Effect of Heavy Metals (Mercury, Lead, and Cyanide) Present in the Osun River on the Testes of Male Wistar Rats

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ABSTRACT

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.Thirty adult male Wistar rats, averaging 160g 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 duration of four weeks. 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. 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 system;s.

KEYWORDS: Osun river, Heavy metals, Toxic effects, Testicular damage.

1.0 Introduction

The Ọṣun River is a river that flows southwards through central Yoruba land in southwestern Nigeria into the Lagos Lagoon and the Atlantic 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 Osun River has reported by Anifowose et al., 2023(2) are lead, cyanide, 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 the 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 drink the 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 aftermath 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 overall health.

2.0 Materials and Method

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 acclimate 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 Institute 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 gotten from the Osun River will be used as exposure for polluted sources of 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 was 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 amount of time. 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 last dose, and blood samples were taken from the left ventricle of their hearts. The blood was put into red-top tubes so that the hormones could be examined. For histological analysis, 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 penetration with paraffin wax before being embedded in molten paraffin wax. Using a rotary microtome, the paraffin block was divided into slices that were 4 μm thick. Following the mounting of these sections onto glass slides, they 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, Protamine in batches with the control sera at both physiological and pathological levels by the standard Quantitative Enzyme-Linked Immunosorbent Assay(ELISA) technique with microwell kit which was manufactured by Syngenemed. The manufacturer 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 PCR (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 abnormal flagellar abnormalities.

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

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 immunohistochemistry (IHC) and immunofluorescence microscopy, which reflects 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 quantitative PCR (qPCR), which provide 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 results in worse chromatin packaging and decreased fertility—genetic research employing CRISPR/Cas9 or RNA interference (RNAi) makes it easier to investigate the roles of PRM1 and PRM2.

2.8 Statistical Analysis

For every set of data, the mean and standard error of the mean (S.E.M.) were calculated. Tukey's post hoc test was used for multiple comparisons after a one-way analysis of variance (ANOVA) in GraphPad Prism 8 was used for statistical comparisons of means. It was determined that a P value of ≤ 0.05 was statistically significant.

3.0 RESULTS

3.1 BIOCHEMICAL RESULTS

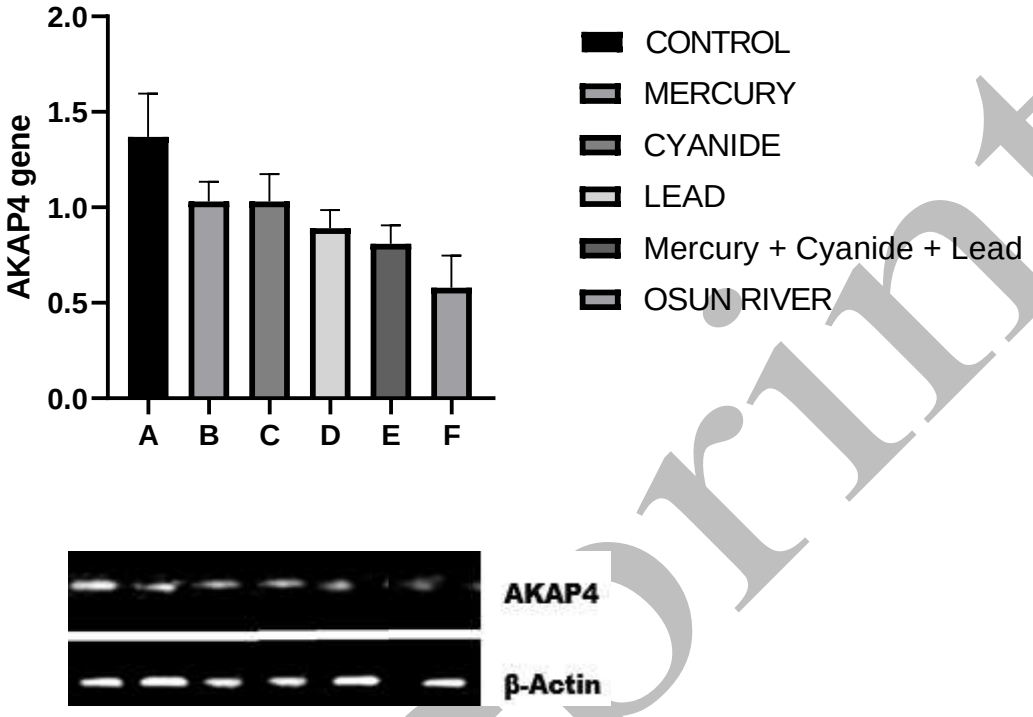


Figure 1: The expression of the A-kinase anchoring protein 4 (AKAP4) gene was compared between the experimental groups in Figure 1. Mean ± SEM is used to express the data ($P \leq 0.05$, $n = 5$).

