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Research Article

Assessment of cadmium and iron exposure on testicular biometry and semen quality in male Albino rats

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Abstract

Exposure to heavy metals such as cadmium (Cd) and iron (Fe) through contaminated water and feed poses significant risks to male reproductive health. This study investigated the effects of Cd and Fe, both individually and in combination, on testicular parameters and semen quality in adult male albino rats. Forty adult male rats were randomly assigned to four groups in a two-phase study: control (Cd- and Fe-free), Cd-exposed, Fe-exposed, and Cd+Fe-exposed. The metals were administered via tainted drinking water (0.229 mg/L Cd; 1.900 mg/L Fe) in the first phase and via tainted feed in the second phase for 4 weeks. Testicular weight, testes-body weight ratio, and semen quality parameters, including sperm motility, viability, count, volume, and morphology were evaluated. Data were subjected to one-way analysis of variance and $p \leq 0.05$ followed by Tukey's post-hoc test. Exposure of rats to Cd and Fe via feed significantly increased testes weight and the testes-body weight ratio compared to the control ($p \leq 0.05$). Fe-exposed rats via water showed reduced sperm motility and count, whereas Fe-exposed rats via feed showed a contrasting effect. Cadmium-exposed rats, especially via water, caused notable sperm morphological abnormalities. The combined exposure of rats to Cd and Fe revealed similar effects of Fe on semen quality via both routes. Rats exposed to Cd and Fe, particularly via contaminated water, showed adverse effects on testicular function and semen quality, whereas Fe exposure showed adaptive effects via feed. This study highlights the differential impacts of exposure routes and underscores the need for environmental control of heavy metal contamination to safeguard reproductive health.

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Cd, Fe, testicular toxicity, semen quality, heavy metal exposure.

1. Introduction

Heavy metal contamination of food and water sources has emerged as a pressing public health concern globally, particularly in developing regions where industrial activities, mining operations, and inadequate environmental regulations contribute to increased exposure [1, 2]. Among these metals, cadmium (Cd) and iron (Fe) are of particular interest due to their widespread environmental presence and potential to adversely affect biological systems, including the reproductive system [3, 4].

Cadmium (Cd) is a non-essential and highly toxic metal that accumulates in biological tissues and has been implicated in reproductive toxicity, particularly in males. It exerts harmful effects through oxidative stress, disruption of the hypothalamic-pituitary-gonadal axis, and direct damage to testicular tissues, resulting in impaired spermatogenesis and hormonal imbalance [5, 6]. In contrast, iron is an essential trace element that is critical for various physiological processes, including oxygen transport and cellular metabolism. However, excessive iron intake or accumulation can induce oxidative damage and interact with other metals such as cadmium, potentially modifying their toxicological effects [7, 8].

Although, the individual toxicities of cadmium and iron have been widely studied [9, 10], there remains limited understanding of their combined effects, especially when exposure occurs via different environmental routes such as drinking water and contaminated feed. Moreover, the influence of exposure route on testicular integrity and semen quality has not been thoroughly investigated. Understanding these interactions is crucial, as environmental exposure to such metals often occurs simultaneously rather than in isolation [7, 11]. However, this study aimed to evaluate the effects of cadmium and iron, administered singly and in combination through tainted drinking water and feed, on testicular weight, testes-body weight ratio, and semen quality in adult male albino rats.

2. Materials and methods

2.1. Materials

2.1.1. Animals

Forty (40) adult male albino rats (Wistar strain) were procured from the Department of Animal Science, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

2.1.2. Chemicals and reagents

All the chemicals and reagents used were of analytical grade.

2.2. Methods

2.2.1. Animal care and treatment

The rats were housed in wooden cages under standard laboratory conditions (temperature 25 ± 2°C, 12h–light/dark cycle) with adequate ventilation and access to standard rat chow and water *ad libitum*.

2.2.2. Preparation and treatment of catfish (Clarias gariepinus) for feed formulation

Preparation and treatment of catfish was carried out as described by Ogunbiyi and Obi [7]. Feed formulations were also compounded as outlined by Ogunbiyi and Obi [7, 11].

