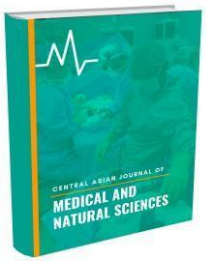

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Article

Evaluation of Oxidative Indicators and Sex Parameters for Male Rats Exposed To Cadmium Chloride and Lead Acetate

Ahmed Salim Atea Ahmed[1] , Qasim Azeez Razooqi[2]

1,2 Department of Biology, College of Education for Pure Sciences, Tikrit University, Iraq
* Correspondence: Ahmed.sa.Atea@st.tu.edu.iq , razooqi.aasim@tu.edu.iq

Abstract: The study included 36 male Wistar rats aged between (90-110 days) and weighing between (200-260 grams). They were randomly divided into 6 groups, with each group consisting of 6 males. The first group served as the control group, administered regular water. The second group was treated with cadmium chloride at a dose of 5 mg/kg of body weight. The third group was administered cadmium chloride at a dose of 10 mg/kg of body weight. The fourth group received lead acetate at a dose of 50 mg/kg of body weight. The fifth group was given lead acetate at a dose of 100 mg/kg of body weight. The sixth group was treated with 5 mg/kg of cadmium chloride and 50 mg/kg of lead acetate. The treatment lasted for 30 days. The results showed a significant increase (P≤0.05) in oxidative products, particularly Malondialdehyde (MDA) concentration, and a significant decrease in the total antioxidant content in the serum, including Superoxide Dismutase (SOD), Catalase Enzyme (CAT), and Glutathione (GSH) compared to the control group. A significant increase (P≤0.05) was also recorded in oxidative stress markers of DNA, specifically Hydroxy-2-deoxyguanosin-8, compared to the control group. Moreover, negative changes in some biochemical and hormonal characteristics of the serum were observed compared to the control group, represented by a significant decrease in sex hormones, particularly the male testosterone hormone. Additionally, the study indicated that treating the animals with different concentrations of cadmium chloride and lead acetate led to negative effects on sperm characteristics. This manifested as a significant decrease in sperm count and its normal form compared to the control group. The treatments resulted in deformities in sperm shape, such as alterations in the head and tail morphology, fusion of the midpiece, tail twisting, and occasionally tail breakage.

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1. Introduction

Heavy metals are one of the environmental pollutants that are widespread in various environmental sites (air, water, soil). Through these components, heavy metals are directly and indirectly linked to the health of humans and animals by affecting the growth of plants that living organisms feed on (Akbarpour et al., 2012). Heavy metals are among the prevalent hazardous problems these days, and pollution with them causes many serious diseases due to the tendency of these compounds to accumulate and accumulate within living environmental systems (Priya et al., 2023). The massive technological advancement has accompanied the excessive use of these metals, and pollution with heavy metals has reached a high level of danger, leaving a significant burden on the environment. Humans

themselves are victims of environmental stress; prolonged exposure to these environmental pollutants results in severe health risks and conditions (Bamgbose et al., 2007).

Cadmium is a toxic heavy metal that poses a serious threat to the health of living organisms. Its presence in the body is hazardous and is entirely undesirable even in very low concentrations. Cadmium can enter the body through the atmosphere, water, soil, and food, accumulating significantly in the kidneys, liver, bones, and other organs. It causes irreversible damage to the targeted organs and poses significant carcinogenic risks. It has a long half-life (10-30 years) (Wang et al., 2021). Cadmium can cause chronic kidney damage, as it is one of the main sites where cadmium accumulates, causing harm within the nephrons and proximal tubules. This accumulation can lead to a functional imbalance in the electron transport chain in mitochondria, resulting in electron leakage and the production of reactive oxygen species (ROS). Additionally, cadmium may also impair the function of NADPH oxidase, leading to apoptosis of epithelial cells and deterioration of kidney functions (Yan and Allen, 2021).

Some studies have shown that cadmium may accumulate in the testes, leading to cell death in testicular tissues, resulting in atrophy and shrinkage of tissues by inhibiting the activity of Glutathione peroxidase (Lovaković, 2020). Research by some scientists has indicated that cadmium induces programmed cell death (Apoptosis) in germ cells in the testes, which is a primary factor preventing spermatogenesis, leading to a significant reduction in sperm formation. Cadmium is one of the causes of this cellular death in spermatozoa, along with an increase in polymerase protein levels. Additionally, cadmium causes testicular atrophy, meaning a decrease in testicular size (Zhou et al., 2021). Cadmium is a non-essential metal with carcinogenic and mutagenic effects on living organisms. It can enter the fetus through the placenta, destroying the morphological structure of the placenta, exposing it to high toxicity levels based on the cadmium concentration. This leads to restricted fetal growth, as well as the occurrence of cancer in the placenta and fetal deformities (Geng and Wang, 2019). Several studies have shown that cadmium enters the female body more than in males. Cadmium can cross the placenta, affecting its growth and function. Cadmium partially crosses the placental barrier, influencing fetal growth. It accumulates in various organs and systems, leading to disturbances in gene expression associated with the fetus's body, potentially causing fetal deformities. Cadmium can be secreted in breast milk and transferred during breastfeeding, accumulating in infants' bodies, resulting in learning difficulties and memory impairment (Dharmadasa and Thunders, 2017).

