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Full-Length Research Article

Zinc Supplementation Reverses Lead-induced Anxiety-like Behaviour and Social Deficits in Male Wistar Rats

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Summary: Lead is a highly pervasive environmental toxin that exerts harmful effects on the body systems, especially the nervous system. Zinc (Zn) is an essential trace element found in eukaryotic cells. Lead has a serious neurotoxic effect via mechanisms such as disruption of the antioxidant defense system and activation of inflammatory mediators. Despite the extensive knowledge on the neurotoxic effects of lead, effective interventions in mitigating its effects are limited. This study aims to assess the effects of zinc supplementation on lead-induced neurotoxicity in male Wistar rats. Twenty-one male Wistar rats were randomly assigned to three groups with seven rats in each group. Group I, the control group, received distilled water; group II, the lead (Pb)-treated group, received 0.5% of lead in drinking water for 4 weeks; group III, Pb + Zn group, received 0.5% of lead in drinking water and 25 mg/kg of Zinc supplement by oral gavage for 4 weeks. Behavioural assessments were conducted using the Open Field Test (OFT), Elevated Plus Maze (EPM), and three-chamber sociability test to evaluate anxiety and social behaviour. Biochemical and haematological analyses were performed to determine the serum levels of proinflammatory cytokines (IL-1β and TNF-α), total antioxidant capacity (TAC), haematological indices, and electrolytes. The results revealed a significant increase in anxiety-like behaviour and a decrease in social behaviour in the Pb-only treated group. Biochemically, there was a marked elevation in IL-1β and TNF-α, with a significant reduction in TAC in these rats. No marked changes were observed in serum electrolyte levels across all experimental groups. Contrastingly, zinc supplementation effectively ameliorated these effects. Rats in the Pb + Zn group demonstrated improved neurobehavioral outcomes, decreased levels of pro-inflammatory cytokines, restored antioxidant capacity, and an increased Neutrophil-to-Lymphocyte Ratio (NLR). These findings suggest that zinc confers neuroprotective effects against lead-induced neurotoxicity, likely by modulating oxidative stress and suppressing inflammatory and neuroimmune responses.

Keywords: *Lead, Neurotoxicity, Inflammation, Zinc, Neuroprotective*

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INTRODUCTION

The persistence and widespread presence of lead in the environment have become increasingly alarming. Once extensively used in paints, gasoline, and pipes, and abandoned in mine wastes (Glass *et al*., 2009; Yamada *et al*., 2020), lead now lingers as a toxic remnant, disproportionately affecting communities with poor environmental safeguards and posing as a major global health issue (Obeng-Gyasi, 2019). Lead is a toxic metal and a recognised silent killer whose danger lies not only in its presence but also in its insidious effect on the body systems, particularly the nervous system. It has been identified as the second most hazardous heavy metal according to the Agency for Toxic Substances and Disease Registry (ATSDR) (Virgolini & Aschner, 2021).

Lead exposure produces a range of neurotoxic effects, from developmental delays, behavioural disturbances, and cognitive and fine motor impairments in children (Hou *et al*., 2013) to deficits in memory, attention, and reasoning abilities in adults (Ramírez Ortega *et al*., 2021). Beyond cognitive and behavioural sequelae, lead-induced neurotoxicity has been associated with a broader spectrum of neurological and systemic manifestations, including encephalopathy, seizures, cerebral palsy, and neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Ramirez Ortega *et alet al*., 2020; Reuben, 2018).

Empirical studies over the past decades have consistently established that lead disrupts the nervous system through multiple interrelated cellular and molecular pathways (Garza *et al*., 2006; Ramírez Ortega *et al*., 2021; Silbergeld, 1992; Virgolini & Aschner, 2021). It generates reactive oxygen species, leading to oxidative stress and disruption of the brain's antioxidant defense systems (Pottier *et al*., 2013). In addition, Lead induces DNA and chromosomal damage, impairs mitochondrial function, disrupts the blood-brain barrier and the blood-cerebrospinal fluid barrier, and triggers neuroinflammation (Virgolini & Aschner, 2021). These changes collectively damage neuronal integrity and function (Ramírez Ortega *et al*., 2021).

In the quest for therapeutic and preventive interventions, considerable attention has turned to the role of micronutrients, such as zinc, in mitigating lead's neurotoxic damage. Zinc is an essential trace element with potential neuroprotective properties due to its integral role in numerous enzymatic processes, antioxidant defense, immune modulation, and the maintenance of cellular integrity (Ajibare *et al*., 2024; Akintoye *et al*., 2023). As a cofactor for key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), zinc has been shown to counteract oxidative stress and modulate inflammatory responses (Sacan *et al*., 2021), two major mechanisms of lead-induced neurotoxicity.

While previous studies have confirmed zinc's protective role against oxidative stress and neuronal degeneration, its specific effects on inflammatory mediators, haematological parameters, electrolyte homeostasis, and associated neurobehavioral outcomes remain inadequately explored. Accordingly, this study assessed the effect of zinc supplementation on neurobehavioral parameters and biochemical markers, including inflammatory mediators, haematological indices, and electrolyte balance, in male Wistar rats exposed to lead, thereby addressing a critical gap in the existing literature.

