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Original Article

Effect of Osun River Heavy Metal Contamination on Testicular Function in Wistar Rats

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ABSTRACT

Introduction: The Osun River is prone to contamination from industrial, agricultural, and domestic activities, resulting in heavy metal pollution. Widespread contaminants such as lead, mercury, cadmium, and arsenic can build up in aquatic ecosystems, presenting serious health hazards to both wildlife and humans. Even at low concentrations, heavy metals are toxic, with the testes being particularly vulnerable given their essential functions in reproduction and hormone regulation. This study aims to examine the potential testicular damage resulting from prolonged exposure to heavy metal-contaminated Osun River water.

Materials & Methods: Thirty adult male Wistar rats, averaging 160 g in weight, were randomly divided into six groups (A–F), with each group consisting of five rats. Group A functioned as the control, whereas groups B, C, and D were exposed to mercury (6.8 mg/kg), cyanide (25.8 mg/kg), and lead (47 mg/kg), respectively. Group E received a combination of two heavy metals (lead and mercury) and a toxic compound (cyanide), while group F was given unrestricted access to Osun River water. All substances were administered orally via an oral cannula for a duration of four weeks.

Results: Statistical analysis revealed no significant differences among the groups exposed to mercury, cyanide, lead, and Osun River water. Toxic effects on the testes included disorganization of seminiferous tubules, altered spermatogenic cell arrangement, structural changes in the basal membrane, testicular stroma abnormalities, and reduced sperm count, motility, and viability. These effects were dose- and time-dependent, occurring even at low concentrations.

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Archives of Razi Institute Journal

Conclusion: The findings demonstrate that exposure to heavy metals, whether individually or through contaminated Osun River water, leads to significant testicular damage. The observed alterations in testicular architecture and sperm parameters emphasize the toxic impact of mercury, cyanide, and lead on reproductive health. This study underscores the importance of addressing environmental contamination to safeguard both human and animal reproductive systems.

1. Introduction

The Oṣun River is a river that flows southward through central Yoruba land in southwestern Nigeria into the Lagos Lagoon and the Atlantic Ocean Gulf of Guinea [1]. In 2018, the river experienced an abrupt change in color, and an investigation by Anifowose et al. (2023) [2] identified illegal and unregulated gold mining activities upstream as the primary cause. These mining operations have introduced heavy metal contaminants into the river, posing a threat to both the water body and the Osun-Osogbo Sacred Grove. Some of the topmost heavy metals present in the Osun River as reported by Anifowose et al. 2023 [2], are lead, cyanide, and mercury. In many mining communities in Osogbo, surface water has become unsuitable for human consumption due to chemical pollution from gold mining and processing. Artisanal and small-scale gold mining can cause spills and runoff, contaminating rivers, ponds, streams, wells, and borehole water sources. Consequently, residents relying on the Osun River may face exposure to heavy metals and/or metalloids through water consumption. This includes, but is not limited to, heavy metals such as mercury, lead, arsenic, and nickel, among others. Mercury is among the most dangerous elements. The use of mercury in gold mining can cause general contamination of the area, including exposure of the population to mercury and contamination of aquatic environments [3]. The latter results in the formation of methylmercury (MeHg), a bioaccumulative environmental toxicant, which is a health risk for fish consumers. Human health risks due to mercury exposure are well known, with renal and neurological effects as possible health outcomes [4]. The neurodevelopmental effects of lead on children, even at minimal exposure levels, are well documented [5]. Population and toxicokinetic modeling studies have demonstrated a direct correlation between lead concentrations in drinking water and blood lead levels in children, even at low exposure levels [6]. This poses serious risks for residents of communities that rely on the Osun River for their daily needs. Concerns have been raised by scholars about the poor health of individuals who consume water from the river. In fact, certain health hazards have been linked to the consumption of water from the Osun River. Cyanide is a highly toxic chemical that poses significant health risks to both humans and animals. It can enter the environment through industrial activities such as mining, metal processing, and improper waste disposal [7]. Cyanide exposure primarily occurs through contaminated water, food, or inhalation of its gaseous form, leading to severe toxicological consequences.