Lead is one of the most important naturally occurring toxic metals in the Earth's crust. Its widespread use has led to general health problems in all countries around the world. Significant sources contributing to environmental lead pollution include mining, smelting, manufacturing, and recycling activities. In some countries, the continuous use of lead-containing paints, as well as leaded gasoline and aviation fuel, are also important sources of lead pollution (Al-Safar, 2005).

Research has found that even low-level exposure to lead is associated with an increased risk of miscarriage, and may lead to stillbirth, low birth weight, and developmental delays in children. When lead levels rise to certain thresholds, they can cause mutations and congenital deformities in the fetus (Edwards, 2014). Acute lead poisoning in children can lead to dementia, irritability, headaches, muscle spasms, hallucinations, memory disorders, learning or behavioral problems, issues with concentration and attention, decreased intelligence quotient, hearing loss, insomnia, or hyperactivity. Acute poisoning can also lead to seizures, paralysis, and coma in fatal cases. Brain damage can occur due to edema and changes in blood vessels (Tamayo et al., 2016).

The current study aims to determine the effects resulting from administering male rats with different levels of cadmium chloride and lead acetate on antioxidant levels, testosterone hormone, and sperm morphology.

2. Materials and Methods

Experimental Animals Preparation: In the study, 35 male and 30 female Albino Rats were used. These rats were obtained from the animal house of the College of Veterinary Medicine, University of Tikrit. They were aged between 14 to 16 weeks and weighed between 190 to 230 grams. They were bred in the animal house affiliated with the College of Veterinary Medicine, University of Tikrit.

Experiment Design: The experiment was conducted according to a Completely Randomized Design (CRD) with three replicates, 3 animals per group:

1. Control group: orally administered tap water for 30 days.
2. Group administered with Cadmium Chloride at 5 mg/kg body weight for 30 days.
3. Group administered with Cadmium Chloride at 10 mg/kg body weight for 30 days.
4. Group administered with Lead Acetate at 75 mg/kg body weight for 30 days.
5. Group administered with Lead Acetate at 150 mg/kg body weight for 30 days.
6. Group administered with Cadmium Chloride at 5 mg/kg and Lead Acetate at 75 mg/kg body weight for 30 days.

Collection of Blood Samples: After the experiment, which lasted for 40 days, three male animals were euthanized, leaving two for mating with healthy females. After mating the affected males with healthy females and upon completion of the gestation period and at birth, the pregnant females were euthanized. Blood samples were collected from the animals using cardiac puncture into plain plastic tubes free from anticoagulants. The samples were then transferred to a centrifuge machine to separate the serum at a speed of 3000 revolutions per minute for 15 minutes. Using micropipettes, the obtained serum was divided into four portions in Eppendorf tubes to avoid repeated freezing and thawing of the sample. It was stored at a temperature of (20°C) for conducting physiological, biochemical, and enzymatic tests.

Estimation of serum malondialdehyde concentration (MDA): I used the Thiobarbituric acid (TBA) reactive method followed by researchers (Guidet & Shah, 1989) to measure MDA. MDA represents one of the final products of the lipid peroxidation process, and its level serves as an indicator of this process. The measurement relies on the reaction between lipid peroxides, especially malondialdehyde, and TBA in a medium dependent on the acidic pH function.

Estimation of Super Oxide Dismutase (SOD) Enzyme Activity in Serum: The activity of the superoxide dismutase enzyme was estimated using the Modified method photochemical Nitroblue Tetrazolium (NBT). This method involved the use of sodium cyanide as an inhibitor of the peroxidase enzyme. The method relies on estimating the SOD enzyme activity indirectly through changes in the optical density of the formazan formed by reducing O2 to the nitroblue tetrazolium (NBT) dye, which is generated from serum irradiation. A decrease in the optical density of the formazan indicates an increase in the enzyme activity (SOD) (Zhang et al., 2016).

