comparison to control in agreement with previous studies (Enogieru and Idemudia, 2025; Enogieru and Momodu, 2022). However, a significant increase was observed in $ZnSO_4$-pretreated rats, thus highlighting the potent antioxidant activity of $ZnSO_4$ against lead acetate. MDA is a measure of lipid peroxidation, which occurs when reactive oxygen species (ROS) attack polyunsaturated fatty acids in cell membranes, resulting in the generation of lipid peroxides. MDA is a by-product of lipid peroxidation that is commonly used as a biomarker to detect oxidative stress and tissue damage (Halliwell and Gutteridge, 2015). In this study, a significant increase was observed in the rats treated with lead acetate in agreement with previous studies (Enogieru and Idemudia, 2024; Inwang et al., 2024). A significant reduction in MDA levels was observed in rats pre-treated with $ZnSO_4$, thus demonstrating the antiperoxidative effect of $ZnSO_4$.

Granule cells, particularly in the internal granular layer (layer IV) of the cerebral cortex, are involved in learning and memory, receiving inputs from the thalamus and other cortical regions, and sending connections to other layers (Chan, 2018). Cytoplasmic vacuolization, the formation of vacuoles within the cytoplasm, is a well-known morphological phenomenon observed in granule cells, often occurring after exposure to heavy metals (Aki et al., 2012). Cytoplasmic vacuolization has been linked to reactive oxygen species generation and endoplasmic reticulum stress, ultimately leading to significant impairment of cognition and memory (Khan et al., 2013). Findings from this study show the presence of cytoplasmic vacuolization in the cortex of rats treated with lead acetate, indicating its neurotoxic effects in consistence with previous studies (Ebhodaghe and Enogieru, 2024; Enogieru and Williams, 2024). However, in the cortex of $ZnSO_4$ pre-treated rats, there were relatively normal granular cells and little or no vacuolization. This demonstrates that $ZnSO_4$ attenuated the effects of lead acetate in the cerebral cortex of experimental rats.

CONCLUSION

Taken together, the findings revealed that $ZnSO_4$ was not harmful to the rats but inhibited lead acetate-induced cerebral toxicity primarily through its potent antioxidant activity. Further studies on its mechanisms of action are recommended for investigation. This will be vital for the development of novel neuroprotective agents from $ZnSO_4$ in addition to other compounds.

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