MATERIALS AND METHOD

Animal Selection and Care: The experiment was conducted using twenty-one (21) adult male Wistar rats with body weights ranging from 98 g to 208 g. The rats were obtained from a credible animal house, McTemmy Concepts in Ogbomosho, Oyo State, Nigeria. Upon arrival at the site of the experiment, the rats were housed in well-ventilated plastic cages with wire covers in an animal house facility within the Physiology Department, College of Health Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria . The rat cages were lined with rat bedding (wood shavings), which was changed daily to ensure cleanliness. The rats were maintained under standard laboratory conditions with normal room temperature ($23^{o}C \pm 4^{o}C$) and a natural photoperiod of a 12-hour light-dark cycle. All the rats were fed with standard rat pellets obtained from Ogo-oluwa Feeds, Sango Road, Ilorin, Kwara State, Nigeria, and water *ad libitum*. The rat feed and water were changed, and the bowls were washed every day to keep them clean and sanitized. The rats were allowed to acclimate to their new environment for 14 days before the commencement of the experiment. Experimental protocol and animal handling were observed following the guidelines provided by the University Ethical Review Committee (UERC) of the University of Ilorin, Ilorin, Kwara State, Nigeria.

Experimental Design: The twenty-one male Wistar rats were randomly divided into three groups of seven (7) rats each. The rats in group I served as the control and were administered 0.2 ml of distilled water. The rats in group II, designated as the 'lead-treated group', were allowed *ad libitum* access to water containing 0.5% of lead acetate for 4 weeks. The rats in group III, designated as 'Zn-treated group', were provided with water containing 0.5% lead with daily administration of 25 mg/kg of ZnCl by oral gavage for 4 weeks. The drinking water was refilled upon exhaustion by the rats or replaced twice daily with freshly prepared lead solution. At the end of the experiment, all rats were euthanized by ketamine overdose (50 mg/Kg) and blood samples were collected through cardiac puncture.

Preparation of 0.5% Lead Solution: Using a sensitive electronic weighing scale (Model: FA2104A**,** Shanghai Jingtian Electronic Instrument Co., Ltd., China) in the Physiology Laboratory, University of Ilorin, 5g of lead chloride ($PbCl_2$) (Tianjin Kemio Chemical Reagent Co., Ltd., China) was weighed and added to 1 liter of distilled water. The solution was shaken for proper mixture. This procedure was performed multiple times throughout the experiment. The 0.5% Pb solution was administered, orally via drinking water to rats (in groups II and III) for 4 weeks.

Administration of Zinc Supplementation: 100 mg of zinc chloride (Tianjin Kemio Chemical Reagent Co., Ltd., China) was dissolved in 4 mL of distilled water to achieve a dose of 25 mg/kg (Ashraf *et al*., 2013). Zinc (25 mg/kg) was administered orally once daily to rats in the Zn-treated group for 4 weeks.

Neurobehavioral Assessments: Neurobehavioral assessments were carried out to evaluate the effects of lead exposure and zinc intervention on various behavioural domains, including anxiety-like behaviour and social interaction. Behavioral tests were conducted in a quiet, controlled environment under uniform lighting and temperature conditions. All tests were performed during the light phase of the light/dark cycle to ensure consistency. The neurobehavioral tests were done an hour after administration for each rat. Between trials, all neurobehavioural apparatuses were cleaned thoroughly with 70% ethanol to eliminate olfactory cues (Hershey *et al*., 2018) and prevent influence from previous subjects.

Open Field Test: The Open-Field Test was used to assess general anxiety-like (Kraeuter *et al*., 2019) behavior. The apparatus consisted of a square arena (typically 100 cm × 100 cm) with high opaque walls (45 cm in height) and a non-reflective floor divided into equal squares. Each rat was placed in the centre of the arena and allowed to explore freely for 5 minutes. All sessions were video recorded for subsequent analysis. Parameters recorded included the number of line crossings, time spent in the centre versus periphery, and grooming behaviour.

Elevated Plus Maze (EPM): The Elevated Plus Maze is a widely validated behavioral test used to evaluate anxiety-related behavior in rodents (Shvachiy *et al*., 2018). The apparatus consisted of two opposite open arms (50 cm × 10 cm) and two opposite closed arms (50 cm × 10 cm × 40 cm high), arranged perpendicularly to form a plus shape and elevated 50 cm above the floor. The maze was made of non-reflective material to reduce light and surface glare. Each rat was placed at the center of the maze, facing one of the open arms, and allowed to explore for 5 minutes. Behaviour was video-recorded and analysed for the following parameters: number of entries into the open and closed arms, time spent in the open and closed arms, and time spent at the centre**.** A decrease in open arm exploration is considered a measure of anxiety-like behavior.