One of the primary mechanisms of cyanide toxicity is its ability to inhibit cytochrome c oxidase, a crucial enzyme in the mitochondrial electron transport chain, thereby disrupting cellular respiration [8]. This results in decreased ATP production, leading to cellular hypoxia and oxidative stress. The reproductive system, particularly the testes, is highly susceptible to oxidative damage, which may lead to impaired spermatogenesis, hormonal imbalances, and testicular atrophy [9]. Chronic exposure to cyanide has been associated with testicular degeneration, reduced sperm quality, and endocrine disruption, which may contribute to infertility [10]. These detrimental effects highlight the need for further investigation into the reproductive toxicity of cyanide, particularly in regions where water contamination is a major concern.

In recent times, we have seen a steady increase in mining activities in Osun State, particularly in the Osogbo-Ijesha axis. Following the increase in mining activities in the state, it is expected that the rate of illnesses in communities dependent on the Osun River will also increase. However, no studies have been conducted to ascertain this speculation or the extent of the effects of consuming water from the Osun River on the health of rats. The effects of consuming water from the Osun River on testicular health remain largely unexamined. This study aims to assess the extent of testicular damage in rats following exposure to Osun River water and to elucidate its impact on their overall health.

2. Materials and Methods

2.1 Compounds procurement and animals procurement

The study's high-purity mercury, cyanide, and lead compounds were purchased from TMJ Chemical Co. Ltd. in China, and the Pharmacology Department at Osun State University, Osogbo, verified their authenticity. The experimental animals were acquired from Adesina Popoola Feed Mills, Osogbo, Osun State, and were given unfettered access to food and water, as well as a two-week acclimatization period to adjust to the laboratory environment before the study began. All procedures followed the ethical guidelines established by the Health Research Ethics Committee, College of Health Sciences, Osun State University, Osogbo, Nigeria, in compliance with the National Institutes of Health's guidelines for the care and use of laboratory animals.

2.2 Osun river

One keg (having a 5-liter capacity) of water collected from the Osun River will be used as a source of exposure to polluted water.

2.3 Experimental design

Thirty male Wistar rats were bought from Adesina Popoola Feed Mills in Osogbo, Osun State. Six groups of five rats each were randomly selected from among the animals. Group B was exposed to mercury (6.8 mg/kg) for four weeks, while group A served as the control. Group D was exposed to lead (47 mg/kg) for four weeks, while group C received cyanide (25.8 mg/kg) for the same duration. Group F was given unlimited access to Osun River water during the trial period, while group E was given a combination of lead, cyanide, and mercury for four weeks.

2.4. Sacrifice of experimental animals, sample collection, and hormonal assay

Thirty adult male Wistar rats were given ketamine hydrochloride (80 mg/kg) anesthesia twelve hours after the final exposure, and blood samples were taken from the left ventricle of their hearts. The blood was placed into red-top tubes for hormonal analysis. For histological examination, the testes were removed after an abdominal incision and preserved in neutral-buffered formalin. The tissue underwent a stepwise dehydration process using increasing concentrations of alcohol, followed by cleaning in xylene and infiltration with paraffin wax before being embedded in molten paraffin wax. Using a rotary microtome, the paraffin block was sectioned into slices that were four µm thick. Following the mounting of these sections onto glass slides, they were floated in a water bath kept at 40 ˚C and were stained with hematoxylin and eosin dyes. Further, blood samples were drawn into red-top tubes via cardiac puncture, and the serum was separated by centrifugation at 4000 rpm at 4 ˚C. The samples were then stored at -20 ˚C until analysis.

2.5. Hormonal measuring assay

Serum samples were assayed for testosterone and protamine in batches with the control sera at both physiological and pathological levels by the standard quantitative enzyme-linked immunosorbent assay (ELISA) technique with a microwell kit manufactured by Syngenemed. The manufacturer's instructions that accompanied the assay kits were strictly adhered to.

2.6. Akap4 (A-kinase anchor protein 4)

Usually, samples are taken from testicular tissue or sperm, and then protein or RNA is extracted. Western blotting confirms the protein expression of Akap4, detecting a specific band (~82 kDa), while immunohistochemistry (IHC) and immunofluorescence microscopy are used to localize Akap4 specifically along the fibrous sheath of the sperm tail. Quantitative polymerase chain reaction (qPCR) assesses Akap4 mRNA levels to determine changes under different experimental conditions. Functional studies, like sperm motility assessments using computer-assisted sperm analysis (CASA), help connect Akap4 expression to sperm functionality. Genetic models, such as CRISPR/Cas9 knockouts or RNA interference (RNAi), allow investigation of the effects of Akap4 loss or suppression, such as impaired motility or flagellar abnormalities.