Estimation of the Activity of the Enzyme Catalase (CAT) in Serum: The principle of the method involves the reaction of ammonium metavanadate with hydrogen peroxide under acidic conditions, reducing vanadium (V) to (III). Hydrogen peroxide is a strong oxidizing agent that forms a red-orange peroxovanadium complex. This complex absorbs at 452 nanometers (Hadwan & Kadhum, 2018).

Determination of the Activity of Glutathione Peroxidase (GSH) in Serum: The level of glutathione in serum was measured using the method adopted by researchers

(Ramadan et al., 2016). This method relies on Ellman's reagent, which contains Nitrobenzoic acid (DTNB) 5,5-dithio bis2. The reagent quickly reacts with glutathione and is reduced by the sulfhydryl (-SH) group of glutathione, producing a yellow-colored compound. The absorbance of this compound is read at a wavelength of 412 nanometers. The concentration of the resulting compound depends on the concentration of glutathione present in the serum.

Estimation of Hydroxy-2-deoxyguanosine-8 (8-OHdG) Concentration: The concentration of 8-OHdG was determined using kits produced by Sunlong, a Chinese company, following the instructions provided with them.

Determination of Testosterone in Blood Serum: Kits prepared by Monobind Inc., USA, were used to determine the concentration of testosterone using the ELISA technique and the Competitive Enzyme Immunoassay method (Tietz, 1995).

Sperm Morphology Test: After blood collection from the animals, the abdomen of the animal was opened using dissection tools to obtain the testes to which the epididymis is attached. The epididymis was then separated from the testes. They were placed in a petri dish, and using a sharp scalpel, the epididymis was minced. Three drops of normal saline solution were added to it, and a drop was taken and placed on a glass slide. Two drops of Eosin stain and two drops of Nigrosin stain were added, then mixed well and spread on the slide. It was left to dry for five minutes, then gently rinsed with tap water to create a thin smear for observing sperm morphology under a light microscope.

Statistical Analysis: Statistical analysis of the results was performed using Analysis of Variance (ANOVA). Significant differences were determined using Duncan's multiple range test at a significance level of ($P \leq 0.05$).

3. Results and discussion

The results of the current study showed an increase in oxidative products and a decrease in antioxidants, as well as an elevation in oxidative stress to DNA in the blood serum of male albino rats when administered varying amounts of cadmium chloride and lead acetate. This is as presented in Table (1):

Table (1): Oxidative balance results for male albino rats.

Variable / Tretement	-8OHDG µm/L	GSH µm/L	CAT µm/L	SOD µm/L	MDA nmol/ml
1	63.533 e ±8.208	13.682 a ±0.176	0.248 a ±0.025	10.220 a ±0.114	34 e ±4.4
2	81.067 d ±2.843	10.518 b ±0.315	0.121 b ±0.004	7.86 b ±0.092	55 d ±2.1
3	91.767 c ±3.583	9.657 c ±0.218	0.101 bc ±0.007	6.682 c ±0.034	66 c ±3.2
4	97.567 bc ±2.361	8.251 d ±0.361	0.085 cd ±0.004	6.464 c ±0.126	76 b ±1.7
5	107.633 b ±5.369	7.388 e ±0.236	0.073 d ±0.002	5.452 d ±0.250	83 a ±2.9
6	123.467 a ±4.167	6.343 f ±0.299	0.039 e ±0.003	3.477 e ±0.374	88 a ±2.9

- Values followed by the same letter do not differ significantly within each variable based on Duncan's multiple range test ($P \leq 0.05$).

Malondialdehyde *(MDA):* The results of the current study in Table (4-1) showed a significant increase in MDA levels in groups treated with cadmium chloride and lead

acetate compared to the control group. In the first group (control group), the MDA level was recorded at 34 nmol/ml. In the second group treated with a low dose of cadmium chloride (60 mg), it was 55 nmol/ml. The third group treated with a higher dose of cadmium chloride (120 mg) recorded 66 nmol/ml. The fourth group treated with lead acetate (600 mg) had a level of 76 nmol/ml. The fifth group treated with a high dose of lead acetate (1200 mg) showed 83 nmol/ml. The sixth group treated with a combination of cadmium chloride and lead acetate, with 60 mg of cadmium chloride and 600 mg of lead acetate, had a level of 88 nmol/ml.

The MDA level in the blood serum of male albino rats showed a statistically significant increase in all groups compared to the control group based on Duncan's multiple range test at a significance level of ($P \leq 0.05$). This indicates oxidative stress in the blood serum, which increases with the quantity of the pollutant substance. The higher the quantity of the substance, the higher the oxidative stress level, as MDA is a product of oxidation and is considered the best indicator of oxidative stress.