Three-Chamber Box: The Three-Chamber Box was used to assess social approach behavior and sociability, important indicators of social cognition and neurobehavioral development (Yang *et al*., 2011). The apparatus was a rectangular box (60 cm × 40 cm × 22 cm) divided into three equal-sized chambers (20 cm × 40 cm each), with two rectangular glass panels of equal length and height. Each glass has a small doorway leading to each chamber. Two identical iron cages were placed in the two side chambers. The test was conducted in two phases. In phase 1, habituation was done by allowing the rat to freely explore all three empty chambers for 10 minutes to acclimate to the environment. Phase 2 involves the sociability test. A strange rat was enclosed in an iron cage in one side chamber, while the other side cage contained a small object. The test rat was reintroduced into the center chamber and allowed to explore for 10 minutes. The time spent in each chamber and the time spent sniffing each cage were recorded to evaluate how sociable the rat is. The sociability index was calculated as:

$$\text{S}\\ \text{sociability Index} \ \frac{\text{Time spent with srange rat} - \text{Time spent with strange rat}}{\text{Time spent with srange rat} + \text{Time spent with strange rat}}$$

Sample Collection: At the end of the experiments, the rats were sacrificed on the 29th day. Each rat was anaesthetized with chloroform. The heart was exposed through a thoraco-abdominal cut, and blood samples were collected via cardiac puncture (Parasuraman *et al*., 2010) using a 5 mL sterile syringe and needle. The blood samples were collected into EDTA bottles and plain bottles for hematological and biochemical analysis, respectively. The blood samples in the plain bottles were then centrifuged at 3000 rpm for 15 minutes to separate the serum, which was stored -20 °C until used for further study.

Biochemical Analysis

Quantification of Serum Pro-Inflammatory Cytokines (TNF-α and IL-1β): The levels of Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-1 beta (IL-1β) in serum were determined using the sandwich enzyme-linked immunosorbent assay (ELISA) technique using TNF-α ELISA Kit (Cat. No. E-EL-R0019) and IL-1β ELISA Kit (Cat. No. E-EL-R0012)**,** respectively, both obtained from Elabscience Biotechnology Co., Ltd., Wuhan, China**.** Briefly, micro-ELISA plates pre-coated with monoclonal antibodies specific to rat TNF-α or IL-1β were used. Standards and serum samples were pipetted into the wells, allowing the cytokines to bind to the immobilised antibodies. A biotinylated detection antibody was then added, followed by an Avidin-Horseradish Peroxidase (HRP) conjugate. Following incubation and thorough washing to remove unbound components, a chromogenic substrate solution was added to each well. A blue color developed in wells containing the cytokine-antibody-HRP complex, which turned yellow after the addition of stop solution. The optical density (OD) was measured at 450 nm ± 2 nm using a microplate reader. The concentrations of TNF-α and IL-1β in the serum samples were calculated by comparing the OD values to a standard curve constructed from known concentrations of each cytokine.

Measurement of Serum Total Anti-Oxidant Capacity (TAC): The Total Antioxidant Capacity (TAC) of serum samples was measured using the Total Antioxidant Capacity Assay Kit (Cat. No. E-BC-K136) obtained from Elabscience Biotechnology Co., Ltd., Wuhan, China. This assay is based on the colorimetric principle in which antioxidants in the sample reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions. The Fe^{2+} then forms a stable complex with a chromogenic agent, producing a colored solution whose intensity is directly proportional to the antioxidant capacity of the sample. Serum samples were mixed with the chromogenic working reagent and incubated at the recommended temperature and time conditions. The resulting- colored complex was measured spectrophotometrically at 520 nm using a microplate reader. A standard curve was generated using known concentrations of Trolox (a water-soluble vitamin E analog), and the TAC values of the samples were extrapolated and expressed in mmol Trolox equivalents per liter (mmol/L).

Haematological Analysis: White blood cells (WBC), platelets (PLT), red blood cells (RBC), and packed cell volume (PCV) were analysed using an autohematology analyzer machine, and differential cell count was estimated using an electronic coulter (advia-tm-60) according to Otitoloju *et al.* (2012). Other haematological indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were also calculated using standard formulae according to Jain and Schalm (1986).

Determination of Serum Ion Electrolytes: The serum Na^+, Cl^- and K^+ were quantified by the Ion Selective Electrode method using the Genius Electrolyte Analyser Model:200. The Electrolyte Analyser applies to advanced Ion Selective electrode (ISE) technology according to the Nernst formula as shown below;

$£ = £o + (RT/\mu F) * \text{Ln}(ax)$

Where;

£: Electric potential of ion selectivity electrode during test
£o: Standard electric potential of ion selective electrode
R: Gas constant (8.314 j.mol)
T: Absolute temperature (t + 273 degree Celsius)
F: Faraday constant (96487 C/mol)
ax: Tested ion's consistency in liquid

Data Analysis

All experimental data were analysed using GraphPad Prism software version 8.4.2 (GraphPad Software Inc., San Diego,

California, USA). Results were expressed as mean ± standard error of the mean (SEM). Differences between groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test for multiple comparisons. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Zinc supplementation mitigates lead-induced anxiety-like behaviour: Figure 1 (a-d) presents the neurobehavioral outcomes from the Open Field Test across the experimental groups. Rats in the Pb-only group exhibited a significant reduction in time spent at the centre and an increase in time spent at the periphery compared to the control group. In contrast, the Pb + Zn group demonstrated a reversal of these effects**,** with increased centre time and decreased peripheral time. No significant difference in line-crossing frequency was observed between the Control and Pb-only groups. However, rats in the Pb + Zn group showed an increase in line crossings compared with the control group. Additionally, grooming was elevated in the Pb-only group compared with the control group and reduced in the Pb + Zn group. These results show that zinc supplements ameliorate anxiety-like behaviour induced by lead exposure in the experimental rats.

