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# Ameliorative Effects of Zinc on Lead-Induced Oxidative Stress and Semen Parameters in Testicular Cells in Male Wistar Rats

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## ABSTRACT

Testicular cell toxicity can be a resultant effect of exposure to environmental pollutants such as lead (Pb) through mechanisms including oxidative stress, inflammation, and endocrine disruption, thereby impairing male reproductive function. This study evaluated the protective effect of zinc on lead-induced oxidative stress and its impact on testicular histoarchitecture and sperm parameters in male Wistar rats. Sixteen adult male Wistar rats were randomly divided into four groups (n=4/group): Control (2 ml/kg distilled water), Lead (20 mg/kg lead acetate), Zinc (20 mg/kg zinc sulfate), and Lead + Zinc (20 mg/kg of each). Treatments lasted 8 weeks. At the termination of the study, animals were euthanized according to ethical guidelines, blood samples were collected via cardiac puncture, and testes were subsequently excised for semen analysis. Lead exposure significantly reduced all seminal parameters ($p < 0.0001$), while increasing lipid peroxidation (malondialdehyde) and reducing antioxidant enzyme activity (superoxide dismutase) ($p < 0.05$). Zinc supplementation alone or with lead significantly improved these parameters ($p < 0.05$). Histological analysis showed disrupted testicular architecture in the lead-only group, while zinc co-treatment preserved normal seminiferous tubule structure. Zinc may have a therapeutic effect in the management of lead-induced oxidative stress and reproductive damage in male Wistar rats, through its antioxidant effect.

**Keywords:** Lead toxicity; Zinc; Oxidative stress; Sperm parameters; Wistar rats

## INTRODUCTION

Exposure to heavy metals, particularly lead (Pb), is detrimental to health and potentially poses significant risks to both human and animal wellbeing[1]. Lead toxicity has been documented to disrupt the systems involved in a balanced physiological stage of being, with the male reproductive system being particularly vulnerable[2-3]. Lead is similarly one of the most hazardous environmental pollutants that impacts various biological systems through exposure via water, air, and food sources[4]. Lead-induced toxicity of the reproductive system is complex and encompasses impaired spermatogenesis, hormonal disturbances, and structural damage to reproductive organs [5,6]. Recent reports have demonstrated that even at low-level lead exposures (< 10 µg/dL), the sperm quality as well as the DNA methylation patterns in adult men may become significantly impaired[7].

Lead, an oxidative toxicant, stimulates the overproduction of reactive oxygen species (ROS), which overwhelms the cellular antioxidant defense mechanisms[8]. This disruption in the balance between pro-oxidants and antioxidants within cells ultimately results in oxidative stress[9]. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense systems within cells[10]. The testes are particularly susceptible to oxidative stress due to their high mitochondrial activity, abundant polyunsaturated fatty acids, and relatively low antioxidant capacity[11-12]. Under stress conditions such as exposure to toxins, inflammation, or ischemia, ROS production overwhelms endogenous antioxidants like superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), resulting in cellular damage [13].

This oxidative damage triggers apoptotic and autophagic pathways in germ cells, leading to cell death and loss of viable spermatozoa[14]. The oxidative stress impairs Leydig cell function, reducing testosterone synthesis and further disrupting spermatogenesis[15]. Meta-analyses have confirmed

that lead exposure significantly affects multiple semen parameters, including concentration, motility, and morphology across both occupational and environmental exposure scenarios[16].

Zinc is a vital trace mineral that plays an essential role in maintaining reproductive health in both males and females, largely through its antioxidant properties[17-18]. It contributes to protecting reproductive cells from oxidative stress caused by reactive oxygen species (ROS), which can otherwise damage sperm and oocytes, impair fertilization, and reduce fertility[19]. In males, zinc is crucial for spermatogenesis, ensuring sperm DNA integrity, motility, and morphology[20]. It also supports testosterone synthesis, which is fundamental for normal reproductive function[21]. Zinc's antioxidant action protects spermatozoa from oxidative damage by scavenging ROS and inhibiting lipid peroxidation, thus preserving sperm quality and function[22].