Superoxide Dismutase (SOD): The SOD values in Table (4-1) showed a significant decrease in groups treated with cadmium chloride and lead acetate compared to the control group based on Duncan's multiple range test at a significance level of ($P \leq 0.05$). In the first group, the control group, it was recorded at 10.220 μm/L. In the second group treated with a low dose of cadmium chloride (60 mg), it was 7.86 μm/L. The third group treated with a higher dose of cadmium chloride (120 mg) recorded 6.682 μm/L. The fourth group treated with lead acetate (600 mg) also recorded 6.682 μm/L. The fifth group treated with a high dose of lead acetate (1200 mg) showed 5.452 μm/L. The sixth group treated with a combination of cadmium chloride and lead acetate, with 60 mg of cadmium chloride and 600 mg of lead acetate, had a level of 3.477 μm/L. The decrease in SOD enzyme in the blood serum indicates oxidative stress as it is an internal antioxidant in the body of living organisms.

Catalase (CAT): The results of the current study in Table (4-1) showed a significant decrease in CAT values in groups treated with cadmium chloride and lead acetate compared to the control group based on Duncan's multiple range test at a significance level of ($P \leq 0.05$). In the first group (control group), it was recorded at 0.248 μm/L. In the second group treated with a low dose of cadmium chloride (60 mg), it was 0.121 μm/L. The third group treated with a higher dose of cadmium chloride (120 mg) recorded 0.101 μm/L. The fourth group treated with lead acetate (600 mg) had a level of 0.085 μm/L. The fifth group treated with a high dose of lead acetate (1200 mg) showed 0.073 μm/L. The sixth group treated with a combination of cadmium chloride and lead acetate, with 60 mg of cadmium chloride and 600 mg of lead acetate, had a level of 0.039 μm/L. The decrease in CAT enzyme in the blood serum indicates oxidative stress as it is an internal antioxidant in the body of living organisms.

Glutathione Peroxidase (GSH-Px): The GSH values in Table (4-1) showed a significant decrease in groups treated with cadmium chloride and lead acetate compared to the control group based on Duncan's multiple range test at a significance level of ($P \leq 0.05$). In the first group (control group), it was recorded at 13.682 μm/L. In the second group treated with a low dose of cadmium chloride (60 mg), it was 10.518 μm/L. The third group treated with a higher dose of cadmium chloride (120 mg) recorded 9.657 μm/L. The fourth group treated with lead acetate (600 mg) had a level of 8.251 μm/L. The fifth group treated with a high dose of lead acetate (1200 mg) showed 7.388 μm/L. The sixth group treated with a combination of cadmium chloride and lead acetate, with 60 mg of cadmium chloride and 600 mg of lead acetate, had a level of 6.343 μm/L. The decrease in the GSH enzyme in the blood serum, like other antioxidants, indicates oxidative stress as it is an internal antioxidant in the body of living organisms.

Oxidative Stress on DNA (Hydroxy-2-deoxyguanosin-8): The results of the current study in Table (4-1) showed a significant increase in 8-OHDG values in groups treated with cadmium chloride and lead acetate compared to the control group based on Duncan's multiple range test at a significance level of ($P \leq 0.05$). In the first group (control group), it

was recorded at 63.533 μm/L. In the second group treated with a low dose of cadmium chloride (60 mg), it was 81.067 μm/L. The third group treated with a higher dose of cadmium chloride (120 mg) recorded 91.767 μm/L. The fourth group treated with lead acetate (600 mg) had a level of 97.567 μm/L. The fifth group treated with a high dose of lead acetate (1200 mg) showed 107.633 μm/L. The sixth group treated with a combination of cadmium chloride and lead acetate, with 60 mg of cadmium chloride and 600 mg of lead acetate, had a level of 123.467 μm/L. The increase in 8-OHDG values in the blood serum indicates oxidative stress on DNA in the blood.

Testosterone Hormone: From the current study in Table (2), it is observed that the testosterone hormone decreased significantly based on the Duncan's multiple range test at a significance level of ($P \leq 0.05$) in the serum of male rats exposed to varying amounts of cadmium chloride and lead acetate. The decrease intensified as the quantity of the pollutant substance increased. In the control group, it was recorded at 1.843 ng/ml. In the second group, which was administered a low dose of cadmium chloride, it was 1.407 ng/ml. In the third group, which was administered a high dose of cadmium chloride, it was 1.220 ng/ml. In the fourth group, which was administered a low dose of lead acetate, it was 1.320 ng/ml. In the fifth group, which was administered a high dose of lead acetate, it was 1.077 ng/ml. In the sixth group, which was administered a combination of cadmium chloride and lead acetate, it was 1.007 ng/ml.