Akap4 is localized using IHC and immunofluorescence microscopy, specifically along the fibrous sheath of the sperm tail. Western blotting verifies the protein's expression by identifying a distinct band (~82 kDa). Akap4 mRNA levels are assessed using qPCR to identify variations under different experimental conditions.

2.7. Protamines 1 (PRM1) and 2 (PRM2)

Usually, samples are taken from sperm or testicular tissue, and then proteins and RNA are extracted. PRM1 and PRM2 are located within the sperm nucleus using IHC and immunofluorescence microscopy, which reflect their function in chromatin condensation. The presence of PRM1 (~6.5 kDa) and PRM2 (~7.6 kDa)

proteins is confirmed by Western blotting, which enables the measurement of their levels.

PRM1 and PRM2 mRNA expression levels are measured using qPCR, which provides information on transcriptional changes under various experimental settings. The effect of PRM1 and PRM2 abnormalities on DNA integrity is evaluated by functional assays, such as DNA fragmentation tests or chromatin structure analysis using chromomycin A3 labeling.

Furthermore, by analyzing the consequences of their deletion or downregulation—which frequently result in poor chromatin packaging and decreased fertility—genetic research employing CRISPR/Cas9 or RNAi facilitates investigation of the roles of PRM1 and PRM2.

2.8. Statistical analysis

For every set of data, the Mean±SEM were calculated. Tukey's post hoc test was used for multiple comparisons after a one-way analysis of variance (ANOVA) in GraphPad Prism software, version 8 was performed for statistical comparisons of means. It was determined that a P≤0.05 was statistically significant.

3. Results

3.1. Biochemical results

All groups exposed to heavy metals, including those given lead, cyanide, mercury, and their combination, showed a significant decrease in *AKAP4* gene expression compared to the control group. Furthermore, *AKAP4* gene expression was significantly and more markedly downregulated in the Osun River water group than in the control group (Figure 1).

All groups exposed to heavy metals showed a significant decrease in *PRM1* gene expression compared to the control group. In a similar vein, *PRM1* gene expression was significantly lower in the Osun River water-treated group than in the control group (Figure 2).

In comparison to the control group, Figure 3 showed a significant decrease in *PRM2* gene expression in all groups exposed to heavy metals. When compared to the control group, a noteworthy and significant decline was also noted in the group that received Osun River water treatment.

All groups exposed to heavy metals had significantly lower serum testosterone concentrations than the control group, as seen in Figure 4. Furthermore, compared to the control group, the Osun River water group showed a significant decrease in serum testosterone levels.

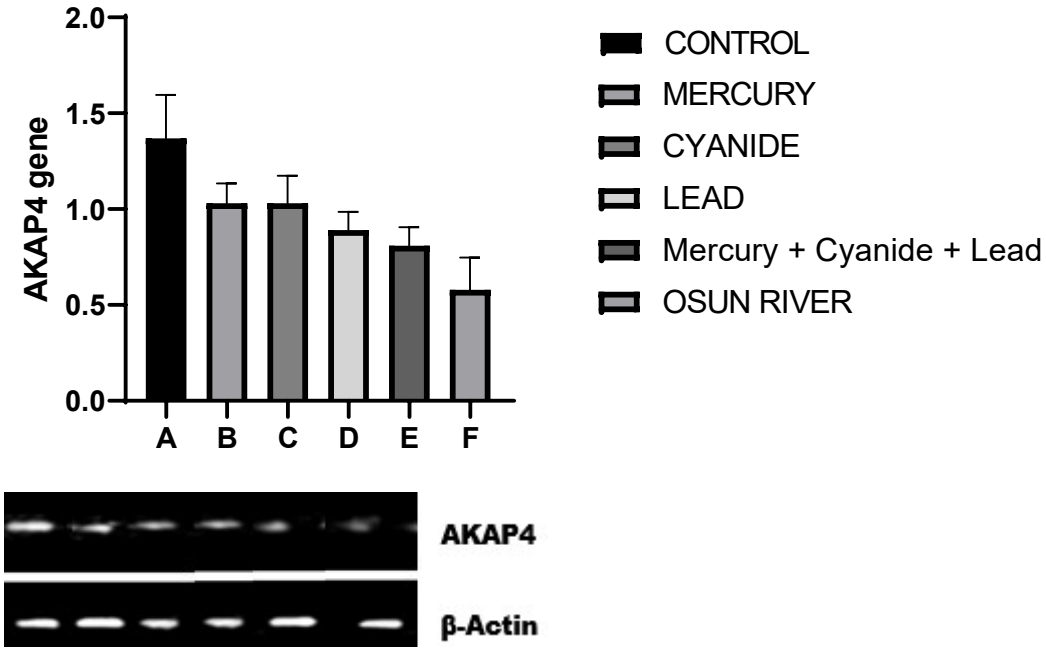

Figure 1. The comparison of the *AQP4* gene expression between the experimental groups