The behavioural outcomes following lead exposure and zinc supplementation were also assessed using the Elevated Plus Maze, as shown in Figure 2. In the Elevated Plus Maze performance, there was a reduction in the time spent in the open arm in the Pb-only group compared to the control. Zinc administration increased time spent in the open arm relative to the Pb-only group. Conversely, there was no significant difference among groups in the time spent in the closed arm**.** The lead-exposed rats spent more time in the centre than the controls. However, rats in the Pb + Zn group spent significantly less time at the centre than the control group.

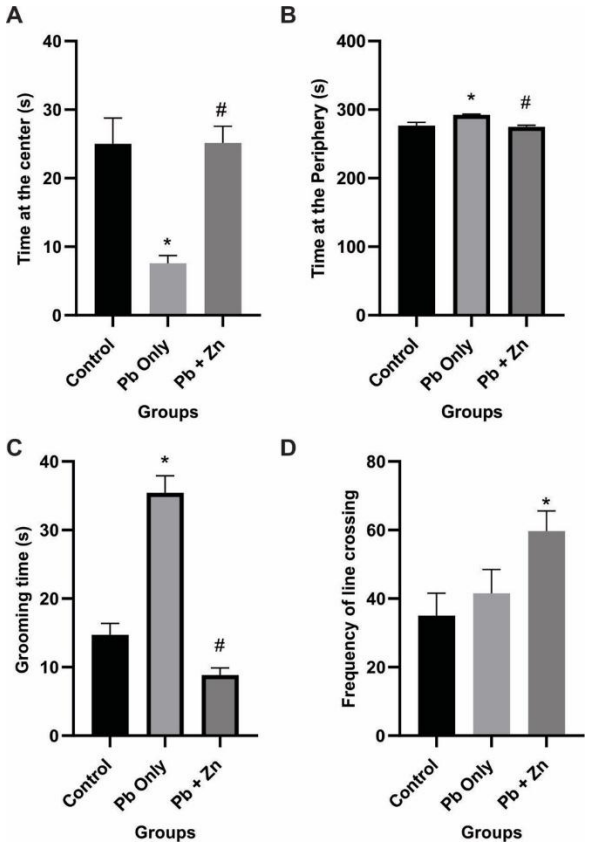

Figure 1:
Open field behavioural tests show that zinc supplementation reduces anxiety-like behaviour in lead-exposed rats.
(A) Average time spent at the centre of the open field arena. (B) Average time spent in the periphery of the open field arena. (C) Average number of lines crossed within the open field arena. (D) The average number of times the rats showed grooming behaviour. Each bar represents mean ± SEM (n=7). Bars marked with asterisks (*) are significant ($p<0.05$) compared to the control, while bars marked with hashtags (#) are significant ($p<0.05$) compared to the Pb only group

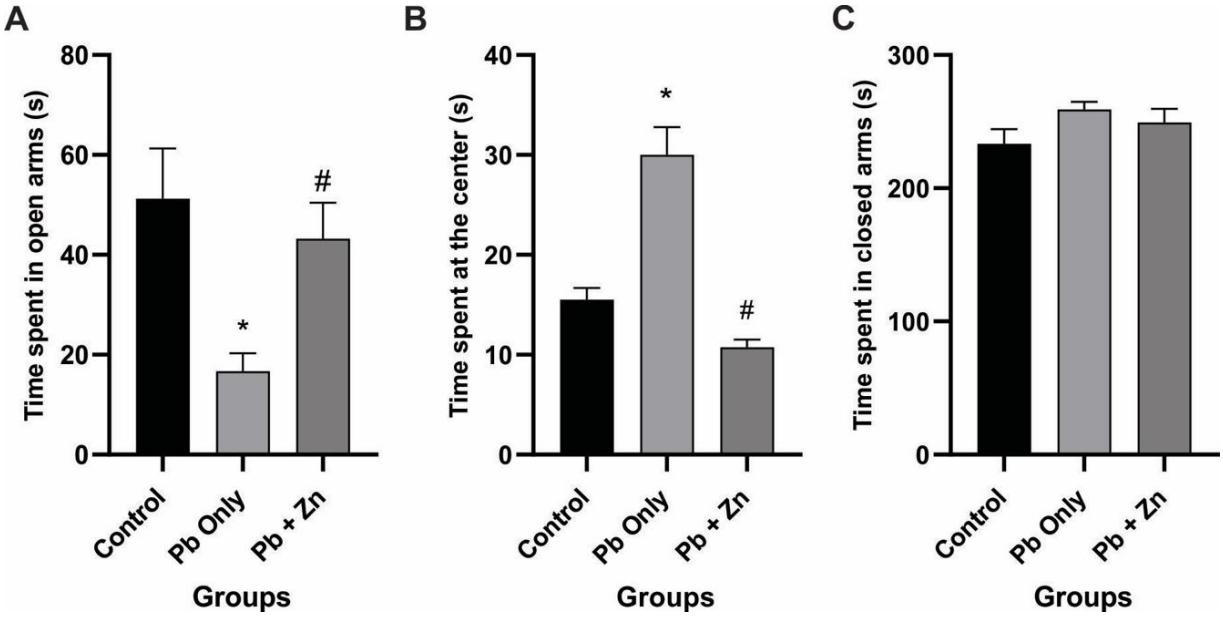

Figure 2:
Elevated plus maze performance in lead-exposed rats treated with zinc supplement

(A) Average time spent in the open arm of the EPM. (B) Average time spent in the close arm of the EPM. (C) Average time spent at the center of the EPM. Each bar represents mean ± SEM (n = 7). Bars marked with asterisks (*) are significant (p < 0.05) compared to the control, while bars marked with hashtags (#) are significant (p < 0.05) compared to the Pb only group.