Table (2): Results of Male Sex Hormones.

Variable / Tretement	Testosterone (ng/ml)
1	1.843 a ±0.039
2	1.407 b ±0.012
3	1.220 c ±0.073
4	1.320 bc ±0.008
5	1.077 d ±0.084
6	1.007 d ±0.029

- Values followed by the same letter do not differ significantly based on Duncan's multiple range test ($P \leq 0.05$).

The use of cadmium chloride led to a decrease in testosterone hormone levels in the blood serum. This is consistent with many studies, including a study by Zhou *et al.* (2022) who administered cadmium chloride to male mice and observed its impact on offspring. Additionally, they noted a decrease in both testosterone and free cholesterol levels in the serum of treated male mice, an increase in total cholesterol, and a decrease in sperm concentration. Similarly, lead acetate resulted in a decrease in testosterone hormone levels. This is in line with several studies, such as the study by Bentaiba *et al.* (2023) which investigated the effect of lead acetate on male hormone levels in rats. They concluded that lead exposure causes tissue changes in the testes, reduces testosterone levels in the blood serum, and decreases sperm count. Cadmium reduces sperm motility, count, and relative weight, increases the percentage of dead sperm, sperm deformities, causes pathological tissue changes in the testes, increases oxidative products, and reduces total antioxidant levels in the testes, leading to decreased testosterone levels (Ibrahim *et al*., 2023).

The impact of lead acetate is clearly evident in the significant decrease in sperm count and motility, as well as in the levels of testosterone and luteinizing hormone in the blood. Additionally, lead acetate caused a significant increase in inflammatory markers of the testes, TNF-α, IL-1β, and TGFβ, accompanied by a decrease in AKT and mTOR levels.

Lead acetate also induced degenerative changes in the testes, atrophy, and loss of spermatic cords, leading to a decrease in testosterone levels (Elhemiely *et al*., 2023).

Effect of Cadmium Chloride and Lead Acetate on Sperm: When male albino rats were orally administered with varying concentrations of cadmium chloride and lead acetate for one month, and their harvested sperm was examined, we observed the effects of these pollutants leading to various deformities in the sperm. This effect varied depending on the contaminant and its concentration compared to the control group.

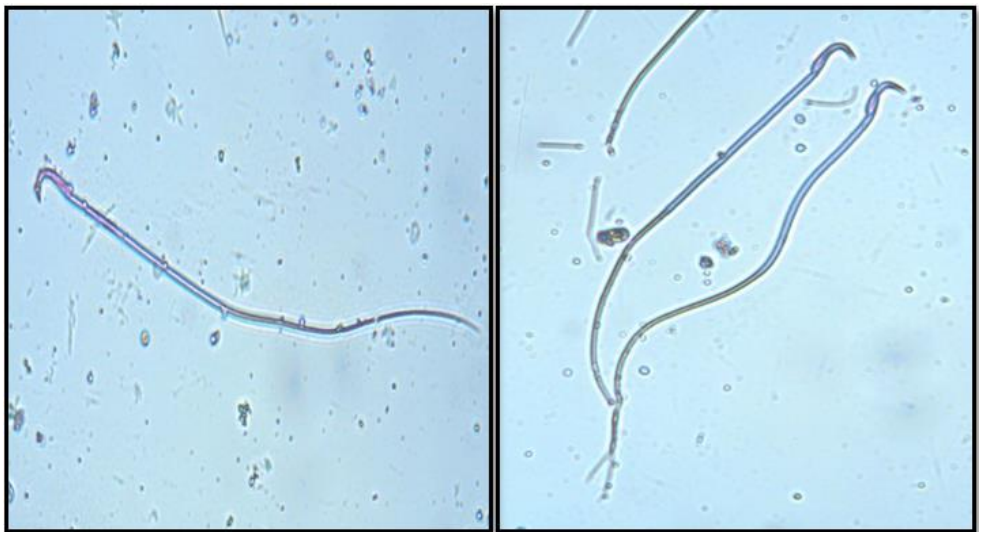

Image (1) illustrates the sperm of the control group of rats.

In image (1), we observe the sperm of the non-administered rats with any pollutants. There is no evidence of deformities in the central piece, the hook shape, or the tail extension.