All groups exposed to heavy metals, including those given lead, cyanide, mercury, and their combination, showed a significant decrease in AKAP4 gene expression as 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.

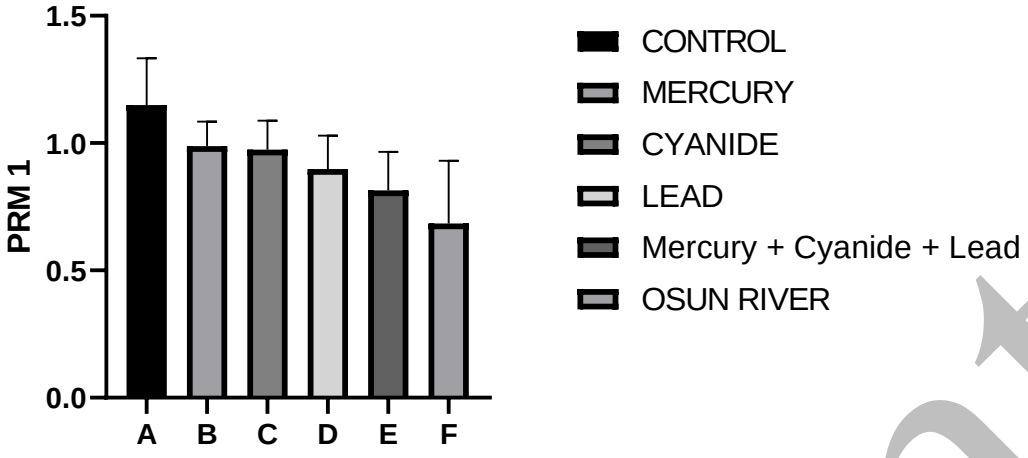


Figure 2: The expression of the Protamine 1 (PRM1) gene was compared between the experimental groups. Mean ± SEM is used to express the data ($P \leq 0.05$, $n = 5$).

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

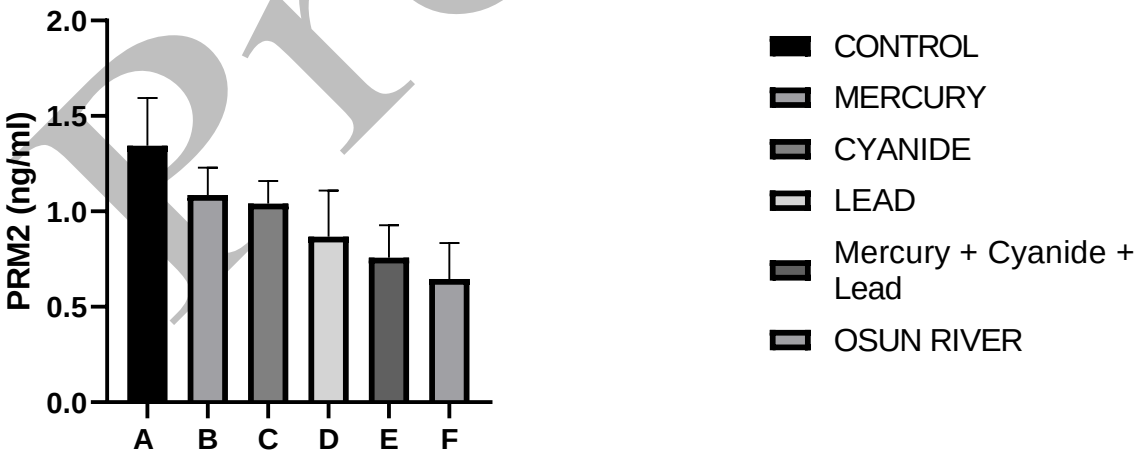


Figure 3: Showed protamine 2 (PRM2) gene expression comparisons between experimental groups. Mean ± SEM is used to display the values ($P \leq 0.05$, n = 5).

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.