Note: The Mean±SEM is used to express the data (P≤0.05, n=5).

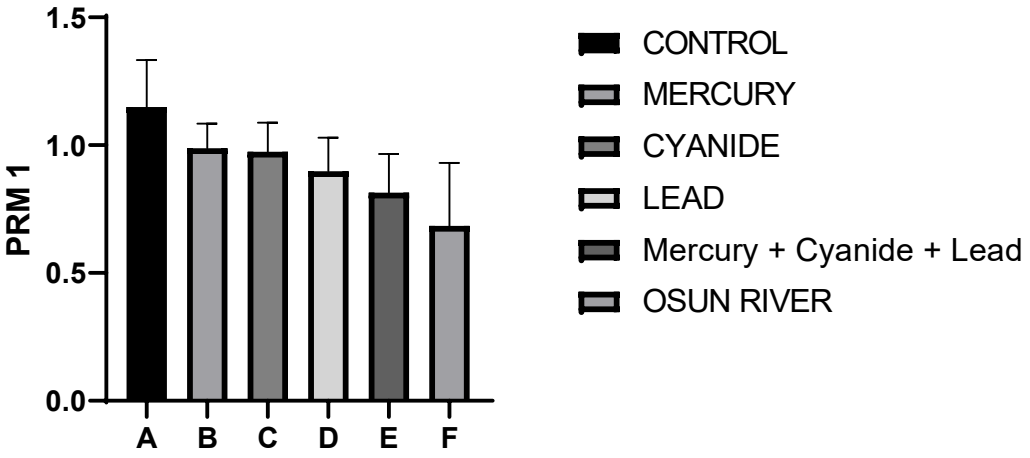

Figure 2. The comparison of the *PRM1* gene expression between the experimental groups

Note: The Mean±SEM is used to express the data (P≤0.05, n=5).

All groups exposed to heavy metals showed a substantial decrease in sperm count concentration compared to the control group. Furthermore, the Osun River water-treated group showed a notable decrease in sperm count concentration compared to the control group (Figure 5).

Figure 6 showed that all groups exposed to heavy metals had significantly lower sperm motility than the control group. Furthermore, the Osun River water-treated group also exhibited significantly reduced sperm motility compared to the control group.

In comparison to the control group, Figure 7 shows a significant decrease in sperm morphology in all groups exposed to heavy metals. In addition, the Osun River water-treated group exhibited significantly reduced sperm morphology compared to the control group.

3.2. Histological observation of the testes

Hematoxylin & eosin (H&E) staining: Figure 8 displays a photomicrograph showing the general cytoarchitecture of the testes of rats across the various experimental groups, stained with H&E at 200× magnification.

The cytoarchitecture of the testes revealed distinct structural components, including the seminiferous tubules, lumen, interstitial spaces, germinal epithelium, and leydig cells. Photomicrographs revealed intact organization and structure of the testes in group A (control). In contrast, group B (mercury) showed testicular damage characterized by degeneration of the seminiferous tubules and irregular lumens. Groups C and D (Cyanide & lead) groups also showed histological evidence of toxicity. In the cyanide-treated group, moderate disorganization of the seminiferous tubules, mildly enlarged lumens,