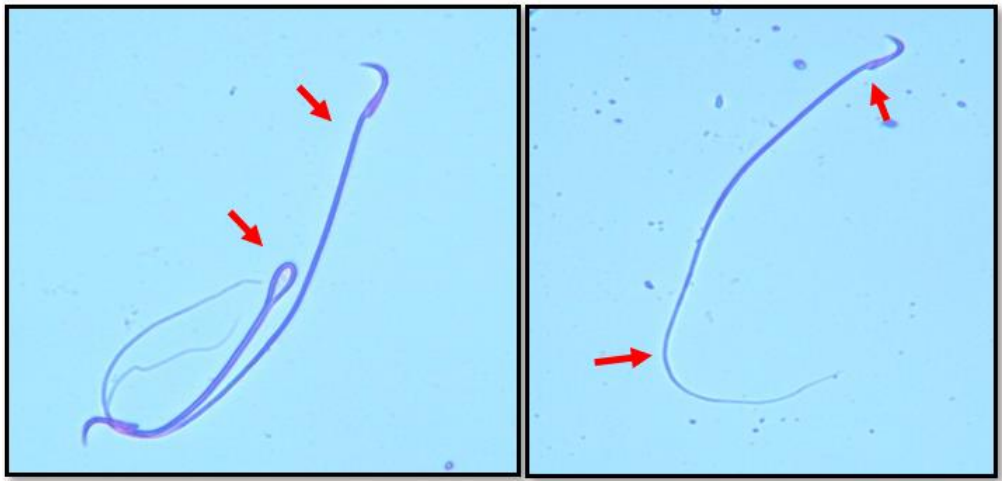

Image (2) illustrates the effect of 60 mg of cadmium chloride on the sperm of white rats.

In image (2), we observe the impact of cadmium pollutants on the sperm morphology. We notice the effect on the hook shape, as well as the fusion of the central piece, and the bending and coiling of the tail.

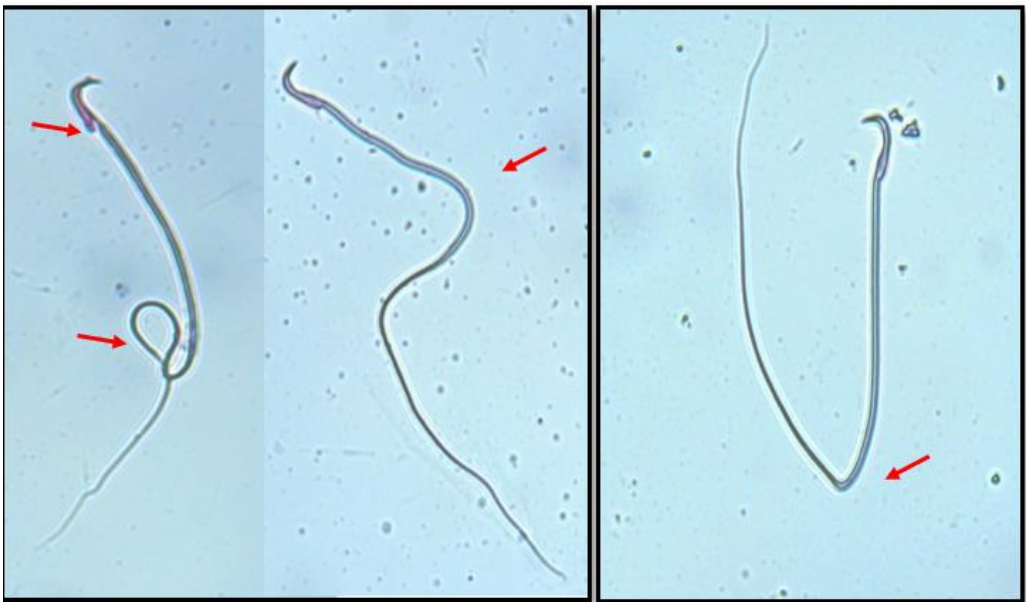

Image (3) illustrates the effect of 120 mg of cadmium chloride on the sperm of male white rats.

In image (3), we observe the impact of a high concentration of cadmium pollutants on sperm morphology. We notice the effect on the hook shape (head of the sperm), as well as the fusion of the central piece, changes in tail shape, and bending and coiling of the tail.

Image (4) illustrates the effect of 600 mg/kg of lead acetate on the sperm of male white rats.

In image (4), we observe the impact of 600 mg/kg of lead acetate on sperm morphology. We notice sperm with large heads, sperm with small heads, decapitated sperm, and sperm with fused central pieces.