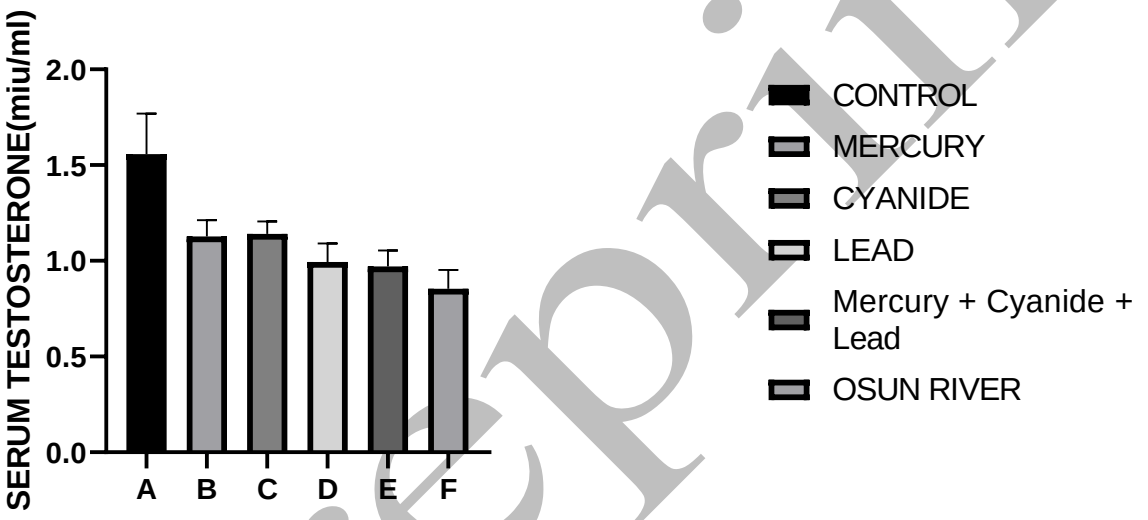


Figure 4: Serum testosterone levels in the experimental groups are compared in Figure 4. Mean ± SEM is used to display the values ($P \leq 0.05$, n = 5).

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 drop in serum testosterone levels.

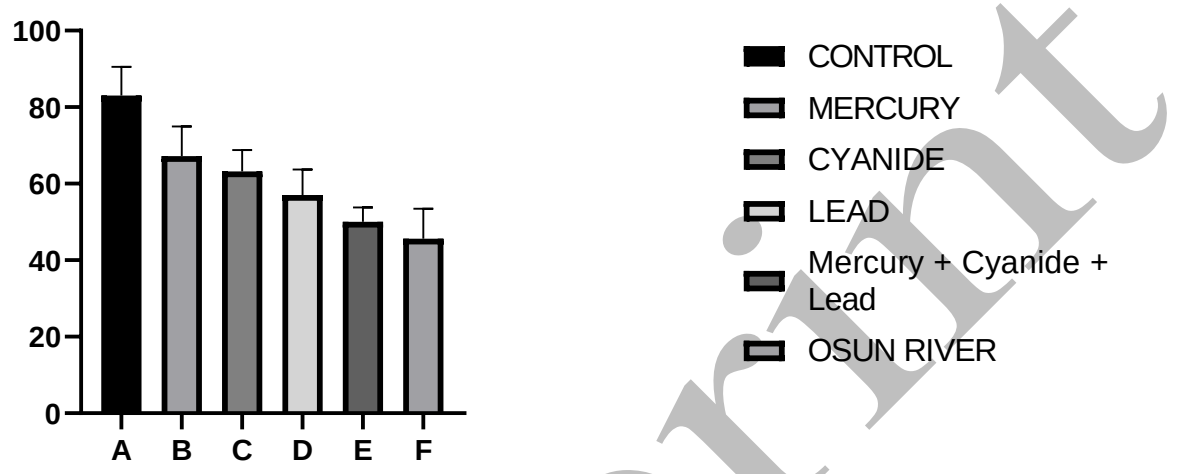


Figure 5: Sperm count concentration comparisons between the experimental groups. Mean ± SEM is used to display the values ($P \leq 0.05$, $n = 5$).

All groups exposed to heavy metals showed a substantial decrease in sperm count concentration when compared to the Control group. Furthermore, compared to the Control group, the Osun River water group showed a notable drop in sperm count concentration.