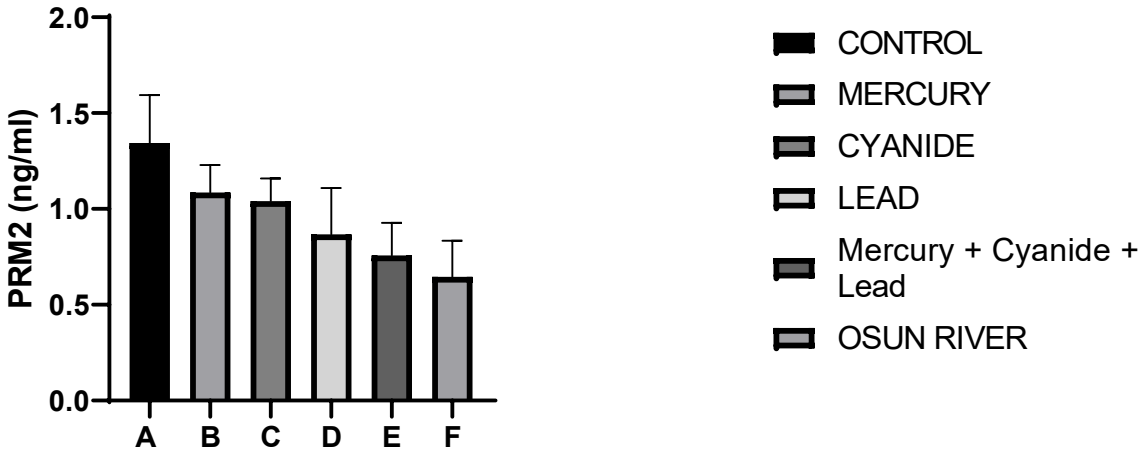

Figure 3. The comparison of the *PRM2* gene expression between the experimental groups

Note: The Mean±SEM is used to display the values (P≤0.05, n=5).

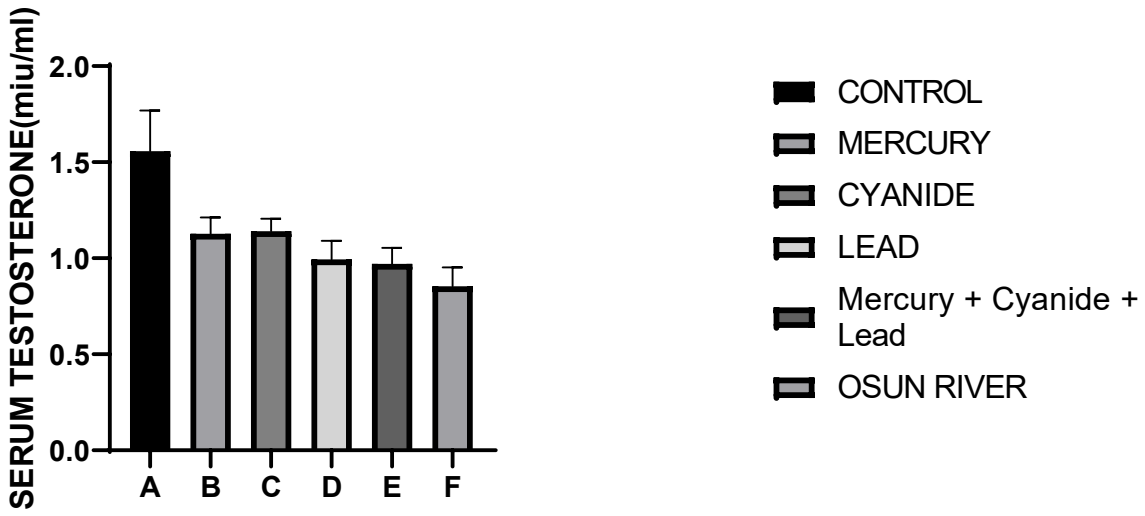

Figure 4. Serum testosterone levels in the experimental groups

Note: The Mean±SEM is used to display the values (P≤0.05, n=5).

and slightly expanded interstitial spaces were observed, indicating moderate spermatogenic disruption. In contrast, the lead-treated group exhibited more pronounced damage, characterized by severe disorganization of the seminiferous tubules, irregular lumens, and prominent interstitial spaces. Groups E and F (PbCnHg and Osun River water) both showed severe testicular damage with marked disruption of the overall cytoarchitecture. The seminiferous tubules were disorganized and irregular, with reduced luminal diameter, and there was disintegration of the interstitial spaces and degeneration of germ cells.