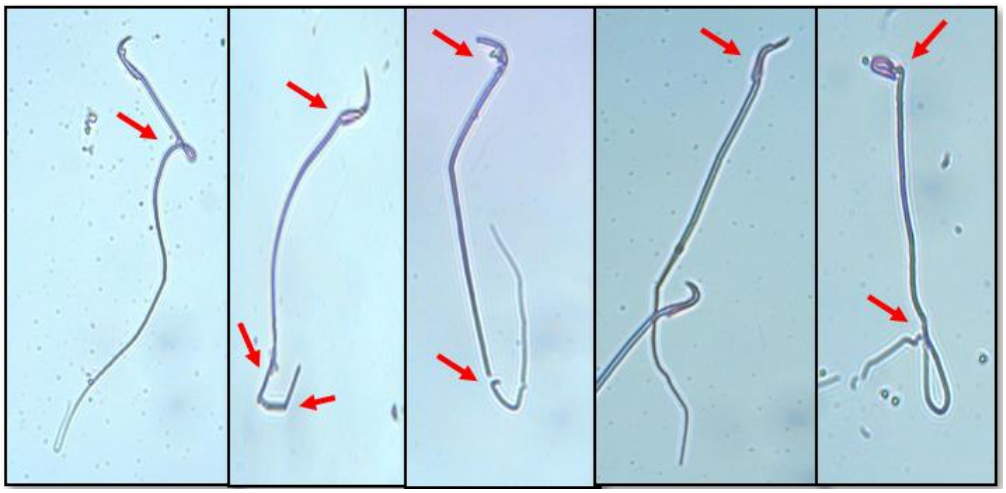

Image (5) illustrates the effect of 1200 mg/kg of lead acetate on the sperm of male white rats.

In image (5), we observe the impact of 1200 mg/kg of lead acetate on sperm morphology. Additionally, we notice sperm with large heads, sperm with small heads, decapitated sperm, sperm with fused central pieces, sperm with twisted central pieces, and sperm with twisted tails. The effect of lead acetate increases with the concentration of the substance.

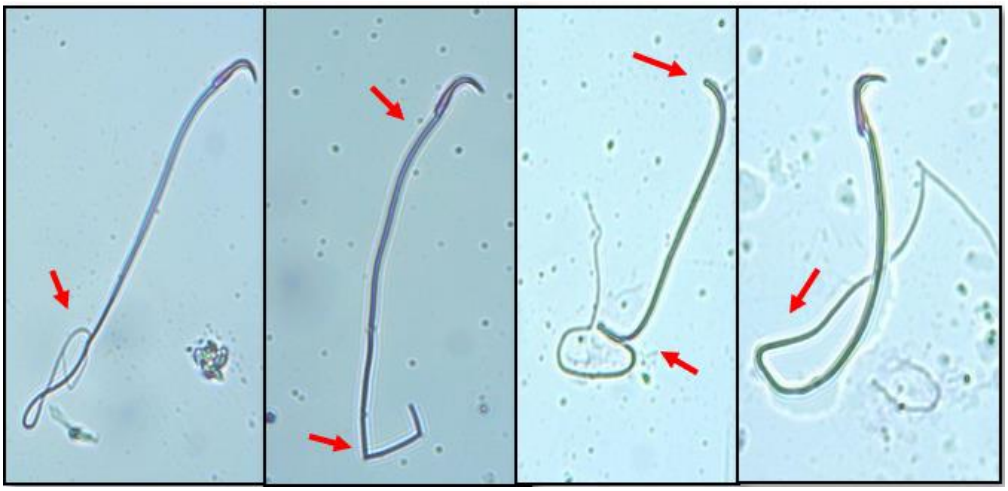

Image (6) illustrates the combined effect of 60 mg/kg of cadmium chloride with 600 mg/kg of lead acetate on the sperm of male white rats.

In image (6), we observe the combined impact of cadmium chloride and lead acetate on sperm morphology. We notice sperm with large heads, sperm with small heads, decapitated sperm with twisted tails, and sperm with fused central pieces.

Treating male white rats with cadmium chloride at a dose of 60 mg/kg of body weight led to significant changes in sperm characteristics compared to the control group. The effect increased with a dose of 120 mg/kg. Cadmium chloride, when administered to rats, reduces the testicular weight relative to body weight and significantly affects sperm parameters by reducing motility, viability, and increasing sperm deformities. It also leads to a decrease in testosterone levels and may result in infertility (El-Sherbiny *et al*., 2022).

Heavy metals, including cadmium, induce oxidative stress by generating reactive oxygen species, including hydroxyl radicals (OH-), superoxide radicals (O-), and hydrogen peroxide (H_2O_2). These reactive oxygen species cause damage to various vital molecules inside the cell, such as DNA, lipids, and proteins, leading to an increase in oxidative products. This results in changes in the sperm cell membranes, affecting sperm vitality and efficiency, which is one of the reasons for sperm deformities (Adeniyi and Paul, 2023).