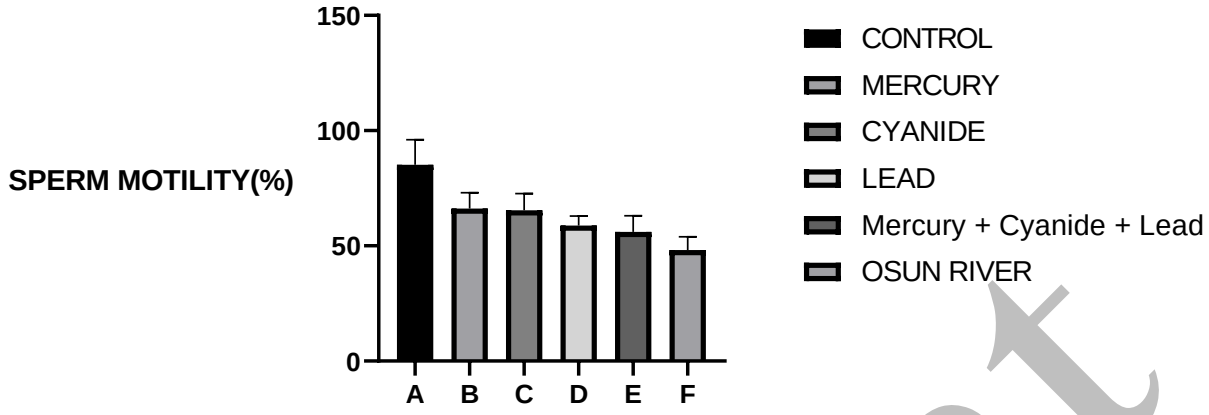


Figure 6: Comparison of sperm motility among experimental groups; values are shown as Mean ± SEM ($P \leq 0.05$, n = 5).

Figure 6 showed that all groups exposed to heavy metals had significantly lower sperm motility than the Control group, and that the group that received Osun River water also had significantly lower sperm motility than the Control group.

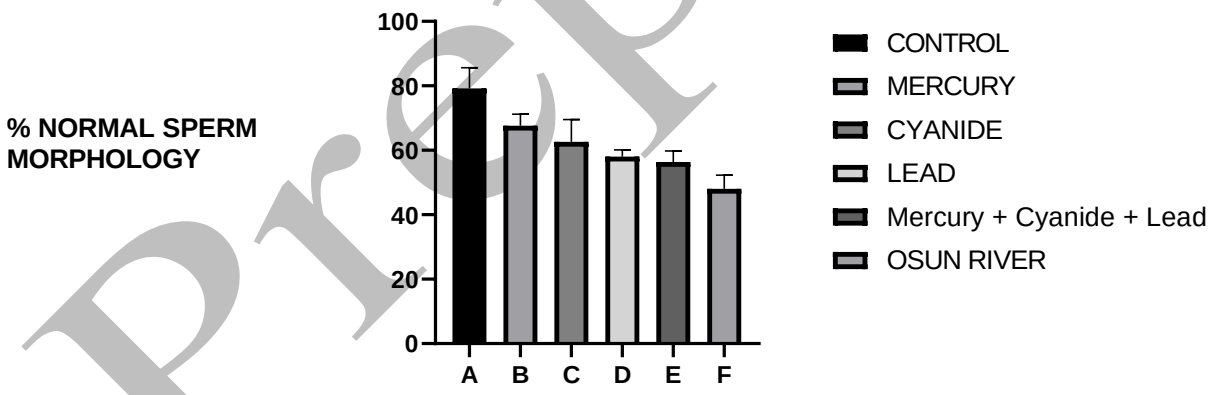


Figure 7: Sperm morphological comparisons between the experimental groups.Mean ± SEM ($P \leq 0.05$, n = 5) is used to display the values.