4. Discussion

This study demonstrates the negative effects of toxic substance such as cyanide and heavy metals including lead and mercury, as well as Osun River water contaminated with these metals, on testicular function in male Wistar rats. The results showed significant biochemical and histological changes in the testes, highlighting the detrimental effects of environmental pollutants on reproductive health. *AKAP4* and *PRM1* gene expression levels were significantly lower in all groups exposed to heavy metals and Osun River water than in the control group. *PRM1* is essential for sperm chromatin condensation and stability, while *AKAP4* is a major regulator of sperm motility and

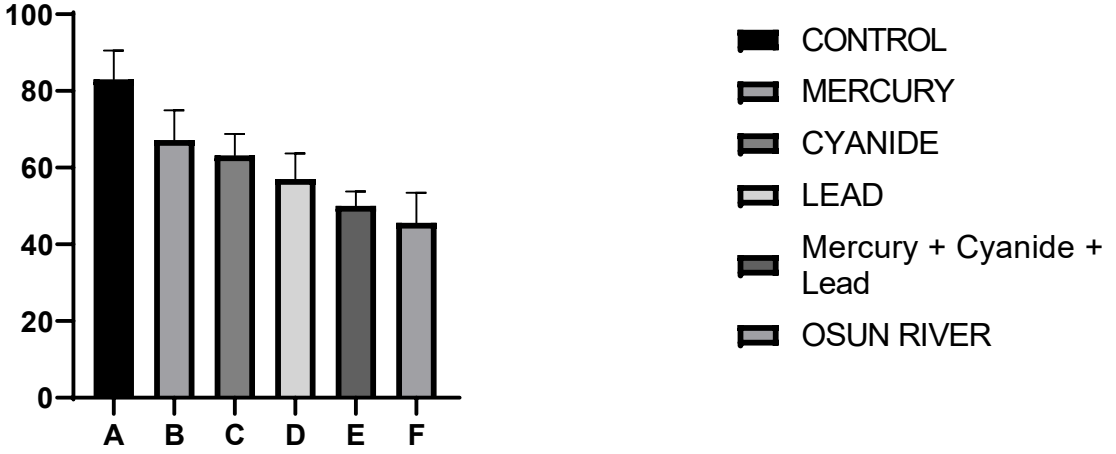

Figure 5. Sperm count concentration comparisons between the experimental groups

Note: The Mean±SEM is used to display the values (P≤0.05, n=5).

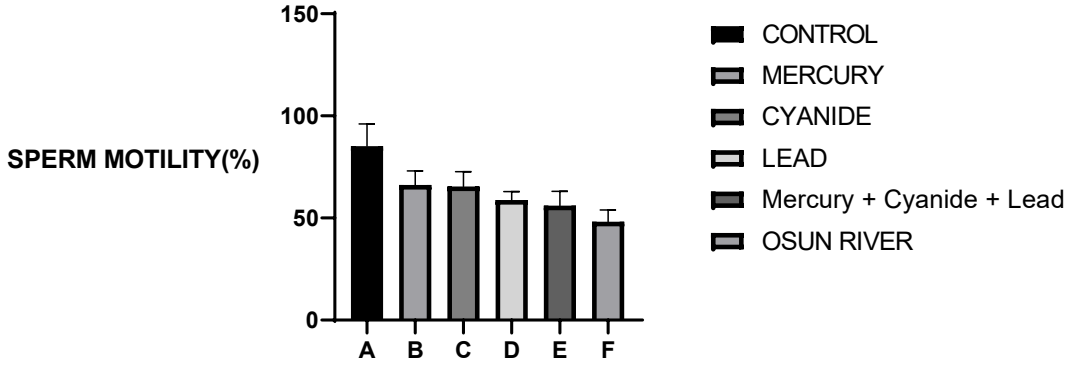

Figure 6. Comparison of sperm motility among experimental groups

Note: The Mean±SEM is used to display the values (P≤0.05, n=5).

integrity. In line with earlier research that described heavy metals as endocrine disruptors and toxicants that can interfere with spermatogenesis and other testicular processes, the suppression of these genes indicates a direct impairment of spermatogenesis and sperm quality as a result of exposure to heavy metals [11].

All exposed groups showed a significant decrease in serum testosterone levels, which is essential for sustaining male reproductive function. The harmful effects of lead, cyanide, and mercury on Leydig cells, which produce testosterone, may be the cause of this observation. The histological observations of decreased interstitial space integrity and Leydig cell degeneration may also be explained by the disruption of Leydig cell function. Heavy metals have been shown in earlier studies to interfere with steroidogenesis, which lowers testosterone synthesis [12].