Despite the established individual roles of lead toxicity and zinc antioxidant properties, the specific protective efficacy of zinc supplementation against lead-induced testicular damage remains insufficiently characterized. Therefore, this study evaluated the ameliorative effect of zinc on lead-induced oxidative stress and semen parameters in male Wistar rats.

## MATERIALS AND METHODS

### Experimental animal care

Sixteen adult male Wistar rats weighing approximately 150 g were used for this experiment. They were housed in well-ventilated wooden wire-gauze cages under standard laboratory conditions (temperature: 27–30°C, 12:12 h light/dark cycle) and fed with standard rat pellets and clean water ad libitum. The animals were allowed to acclimatize for three weeks before the start of the experiment. Ethical approval for animal use was obtained from the University of Ilorin Ethical Review Committee (Approval Number: UERC/ASN/2023/2515), and all procedures conformed to international guidelines for animal care and use.

### Grouping/Treatment

The rats were randomly assigned to four groups (n = 4 per group):

Group A (Control): 2 mL/kg body weight of distilled water orally for 8 weeks

Group B (Lead): 20 mg/kg body weight of lead acetate in PBS administered via oral gavage, every 48 hours for 8 weeks

Group C (Zinc): 20 mg/kg body weight of zinc sulfate in PBS via oral gavage every 48 hours for 8 weeks

Group D (Lead + Zinc): 20 mg/kg lead acetate followed by 20 mg/kg zinc sulfate via oral gavage for 8 weeks

### Preparation of solutions

Lead acetate and zinc sulfate were each prepared in phosphate-buffered saline (PBS) to a working concentration of 6 mg/mL. The average rat weight was 150 g; hence, 3 mg (0.5 mL) of each compound was administered per rat per dose. Fresh solutions were prepared regularly and administered between 8:00 and 10:00 AM.

### Biological sample collection

At the end of the 8 weeks, animals were sacrificed humanely via cervical dislocation. Blood was collected via cardiac puncture using a 5 mL syringe and transferred into plain tubes. The serum was separated by centrifugation and stored for biochemical analysis. Testes and epididymis were harvested and processed for histological and sperm analyses, respectively.

### Sperm parameter analysis

Spermatozoa were collected from the cauda epididymis of the experimental male Wistar rats immediately after sacrifice. The cauda epididymis was dissected out and minced in 1 mL of normal saline pre-warmed to 37°C. The sperm suspension was then used for the analysis of various sperm parameters, including sperm count, motility, viability, and morphology.

Sperm count was determined using a hemocytometer. A small volume of the sperm suspension was diluted appropriately with normal saline, and 10 µL of the diluted sample was loaded into a Neubauer counting chamber while the number of spermatozoa was counted under a light microscope at ×400 magnification. A drop of sperm suspension was placed on a slide and observed under a microscope at ×200 with motility recorded as a percentage of motile sperm. Viability was assessed using the eosin-nigrosin stain; unstained sperm were considered viable, and stained ones non-viable. Sperm morphology was evaluated with sperm smears stained with eosin, examined at ×1000 magnification, and abnormalities were recorded.

### Biochemical assay for oxidative stress markers

Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) method. Superoxide dismutase (SOD) activity was determined spectrophotometrically based on inhibition of epinephrine auto-oxidation.

### Histological study

Testes were fixed in 10% neutral-buffered formalin for 48 hours, processed through graded alcohol, cleared in xylene, and embedded in paraffin wax. Sections of 5 µm thickness were stained with hematoxylin and eosin (H&E) and examined microscopically.

### Photomicrography

Photomicrographs were captured using an Amscope camera (AmScope Inc., Irvine, California, USA) connected to a laptop at ×200 magnification. The

images were used to assess the structural integrity of seminiferous tubules and testicular architecture.

**Statistical analysis**

Data were analyzed using GraphPad Prism version 9. Results were expressed as mean ± SEM. Statistical significance between groups was determined using one-way ANOVA followed by Tukey's multiple comparison test. A p-value < 0.05 was considered statistically significant.