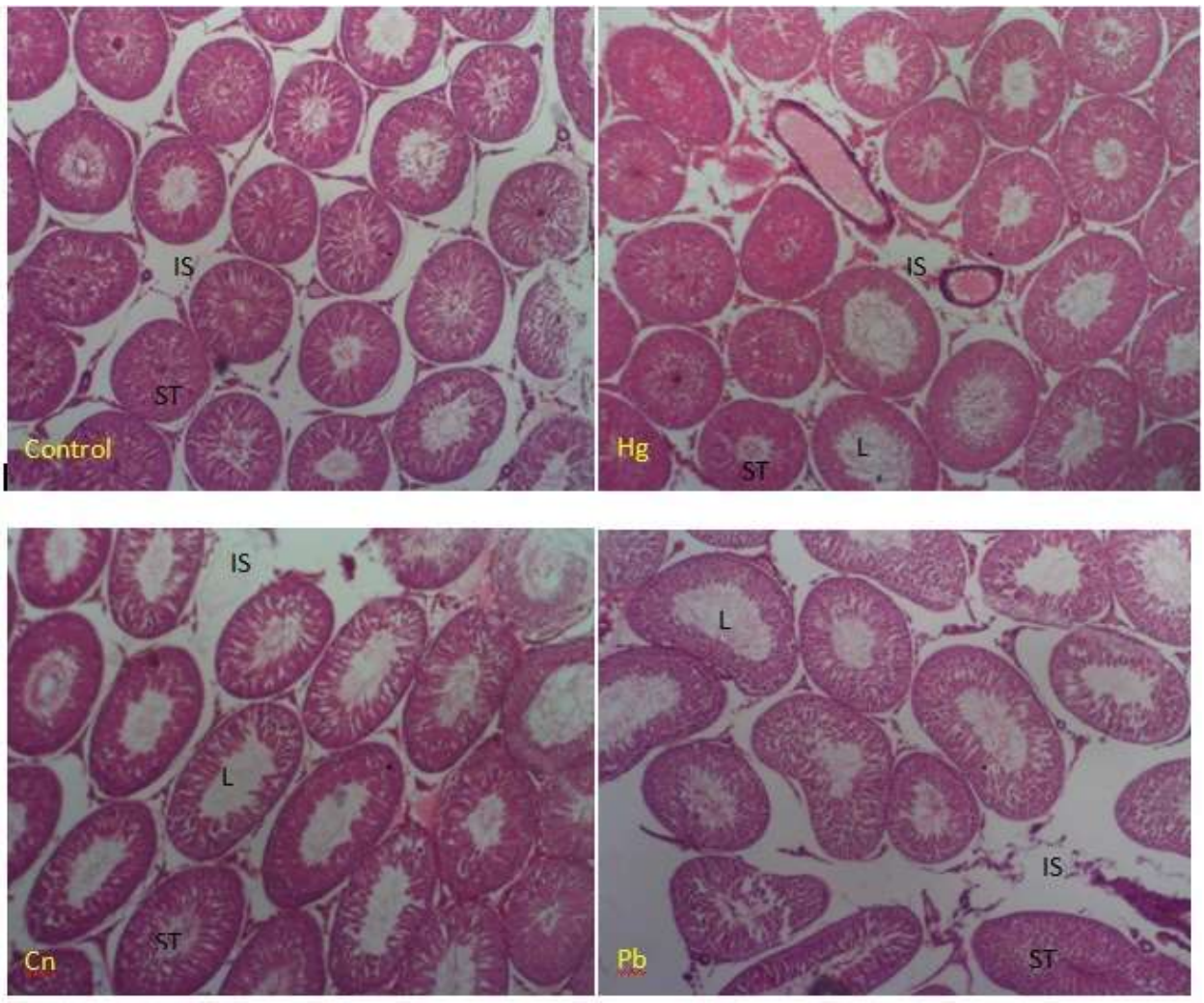
In comparison to the Control group, Figure 7 shows a significant decrease in sperm morphology in all groups exposed to heavy metals. In addition, the sperm morphology of the Osun River water-treated group was significantly lower than that of the control group.

Histological Observation Of The Testes

Hematoxylin & Eosin stain

Photomicrograph showing the general cytoarchitecture of the testes of rats across the various experimental groups using H&E stain (magnification: x200) is displayed in Figure 8.

The cytoarchitecture of the testes revealed the different parts of the testes; the seminiferous tubules, lumen, interstitial spaces, the germinal epithelium and leydig cells. The photomicrographs revealed intact organization and structure of the testes in group A (Control), B (Mercury) group showed testicular damage including degeneration of the seminiferous tubules irregular lumens. Groups C and D (Cyanide&lead) both groups showed evidence of toxicity. In the cyanide group, there is moderate disorganization of seminiferous tubules,mildly enlarged lumens and slightly expanded interstitial spaces which indicated moderate spermatogenic disruption while the lead group, the damage is more pronounced with severe disorganization of the seminiferous tubules, irregular lumens and prominent interstitial spaces. In group E and F (PbCnHg and Osun river), the cytoarchitecture of the testes both showed severe testicular damage. The seminiferous tubules were disorganized, irregular and reduced lumens,disintegration of the interstitial spaces and germ cells.