In contrast to the control group, the exposed groups showed a significant decrease in sperm motility and count, as well as a greater degree of aberrant sperm morphology. The cumulative harmful effects of heavy metals on sperm quality, a sign of reduced reproductive capacity, are highlighted by these observations. The histological evidence of seminiferous tubule degeneration and disrupted germinal epithelium in the testicular tissue of exposed rats may be connected to the decrease in sperm count. These results are in line with research on the toxicity of environmental contaminants to reproduction [13].

Additional proof of the harmful effects of heavy metals was supplied by the histological examination of testicular tissue. Seminiferous tubule degeneration, disrupted germinal epithelium, and decreased interstitial space integrity were observed in the groups exposed to mercury, cyanide, and lead, as well as the group exposed to Osun River water. Complete disarray of the testicular architecture was noted in a few instances. The biochemical results are supported by these changes, which are suggestive of testicular shrinkage and compromised spermatogenesis.

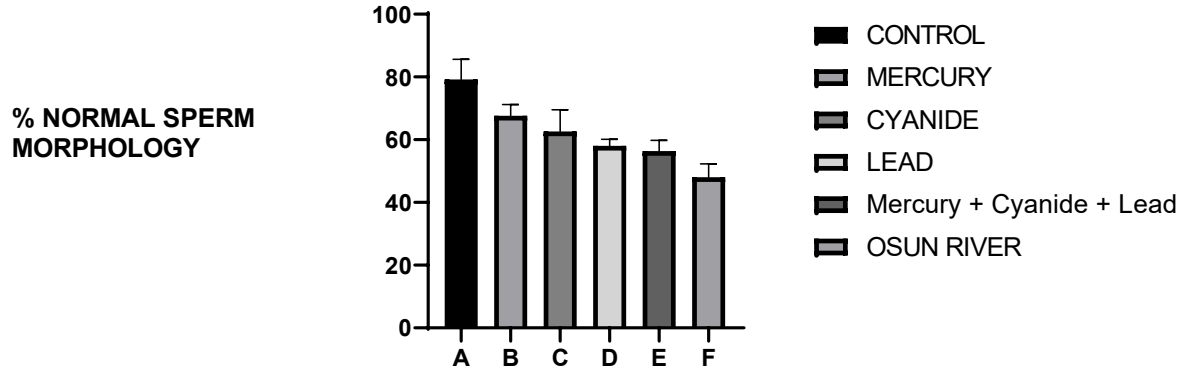

Figure 7. Sperm morphological comparisons between the experimental groups

Note: The Mean±SEM is used to display the values (P≤0.05, n=5).

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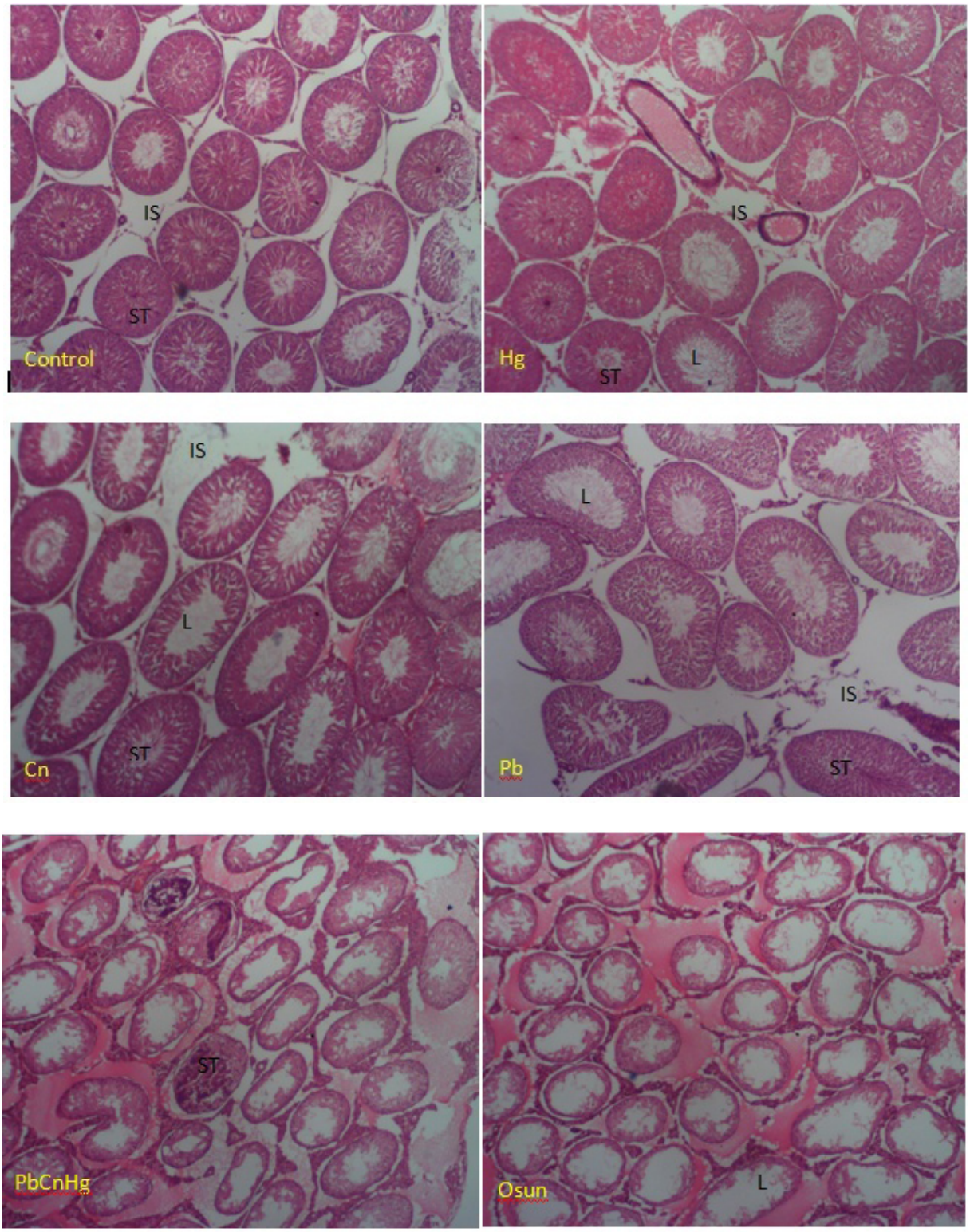