## RESULTS

**Effect of zinc and lead on sperm parameters**

Table 1 and Figure 1 show that sperm count, motility, viability (life/death ratio), and morphology were significantly ($p < 0.0001$) impaired in lead-treated rats compared to the control. Sperm count and motility were significantly reduced in the lead-only group, while abnormal sperm morphology and dead sperm percentage were significantly increased. Treatment with zinc alone maintained values similar to those of the control group. Co-treatment with zinc and lead (Lead + Zinc group) significantly ($p < 0.05$) improved all sperm parameters compared to lead-only, indicating a protective effect of zinc.

**Table 1:** Effect of zinc (Zn) on sperm parameters in lead (Pb)-exposed male Wistar rats

| Variables | Control | Lead group | Zinc group | Lead+Zinc |
|---|---|---|---|---|
| Sperm Count (10^6/mL) | 87.85 ±5.78 | 39.25 ±5.441 | 94.30±3.484 | 66.25±2.105 |
| Sperm motility (%) | 81.95 ±0.36 | 62.90 ±1.834 | 83.07±1.437 | 83.11±2.056 |
| Sperm Morphology(%) | 83.41 ± 1.35 | 66.25±1.402 | 79.85±1.013 | 80.13±1.868 |
| Life and death (%) | 86.66 ± 0.96 | 69.40±1.501 | 82.52± 1.441 | 82.50±2.053 |

Values are expressed as mean ± SEM. Statistical comparison revealed significant differences among groups ($p < 0.05$).

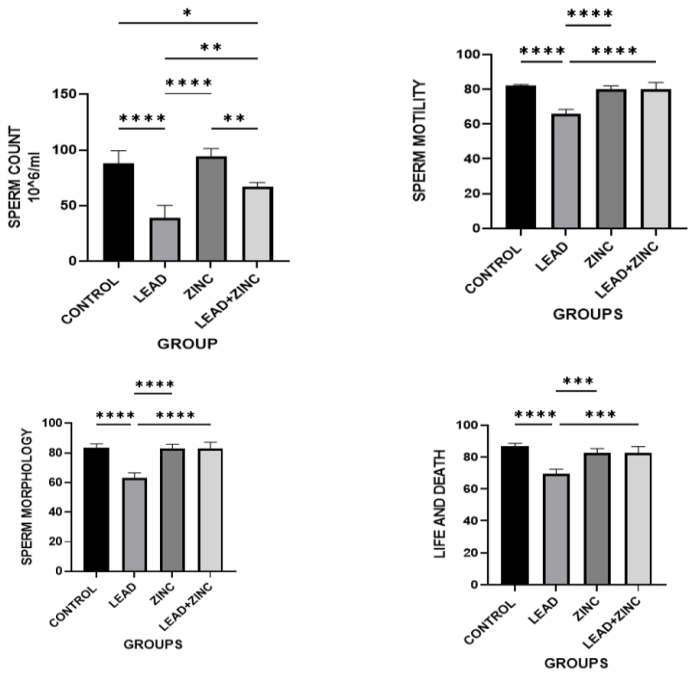


**Figure 1:** The bar graphs show that lead exposure significantly reduced sperm parameters compared to the control group ($p < 0.0001$).

**Effect of zinc and lead on oxidative stress markers**

As shown in Table 2 and Figure 2, lead exposure significantly ($p < 0.05$) increased malondialdehyde (MDA) levels and decreased superoxide dismutase (SOD) activity in testicular tissues, confirming oxidative stress. Zinc treatment significantly ($p < 0.05$) lowered MDA levels and enhanced SOD activity compared to the lead group. The Lead + Zinc group showed marked improvement in antioxidant balance, though not fully restored to control values.