2.2.3. Experimental design

Twenty adult male rats consisting of five rats per group (Table 1) were used for each study. The rats were exposed to 0.229 mg/L Cd and 1.900 mg/L Fe based on the previously reported concentration of these metals in Warri river [12], though these concentrations were found to be below the regulatory limits for drinking water. All procedures were performed in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals [13]. Ethical approval was obtained from the Faculty of Life Sciences Research Ethics Committee (FLSREC), University of Benin (Approval No.: FLSREC-2023-007).

Table 1. Animal grouping.

Experimental groups	Treatment
A (Control)	Received Cd and Fe-free water/feed
B	Received Cd-tainted water/feed
C	Received Fe-tainted water/feed
D	Received Cd+Fe-tainted water/feed

The animals were randomly divided into four groups in the two-phase of study (n = 5 per group).

2.2.4. Sample collection/determination of weight

After four weeks of exposure, the rats were weighed, and the testes were excised, cleaned and weighed also. Testis weight was determined by direct measurement using an electric weighing balance, while the testis-to-body weight ratio was determined by dividing the individual testis weight by the weight of the rat.

2.2.5. Determination of semen quality

The epididymal content of the cauda epididymis was obtained by excising it with surgical blades and squeezing it onto a sterile glass slide. This content was diluted tenfold with 0.9% normal saline, mixed well and then covered with a coverslip. Progressive motility and sperm counts were assessed using a

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Table 2. Testes weight and testes-to-body weight ratio in exposed rats (n = 5).

Groups	Testes wt. via water (g)	Testes wt. via feed (g)	Testes-body wt. ratio via water	Testes-body wt. ratio via feed
A	1.67 ± 0.08	1.27 ± 0.05	0.0252	0.0127
B	1.55 ± 0.10	1.73 ± 0.08[a]	0.0135	0.0145[a]
C	1.77 ± 0.06	1.92 ± 0.04[a]	0.0149	0.0172[a]
D	1.70 ± 0.05	1.95 ± 0.08[a]	0.0147	0.0187[a]

Values are expressed as mean ± SEM. Superscript 'a' indicates values significantly different from the control group ($p \leq 0.05$). Wt. = Weight.

compound microscope. A smear of the semen suspension was stained with Eosin-Nigrosin to ascertain the percentage of live/dead sperm and assess sperm cell morphology [9].

2.3. Data analysis

Data are presented in tables and graphs using GraphPad Prism 6.0 software. One-way analysis of variance (ANOVA) was conducted while Tukey's multiple range comparison test was used as a post-hoc test with a significant level of $p \leq 0.05$.

3. Results

3.1. Testis weight and testis-to-body weight ratio

The results of the analysis of testis weight and testis-to-body weight ratio after 4 weeks of exposure are presented in Table 2. Notably, there was a significant increase ($p \leq 0.05$) in the testis weight and testis-to-body weight ratio in rats exposed to cadmium (Cd), iron (Fe), or a combination (Cd + Fe) via feed, compared to the control group. However, exposure to water did not result in statistically significant changes in these parameters, except for a slight, non-significant variation.

3.2. Semen quality parameters

The effects of Cd and Fe exposure on semen quality are illustrated in Figs 1–6.

3.2.1. Sperm motility

Compared to the control, rats exposed via water had significantly reduced sperm motility in rats treated with Fe and the combination of Cd and Fe. In contrast, rats exposed via feed, particularly to Fe alone and Cd+Fe, exhibited a significant increase in sperm motility (Fig. 1).

3.2.2. Sperm viability

Rats exposed to Fe via both water and feed showed a significant increase in sperm viability compared to the control. However, Cd exposure had no beneficial effects on this parameter (Fig. 2).

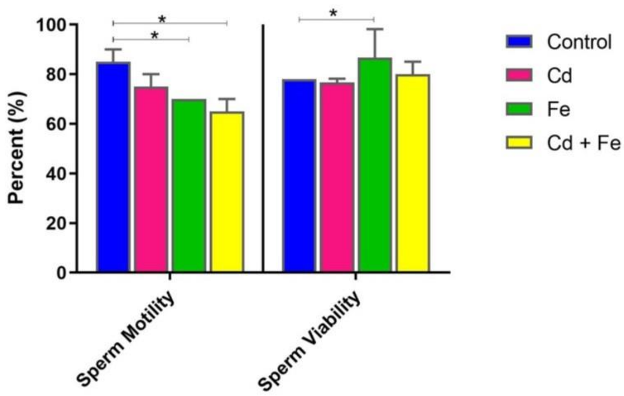

Figure 1. Sperm motility and sperm viability of rats exposed via water.