Exposing male white rats to varying concentrations of cadmium chloride and lead acetate resulted in increased oxidative products in the blood serum and decreased antioxidants. The decrease in antioxidant concentration, the increase in oxidative products, and the rise in reactive oxygen species in the seminal fluid lead to an increase in sperm deformities. This could be due to the fact that the sperm plasma membrane contains high levels of unsaturated fatty acids, which are primary targets for reactive oxygen species. These reactive oxygen species act to degrade the lipid structure of the plasma membrane, leading to deformities in the sperm midpiece, tail coiling, and also affecting testosterone levels (Eyeghre *et al*., 2023).

Hormone deficiency leads to a decrease in fructose in the seminal fluid. Male hormone deficiency is associated with an increase in the production of reactive oxygen species that cause tissue damage to the testes due to exposure to cadmium (Li *et al*., 2016). The reason for the decrease in sperm count and the increase in deformed sperm in rats treated with cadmium chloride may be due to various effects on testicular tissue, such as the breakdown and appearance of blood vessels leading to interrupted blood supply, decreased sperm concentration, reduced motility, atrophy, and testicular degeneration (Zhou *et al*., 2022).

Exposure to cadmium leads to zinc deficiency and may occupy zinc sites in the cells composing the sperm, leading to a decreased sperm survival rate, sperm deformities, reduced sperm quality, alteration in substructure, and also an increase in programmed cell death in the testes (Liu *et al*., 2023). The primary cellular role and mechanism of zinc in sperm are its essential functions in sperm motility, capacitation, and acrosome reaction, which are three crucial functions for successful fertilization. The effect of zinc supplements on assisted reproductive technologies has also been described (Allouche-Fitoussi and Breitbart, 2020).

Lead acetate has been found to cause testicular toxicity, leading to decreased sperm motility, an increase in dead sperm ratio, increased oxidative stress, decreased antioxidants, increased testicular tissue inflammation, and programmed cell death, resulting in reduced sex hormones (Ileriturk *et al*., 2021). Lead acetate, when administered to white rats, led to a significant increase in MDA in the testes, nitric oxide, and lead ion. It also increased abnormal sperm while decreasing CAT and enzyme DOS activity in the testes. Additionally, it resulted in decreased calcium ion, zinc ion, testosterone hormone, sperm count, and motility, along with sperm deformities (Oyeyemi *et al*., 2022).

Exposure to lead leads to a decrease in sperm count and motility, an increase in sperm deformities, disruption of testicular morphology and structure, decreased expression of enzymes associated with steroid hormone synthesis, and a decrease in testosterone concentration in the blood. Additionally, it results in an increase in ROS, significantly elevating MDA oxidative products and decreasing antioxidants T-AOC, SOD, and GSH. Long-term exposure to low concentrations of lead acetate can negatively affect sperm quality and cause inflammatory damage through oxidative stress, leading to decreased testosterone levels and subsequent damage to the blood-testis barrier (BTB) tight junction structure, ultimately affecting sperm formation. Lead exposure results in various abnormal sperm forms, including biflagellate sperm, decapitated sperm, large-headed, small-headed, and sperm with fused midpieces, along with reduced sperm count (Zhao *et al*., 2023). The molecular mechanism of oxidative damage caused by lead in the testes includes increased total leukocytes and lymphocytes in the blood, elevated levels of pro-inflammatory cytokines TNF-α and IL-6, and anti-inflammatory cytokines, indicating that lead stimulates inflammatory responses (Ommati *et al*., 2022).

Oxidative and reductive imbalances severely affect male reproduction. Reactive oxygen species attack the double bonds within the structures of polyunsaturated fatty acids in sperm's outer membranes, leading to the breakdown of the sperm's outer membrane. Unsaturated fats turn into lipid peroxides, potentially reducing antioxidants crucial for male reproductive system mechanisms like SOD, CAT, and GSH, resulting in

an increase in MAD concentration, an end-product of oxidation, weakening sperm quality and DNA integrity. Elevated active oxygen species concentrations may result in immature sperm, deformed sperm, and the presence of white blood cells. Chemical pollutants and smoking are the primary causes of exposure (Sengupta *et al.*, 2024).

4. Conclusion

The current study concludes that chemical pollutants, specifically cadmium chloride and lead acetate, induced negative effects on the animals. These effects manifested as disturbances in the oxidative balance, leading to an increase in oxidative products and a decrease in antioxidants. This negatively impacted the male reproductive system, reducing the levels of male sex hormones and affecting sperm morphology when administered to males at different concentrations.

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