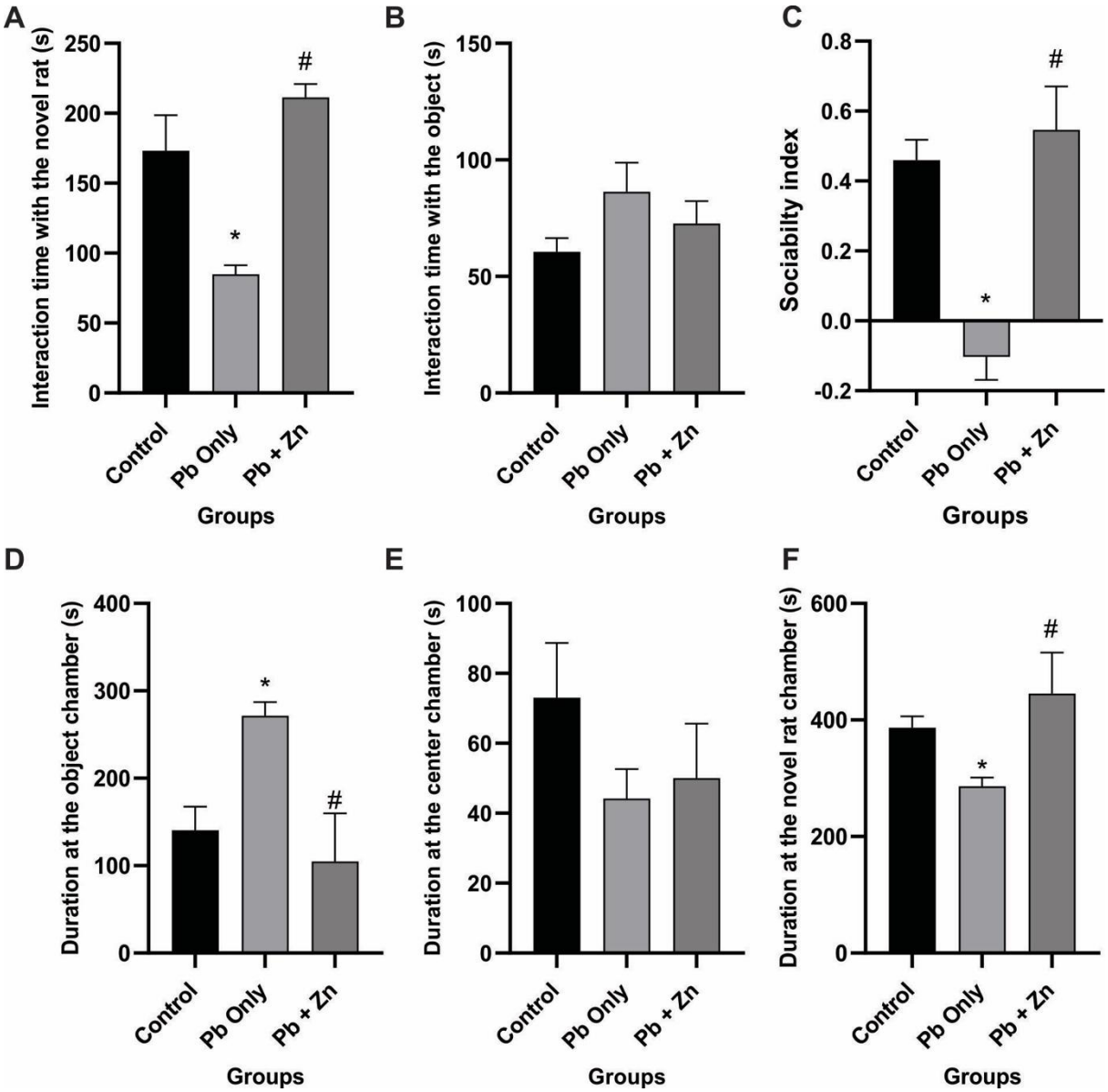

Figure 3:
Social behavioral differences in control and treated groups using a three-chamber box.
(A) Time spent with a strange rat by the experimental rats. (B) Time spent with an object by the experimental rats. (C) Time spent in the centre chamber by the experimental rats. (D) Sociability index. (E) Time spent in the strange rat's chamber. (F) Time spent in the object chamber. (G) Time spent in the centre chamber by the experimental rats. Data presented as mean ± SEM (n=7). Bars marked with asterisks (*) are significant (P<0.05) compared to the control, while bars marked with hashtags (#) are significant (P<0.05) compared to the Pb only group.