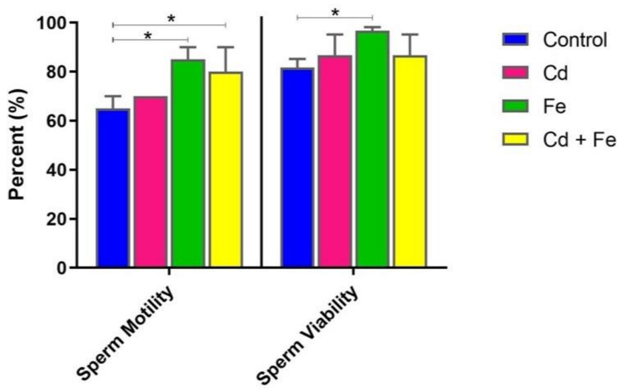

Figure 2. Sperm motility and sperm viability of rats exposed via feed.

3.2.3. Sperm count

Rats exposed to Fe and Cd+Fe via water showed a significant decrease in the sperm count. Conversely, feed exposure to either metal, alone or in combination, resulted in a marked increase (Fig. 3).

3.2.4. Sperm volume

No significant differences were observed in sperm volume across all experimental groups, regardless of the exposure route or metal combination (Fig. 4).

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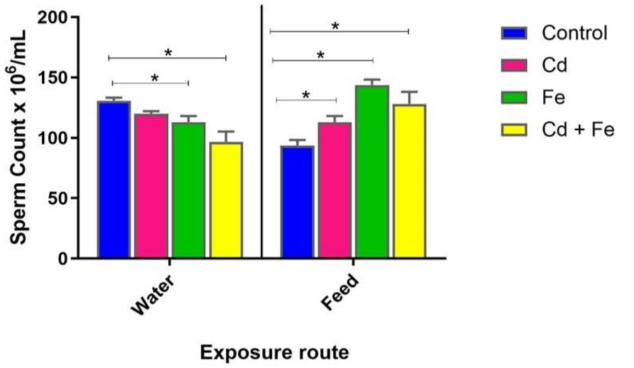

Figure 3. Sperm count of exposed rats.

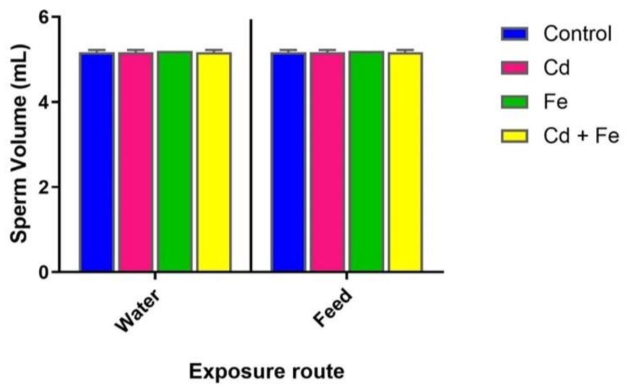

Figure 4. Sperm volume of exposed rats.

*Values carrying asterisks are significantly different ($p \leq 0.05$) from one another, while those without asterisks do not differ significantly from one another.

3.2.5. Sperm morphology

Notable morphological deformities were observed in spermatozoa of rats exposed to Cd, particularly in water. These included head and tail abnormalities, indicating potential structural damage. Exposure via feed showed less pronounced morphological alterations (Figs 5 and 6).

4. Discussion

Cadmium (Cd) has long been recognized as a potent toxicant in both humans and laboratory animals, inducing various biochemical and physiological dysfunctions [6]. One of its primary targets is the male reproductive system, particularly the testes [14]. Similarly, iron (Fe), though essential for biological functions, can act as a pro-oxidant in excess and may exacerbate toxic effects when co-administered with other metals. This study investigated the effects of Cd and iron (Fe), administered singly or in combination via tainted drinking water and feed, on testicular weight and semen quality in adult male rats. These findings indicate that the route of exposure plays a crucial role in the degree and nature of observed reproductive toxicity.