**Effect of zinc and lead on testicular histoarchitecture**

Histological analysis revealed normal testicular cytoarchitecture in the control group, with intact seminiferous tubules and well-arranged spermatogenic layers. The lead-only group showed signs of testicular damage, including degeneration of seminiferous tubules, reduced spermatogenic cells, and disrupted lumen structure. Zinc-treated rats displayed preserved tissue structure comparable to controls. In the Lead + Zinc group, zinc co-treatment markedly restored testicular architecture, with less vacuolation and active spermatogenesis evident under light microscopy (Figure 3).

**Table 2:** Effect of zinc on oxidative stress markers in lead-exposed male Wistar rats

| Variables | Control | Lead group | Zinc group | Lead+Zinc |
|---|---|---|---|---|
| MDA | 2.01±0.85 | 0.90±0.14 | 1.45±0.29 | 1.88±2.43 |
| SOD | 1.51±0.46 | 0.94±0.24 | 0.78±0.34 | 1.86±0.08 |

Values are expressed as mean ± SEM. Statistical comparison revealed significant differences among groups ($p < 0.05$)

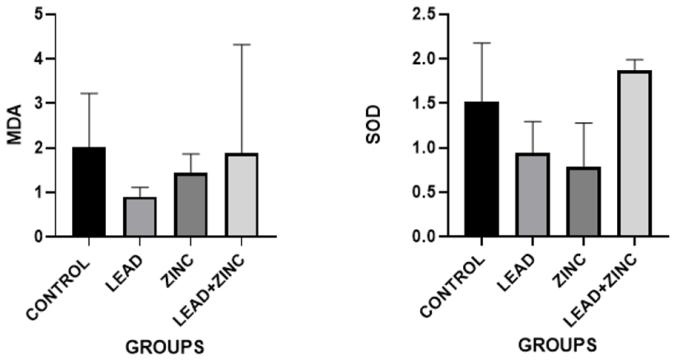


**Figure 2:** The bar graphs show that lead exposure significantly reduced and increased MDA and SOD, respectively, compared to the control group ($p < 0.0001$).