Administration of Zinc Supplement Ameliorates Lead Exposure-Induced Social Interaction Deficit: Figure 3 shows the assessment of social behaviour across experimental groups using the three-chambered box. Rats in the Pb-only group exhibited a reduction ($p < 0.05$) in time spent with the strange rat compared to the control group. Conversely, rats in the Pb + Zn group spent more ($p < 0.05$) time with the stranger rat than the Pb-only group. The time spent with the object and the time spent in the center did not differ significantly among the groups. The sociability index, which reflects the preference for a social stimulus over a non-social one, was significantly lower in the Pb-only group compared to the control ($p < 0.05$). Zinc administration increased the sociability index relative to the Pb-only group ($p < 0.05$). Similarly, across both left and right chambers (rat and object), rats in group II (Pb-only) spent less time in the rat chamber and more time in the object chamber compared to rats in group I (control) ($p < 0.05$), while zinc treatment reversed these patterns ($p < 0.05$). Time spent in the center chamber showed no significant variation across the groups.

Administration of Zinc Supplement Reduces the level of pro-inflammatory cytokines and increases total antioxidant Capacity in lead-exposed rats: Figure 4 illustrates the serum levels of interleukin-1 beta (IL-1β), tumour necrosis factor-alpha (TNF-α), and total antioxidant capacity (TAC) across the experimental groups. IL-1β and TNF-α levels were higher in the lead-only group than in the

control group. Administration of zinc with lead (Pb + Zn group) resulted in a significant reduction in IL-1β levels compared to the Pb-only group, suggesting an anti-inflammatory effect of zinc. For TNF-α, the Pb + Zn group showed a reduced level compared to the Pb-only group, but the difference was not statistically significant relative to the control, further suggesting that zinc can mitigate lead-induced inflammation. Additionally, serum TAC levels declined in the Pb-only group compared with the control group, reflecting reduced antioxidant levels. This was reversed in the Pb + Zn group, where TAC levels significantly increased compared with the Pb-only group and were not significantly different from those in the control group.

Zinc Supplementation Reduces the Neutrophil-Lymphocyte Ratio in Lead-Exposed Rats: Table 1 shows the effect of lead exposure and zinc supplementation on haematological parameters in rats. The result showed that there was no significant difference in red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), white blood cells (WBC), and some of its differential count, such as eosinophils, basophils, and monocytes between the rats in all experimental groups.

However, neutrophil counts were lower in rats co-treated with Pb and Zn than in control rats and in rats treated with Pb only. On the other hand, the lymphocyte level in the Pb-exposed group was lower than in the control group and higher than in the Pb + Zn group. The neutrophil-lymphocyte ratio (NLR) in the Pb + Zn group is also lower than in the control, with no significant change when compared to the Pb-treated group.

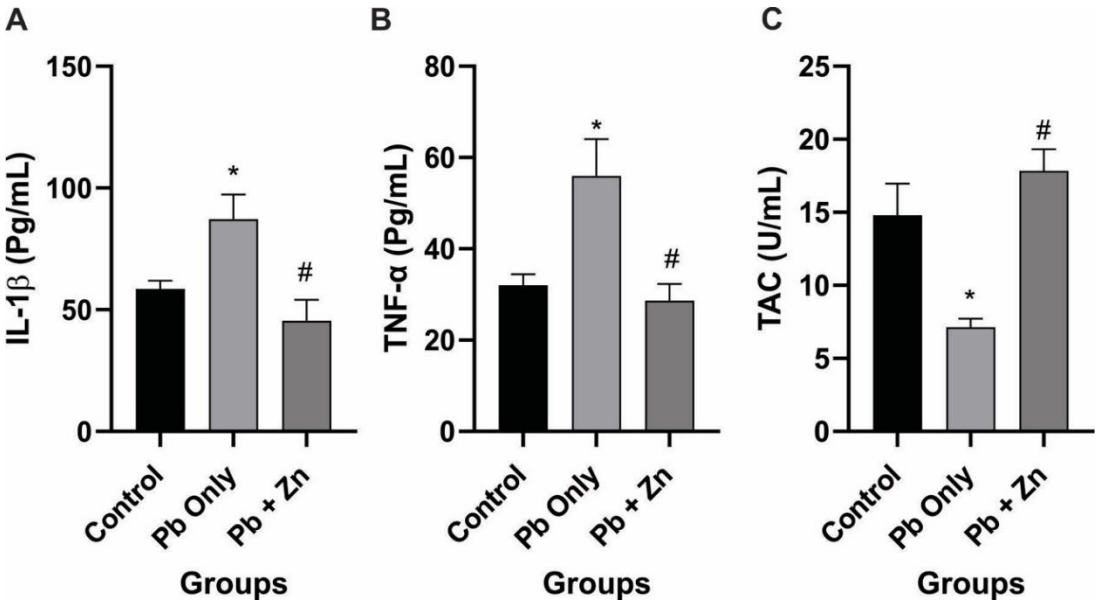

Figure 4:
Assessment of serum levels of interleukin-1 Beta (IL-1β), Tumor Necrosis Factor-alpha (TNF-α), and Total Anti-oxidant Capacity (TAC) in experimental rats.
(A) serum level of IL-1β. (B) serum level of TNF-α. (C) serum total antioxidant capacity. Data presented as mean ± SEM (n = 7). Bars marked with asterisks (*) are significant ($p < 0.05$) compared to the control, while bars marked with hashtags (#) are significant ($p < 0.05$) compared to the Pb only group.