A significant increase ($p \leq 0.05$) in testicular weight and testes-to-body weight ratio was observed in rats exposed to Cd, Fe, or Cd+Fe via tainted feed, but not through drinking water. In fact, rats exposed via water exhibited either reduced or statistically unchanged testicular weights compared to the control group. This suggests that dietary exposure may lead to more prolonged accumulation or localized effects in the reproductive tissues, thereby contributing to the observed weight gain. Previous studies have shown that heavy metal accumulation in organs like the liver, kidney, and spleen can lead to hypertrophy or inflammation [8]. Similar mechanisms may explain the testicular weight increase observed in this study.

Semen quality parameters further reflect this exposure route-dependent effect. Exposure to Fe and Cd+Fe via tainted water led to a significant decrease in sperm motility and count, whereas exposure to Fe alone via water surprisingly improved sperm viability. These findings suggest that while Fe may support certain cellular functions (e.g., mitochondrial activity for viability), its presence, especially when combined with Cd, may disrupt motility and spermatogenesis when ingested drinking water.

Conversely, feed-based exposure to Fe and Cd+Fe resulted in improved sperm motility, count, and viability compared to the control group. This may reflect a more gradual absorption or modulation of systemic distribution when ingested through the feed. The enhancement of sperm quality parameters in the feed-exposed groups also suggests a possible adaptive or hormetic response, particularly with Fe, which is known to be a vital trace element involved in many reproductive functions [15].

Despite these improvements, sperm morphological abnormalities were evident across all the exposed groups. Notably, deformities were more pronounced in rats exposed to Cd via water, with fewer abnormalities observed in Cd-fed groups. This supports previous findings that Cd exposure, especially through water, can adversely affect spermatogenesis and sperm morphology [9]. Cd's known ability to induce oxidative stress and disrupt

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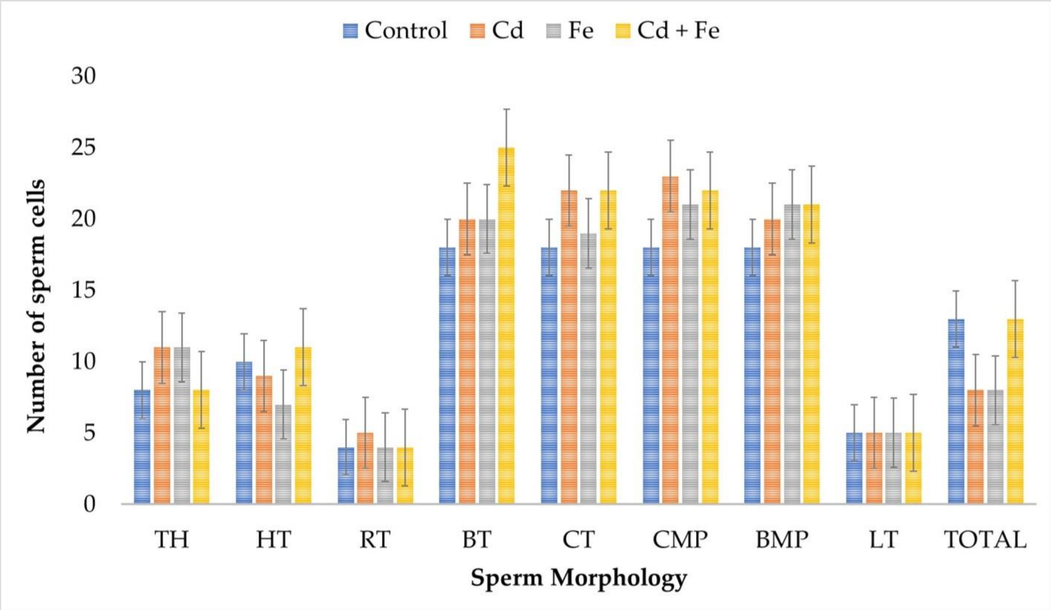

Figure 5. Sperm morphology of exposed rats via water.