Figure 8. Photomicrograph of histological sections of the testes stained with hematoxylin and eosin (H&E, magnification ×200).

Abbreviations: L: Lumen; ST: Seminiferous tubules; IS: Interstitial space.

Note: Groups: A: Control; B: Mercury; C: Cyanide; D: Lead; E: Mercury + lead + cyanide; F: Osun River water.

A synergistic or cumulative toxic effect of the heavy metals found in the river is suggested by the noticeable effects observed in the group exposed to water from the Osun River. This demonstrates the extent of the Osun River's environmental contamination and its possible effects on public health.

5. Conclusion

In conclusion, there is growing concern around the world about how heavy metals affect human health, particularly in relation to reproductive toxicity. Studies have highlighted the detrimental effects of toxic substances such as cyanide, lead, and mercury, which pose serious risks to the environment and public health. These toxins can have long-term effects by interfering with various physiological functions, such as fertility and hormone balance. The study also highlights how crucial it is to evaluate the dangers of heavy metal exposure in various settings, especially in areas like Nigeria's Osogbo Metropolis. The data emphasizes the necessity of ongoing monitoring, regulation, and public health interventions to mitigate the harmful effects of these environmental contaminants. Furthermore, understanding the mechanisms through which heavy metals affect human health can guide the development of effective strategies to reduce exposure and protect vulnerable populations, particularly in regions where these pollutants are widespread.

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Compliance with ethical guidelines

All procedures followed the ethical guidelines established by the Health Research Ethics Committee, College of Health Sciences, Osun State University, Osogbo, Nigeria, in compliance with the National Institutes of Health's guidelines for the care and use of laboratory animals.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

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Authors' contributions

Conceptualization: Babatunde Samuel Adeyemi and Opeyemi Samson Adeleke; Methodology, experiments, and data interpretation: Tolulope Ayodeji Oni; Data curation: Opeyemi Samson Adeleke; Analysis: Opeyemi Samson Adeleke and Tolulope Ayodeji Oni; Babatunde Samuel Adeyemi; Statistical analysis: Babatunde Samuel Adeyemi; Project administrative and technical support: Oluwatosin Abosede Akindehin; Material support and writing the original draft: Oluwatobi Oluseun Adunfe; Review and editing: Opeyemi Samson Adeleke and Olusola Atilade Adeeyo; Supervision: Oluwatobi Oluseun Adunfe.

Conflict of interest

The authors declared no conflict of interest.

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