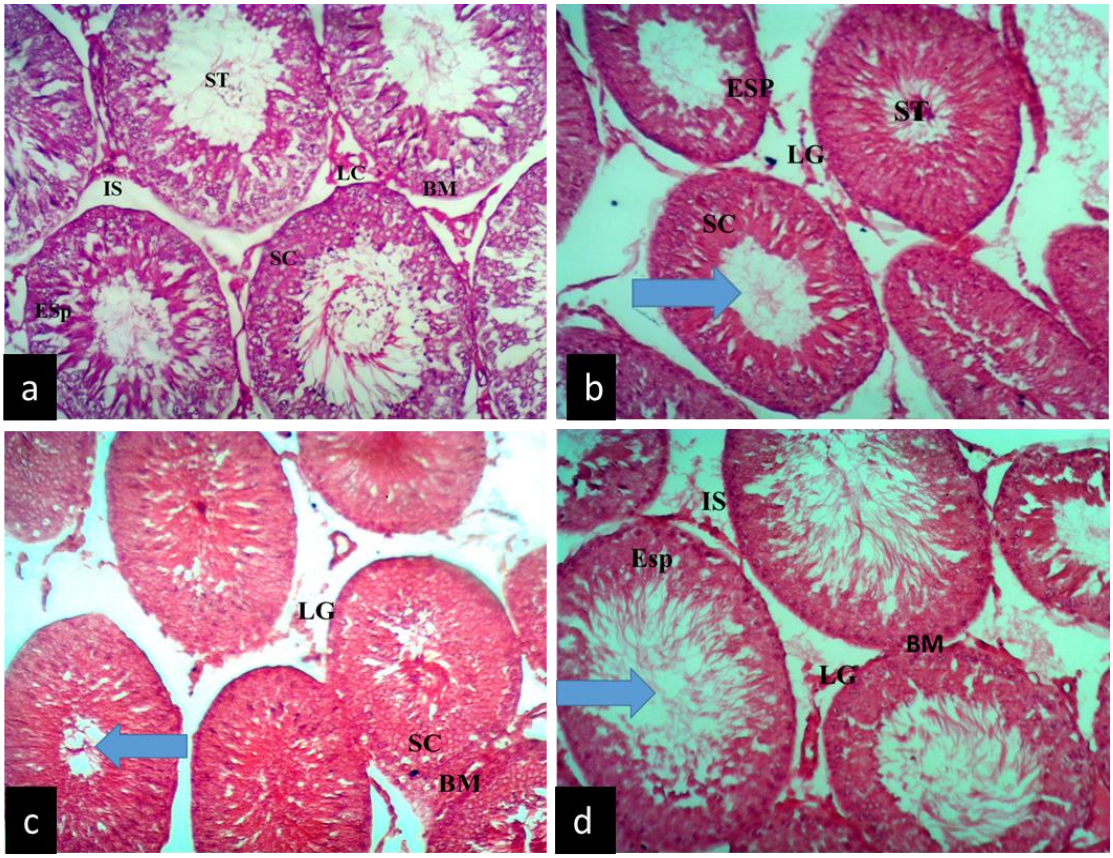


**Figure 3:** Photomicrograph of a transverse section of testes of Wistar rat (H&E ×200). The photomicrograph of the testes shows the seminiferous tubules. Pb treatment led to the lumen of each tubule appearing clear or containing loose spermatozoa. This effect was milder in Zn+Pb treatment. Zinc treatment showed similar testicular cytoarchitecture to the control.

## DISCUSSION

Analysis of sperm parameters in the present study provides critical information on the reproductive toxicity of lead and the mitigating effects of zinc. Lead exposure significantly reduced all seminal parameters, underscoring its potent spermatotoxic effects, which are primarily mediated through oxidative stress, hormonal imbalances, and direct damage to testicular cells[2,23], suppressing spermatogenesis and contributing to male infertility, which is a rather concerning reproductive health concern. Lead concentrations exceeding 40 µg/dL have been consistently associated with decreased sperm concentration, volume, and motility in both human and animal studies. Recent research confirms that lead exposure at various concentrations significantly affects sperm motility and increases the percentage of spermatozoa with abnormal morphology, with higher concentrations (0.5% and 1%) showing more pronounced effects[24].

The study found a significant reduction in normal sperm morphology in the lead-exposed group, compared to controls, consistent with previous reports of lead-induced spermatogenic disruption[25]. This finding aligns with previous studies demonstrating that lead acetate administration adversely affects male fertility through changes in sperm morphology, viability, membrane integrity, and increased intracellular reactive oxygen species[26]. These morphological abnormalities are critical indicators of impaired spermatogenesis and DNA damage, often resulting from lead's interference with germinal cell differentiation and testicular function[27].

The study revealed a significant reduction in testicular cell viability in the lead-exposed group, compared to the control group. This finding is consistent with the known cytotoxic effects of lead, which can induce apoptosis and necrosis in testicular cells, leading to decreased viable cell populations[28-29]. Lead exposure has been shown to cause microscopic and histological changes in testicular tissues, including alterations in testicular morphology, decreased germ cell layer populations, and seminiferous tubule degeneration.

The biochemical analysis in the present study confirms lead-induced oxidative stress through decreased antioxidant enzyme activity (SOD) and

increased lipid peroxidation (MDA). Zinc supplementation effectively restored oxidative balance, supporting its protective role against lead-induced testicular damage[28,29]. This finding is central to understanding how lead impairs reproductive health and how zinc mitigates these effects.

## CONCLUSION

These findings contribute valuable insights into the mechanisms of lead toxicity and the vital role of zinc in maintaining reproductive health, suggesting that zinc supplementation could be a promising strategy for ameliorating lead-induced male reproductive dysfunction.

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**Authors' contribution:** AOA, BAO, RAO: Conceptualization and supervision; OA, BF: First draft preparation; YMM: Editing and proofreading; RAO, BAO, AOA, YMM: Data acquisition and analysis; RAO, BAO, YMM, OA, BF: Manuscript review and revision. All authors approved the final version.

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