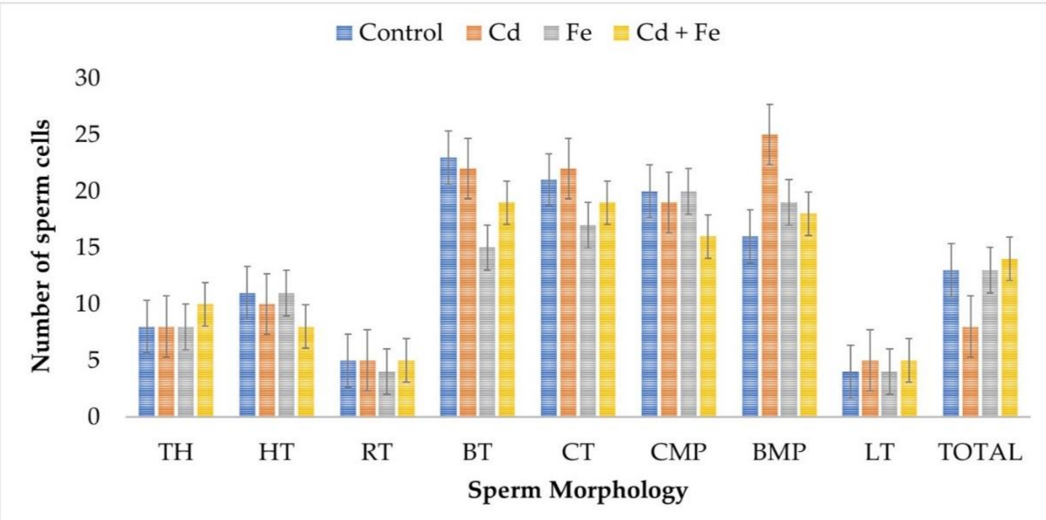

Figure 6. Sperm morphology of exposed rats via feed.
TH= Tailess head; HT= Headless tail; RT= Rudimentary tail; BT= Bent tail; CT= Curved tail; CMP= Curved mid-piece; BMP= Bent mid-piece; LT= Looped tail.

the hypothalamic-pituitary-testicular axis likely underpins these effects [16].

Interestingly, sperm volume remained unaffected across all groups, indicating that while spermatogenesis and morphology were affected, seminal fluid production per se was not altered by Cd or Fe exposure.

In summary, Cd and Fe, administered via different routes, produced distinct effects on testicular and semen parameters in male rats. Feed-based exposure resulted in increased testicular mass and improved semen quality, whereas water-based exposure, especially to Cd and Cd+Fe, was associated with testicular dysfunction, including reduced sperm motility and count. These findings highlight the importance of the exposure route in evaluating the reproductive toxicity of heavy metals and suggest that dietary ingestion of these metals may yield different

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toxicokinetic profiles compared to waterborne exposure.

5. Conclusions

This study demonstrated that exposure to Cd and Fe, either individually or in combination, via the feed leads to a significant increase in testicular weight and testes-to-body weight ratio in adult male rats. However, this increase was not evident through water-based exposure. Semen quality was differentially affected depending on the exposure route. Cd and Fe exposure via tainted water led to a decrease in sperm motility and count, particularly when both metals were combined, while Fe alone via water unexpectedly improved the sperm viability. In contrast, feed-based exposure to Fe and Cd+Fe significantly improved sperm motility, count, and viability. Although sperm volume remained unaffected, variations in sperm morphology were observed across all exposure groups, with more pronounced deformities observed in rats exposed to Cd via water. These morphological changes are indicative of a compromised sperm quality. Overall, Cd and Fe exposure, especially via water, negatively impacted key parameters of reproductive function, including spermatogenesis and sperm morphology, thereby reducing overall semen quality while Fe exposure showed adaptive effects via feed.

Authors' contributions

Conceptualization, O.O.J.; data curation and formal analysis, O.O.J., J.T.A., H.E.I.; investigation, methodology, project administration and supervision, O.O.J, R.J.J., H.E.I.; resources, validation and visualization, J.T.A., E.I., C.A.U.

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Availability of data and materials

All data and materials shall be provided on reasonable request from the corresponding author.

Conflicts of interest

The authors declare no conflict of interest.

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