Table 1:
Hematological parameters across the experimental groups.

Groups/Index	Control	Pb Only	Pb + Zn
RBC ($\times 10^{12}$/L)	5.48 ± 0.65	5.45 ± 0.52	4.27 ± 0.31
HB (g/dL)	10.93± 1.15	10.00± 0.52	8.46 ± 0.31
PCV (%)	32.33± 3.49	31.80± 2.18	26.33± 1.86
MCV (fL)	59.32± 2.45	59.10± 2.59	61.67± 0.09
MCH (Pg)	20.03± 0.38	18.76± 1.28	20.40± 0.21
MCHC (g/dl)	33.98± 1.07	31.58± 0.99	33.10± 0.35
WBC ($\times 10^9$/L)	8.59± 0.60	6.52± 0.98	6.90± 0.59
NEUT (%)	37.33± 1.65	40.60± 1.40	32.33± 1.86$^{\#}$
LYM (%)	59.83± 1.33	55.40±0.87*	66.00± 1.15$^{*\#}$
MON (%)	2.40± 0.24	1.80± 0.37	2.00± 1.00
PLT ($\times 10^9$/L)	353.00±64.78	272.20±61.5	308.67± 10.73
NLR	0.64 (0.54, 0.45)	0.75 (0.68, 0.78)	0.46 (0.45, 0.56)*

*n = 7; $p < 0.05$ was considered significant. * $p < 0.05$ compared with the control group; # $p<0.05$ compared with Pb-group. Abbreviations: NA, not available; RBC, red blood cells; HB, hemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white s cell; NEUT, neutrophil; LYM, lymphocytes; MON, monocytes; PLT, platelets; NLR, neutrophil-to-lymphocyte ratio. Continuous variables with normal distribution are presented as mean ± standard error of mean (SEM); non-normal variables e.g., NLR are expressed as median (interquartile range).*

Lead exposure did not cause a significant shift in electrolyte balance: The effect of lead exposure and zinc supplementation on serum electrolyte levels in the experimental rat is presented in Table 2. Although numerical differences were observed among the experimental groups, statistical analysis showed no significant differences ($p > 0.05$) in the concentrations of sodium (Na^+), potassium (K^+), and chloride (Cl^-) across all the groups. However, these variations were not statistically significant, indicating that lead exposure and zinc treatment did not significantly alter serum electrolyte balance under the conditions of this study

Table 2:
Effect of Zinc Supplementation on Serum Electrolyte Levels in Lead-Exposed Male Wistar Rats

Groups/Index	Control	Pb Only	Pb + Zn

Na^+ (mmol/L)	132.43±4.17	138.68±2.26	140.50±1.96
K^+ (mmol/L)	3.85± 0.40	3.24 ± 0.24	3.53 ± 0.41
Cl^- (mmol/L)	89.20±6.03	75.94±6.95	70.43±8.12

Data are presented as mean ± SEM (n = 7)

DISCUSSION

The outcome of this study showed that lead exposure resulted in significant neurobehavioral, biochemical, and hematological alterations in rats, which were all mitigated by zinc supplementation. It was observed that when subjected to the Open Field and Elevated Plus Maze tests, rats exposed to lead only showed increased anxiety-like behaviors. This was indicated by the reduction in time spent in the center, increase in time spent at the periphery, and increase in grooming time in the open field test, as well as a decrease in time spent in the open arm with an increase in time spent in the closed arm and center of the elevated plus maze. These findings align with previous studies demonstrating that lead disrupts brain regions involved in anxiety regulation, such as the hippocampus and amygdala (Barkur & Bairy, 2016; Zhou *et al*., 2023) and the serotonergic and dopaminergic systems that have been implicated in the regulation of anxiety (Bouyatas *et al*., 2019; Sansar *et al*., 2012; Tamegart *et al*., 2021). These behaviors were ameliorated in rats with zinc supplementation, supporting previous claims of the potential anxiolytic effects of zinc (Prasanthi *et al*., 2006; Azargoonjahromi, 2024).

In the sociability test performed with a three-chambered box, the sociability index, which reflects the preference for a social stimulus over a non-social one, was significantly lower in the rats exposed to lead only when compared with the controls and rats given lead with zinc supplementation. This demonstrates impairment of social behavior in the lead-exposed rats, which was significantly improved by zinc supplementation, thus supporting the role of zinc in mitigating neurobehavioral deficits induced by lead. This implies zinc's involvement in maintaining social behavior networks and preventing social withdrawal, a hallmark of neurobehavioral toxicity.

Lead toxicity has been linked to various molecular and biochemical mechanisms, notably the dysregulation of the antioxidant system (Abdelmonem *et al*., 2022; Abubakar *et al*., 2019; Ahamed *et al*., 2008; El-Tantawy, 2016; Prasanthi *et al*., 2010; Virgolini & Aschner, 2021). Lead (Pb) exposure disrupts redox balance even at low concentrations by promoting the production of free radicals and reducing the activity of antioxidant enzymes (Virgolini & Aschner, 2021). This leads to oxidative stress due to disproportional generation and neutralization of reactive oxygen species (ROS) and reactive nitrogen species (RNS). TAC is one of the most commonly used indicators of the overall oxidant-buffering capacity within a sample (Silvestrini *et al*., 2023). It quantifies the ability of a biological fluid to scavenge free radicals and other reactive oxygen species (Silvestrini *et al*., 2023). In this study, TAC in the rats exposed to lead only is lower compared to the control, reflecting a weakened antioxidant defense system overwhelmed by oxidative stress as a result of lead exposure. This corroborates previous studies that implicated lead exposure in the dysfunction of the body's oxidant-buffering system. However, administration of zinc supplements resulted in a marked increase in TAC, highlighting the potential antioxidant effect of zinc. This further reiterates zinc's role in neutralizing reactive oxygen species and preventing injury caused by oxidative stress (Ajibare *et al*., 2024).