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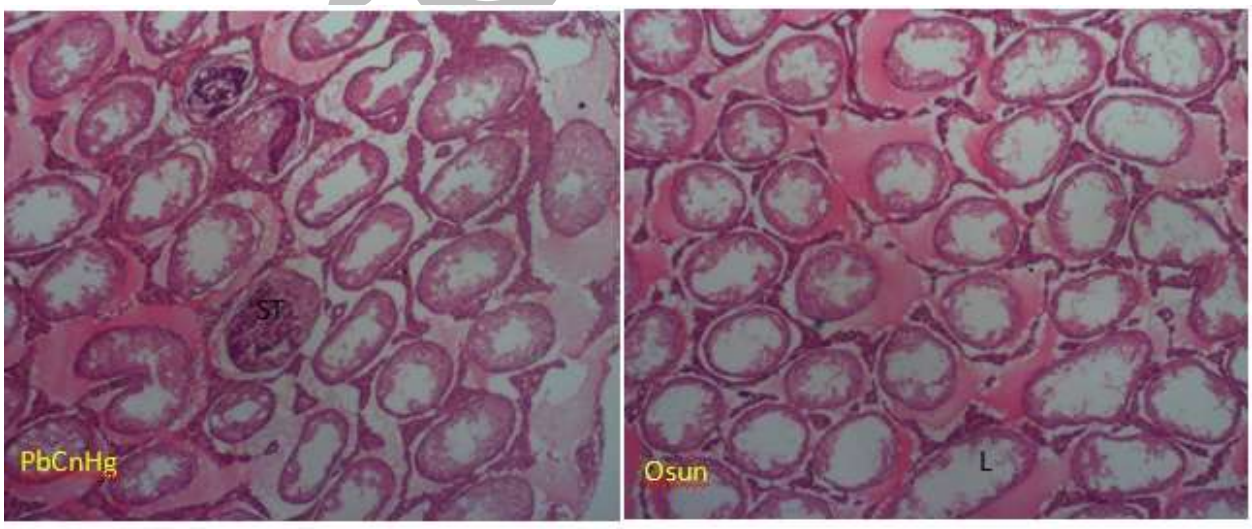

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Figure 8: Photomicrograph of histological section of the testes stained with hematoxylin and eosin x200, done and arranged together using powerpoint software. A=Control; B= MERCURY; C= CYANIDE; D= LEAD; E= MERCURY+LEAD+CYANIDE; F=OSUN RIVER.

Key: (L) Lumen, (ST) Seminiferous tubules, (IS) Interstitial space.

4.0 Discussion

This study demonstrates the negative effects of toxic substance like cyanide and heavy metals like lead and mercury, as well as Osun River water tainted with these metals, on testicular function in male Wistar rats. The results show significant biochemical and histological changes in the testes, supporting the negative 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 mobility and integrity. In line with earlier research that describes 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 drop in serum testosterone levels, which are 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 disturbed 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, disturbed 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 few instances. The biochemical results are supported by these changes, which are suggestive of testicular shrinkage and compromised spermatogenesis.

A synergistic or cumulative toxic effect of the heavy metals found in the river is suggested by the noticeable effects shown 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. 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 a number of 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 prevalent.

Acknowledgment

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Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Data Availability

The data that support the findings of this study are available upon request from the corresponding author.

Conflict of Interest

The authors declare that they have no conflict of interest.

S/N	AUTHORS	CONTRIBUTION
1	Opeyemi Samson Adeleke	Conceptualization, Data curation, Formal Analysis, Review, Editing and Supervision.
2	Tolulope Ayodeji Oni	Analysis, Methodology, Interpretation of data
3	Oluwatobi Oluseun Adunfe	Drafting of the Mnauscript, Material Support
4	Babatunde Samuel Adeyemi	Conceptualization, Statistical analysis
5	Oluwatosin Abosede Akindehin	Technical support and Administrative support
6	Olusola Atilade Adeeyo	Critical revision of the manuscript

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