While lead is known to induce oxidative stress by depleting the body's antioxidant system, a growing body of evidence has shown that lead exposure induces neuroinflammatory injury through activation of glial cells, specifically microglia and astrocytes (Chen *et al*., 2015). Upon activation, these glial cells release pro-inflammatory cytokines such as IL-1β and TNF-α, which exacerbate inflammation in the brain (Chen *et al*., 2015; Kasten-Jolly *et al*., 2011; Strużyńska *et al*., 2007).

In this study, a significant increase in the levels of pro-inflammatory cytokines IL-1β and TNF-α was observed following lead exposure, consistent with findings from previous studies. For instance, Li *et al*. (2014) reported elevated concentrations of IL-1β and TNF-α in the cerebral cortex of mouse pups exposed to lead, while subsequent research revealed significant upregulation of IL-1β and IL-6 in the hippocampus (Chen *et al*., 2015; Li *et al*., 2014, 2015). These observations were further supported by in vitro studies demonstrating increased TNF-α expression in glial cells following lead exposure (Chen *et al*., 2015), as well as gene expression analyses confirming lead-induced dysregulation of IL-6 and TNF-α across multiple brain regions (Kasten-Jolly *et al*., 2011). In contrast, animals co-treated with zinc exhibited markedly reduced levels of IL-1β and TNF-α, approaching those of the control group. This is in line with recent findings indicating that zinc supplementation significantly attenuates inflammatory responses. Specifically, zinc was shown to inhibit the expression of inflammatory cytokines, including TNF-α and IL-6, in lipopolysaccharide (LPS)-stimulated microglial BV2 cells (Hongxia *et al*., 2019). Additionally, zinc enhances the anti-inflammatory zinc-finger protein A20, while suppressing components of the NF-κB signaling pathway that are involved in cytokine production (Hongxia *et al*., 2019). These findings suggest that zinc exerts neuroprotective effects by suppressing pro-inflammatory signaling pathways such as NF-κB.

An important biomarker of neuroinflammation is the neutrophil-lymphocyte ratio (NLR), which is the ratio of circulating neutrophils to lymphocytes in peripheral blood, reflecting a balance between the innate and adaptive immune systems (Buonacera *et al*., 2022). Neutrophils are innate effector cells that serve as first responders, rising during infection, injury, or stress through rapid inflammatory responses by chemotaxis, phagocytosis, and a powerful oxidative burst, releasing reactive oxygen species (ROS), proteases, cytokines, and forming neutrophil extracellular traps (NETs) to contain pathogens (Buonacera *et al*., 2022). In contrast, lymphocytes (T cells, B cells, NK cells) mediate antigen-specific, adaptive immunity over a longer time scale and are often suppressed during chronic inflammation (García-Escobar *et al*., 2023).

Exposure to lead has been shown to cause neuroinflammation, which raises neutrophil levels and lowers lymphocyte counts, thereby increasing the NLR. An elevated NLR indicates a shift toward acute, neutrophil-driven inflammation and relative lymphocyte depletion, often reflecting inflammatory stress and

immunosuppression (Buonacera *et al*., 2022; García-Escobar *et al*., 2023). Conversely, lower NLR values generally signal a more balanced immune state. Elevated NLR has been linked to poor outcomes in various inflammatory and neurodegenerative diseases. Clinically, it correlates with increased oxidative stress and pro-inflammatory cytokines, while low lymphocyte levels may suggest weakened adaptive immunity (Aprile *et al*., 2025). In this study, lead exposure causes a decrease in lymphocyte levels and slightly increases neutrophils, though not significantly, in lead exposed rats. However, with the co-administration of zinc, a significant increase in lymphocytes as well as a decrease in neutrophils and NLR was observed when compared to the control group. These changes support zinc's anti-inflammatory functions and indicate the potential of zinc in restoring immune balance.

While numerical changes were observed in serum sodium, potassium, and chloride levels, these changes were not statistically significant. This suggests that, under the conditions of this study, lead exposure and zinc treatment did not markedly disturb electrolyte homeostasis. It is possible that the duration or dosage used was insufficient to induce observable shifts, or that compensatory mechanisms preserved electrolyte stability. Taken together, the findings of this study demonstrate that lead exposure induces anxiety-like behavior, sociability deficits, oxidative stress, neuroinflammation, and immune functions dysregulation in male Wistar rats. Zinc supplementation significantly mitigated these effects, highlighting its protective potential against lead-induced neurotoxicity. The observed benefits of zinc may be attributed to its antioxidant, anti-inflammatory, and neuroregulatory properties. These results suggest the use of zinc as a possible supplement in environments with high lead exposure risk.

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