



Chromium (III) Chloride Partially preserves Testicular Morphology but fails to Attenuate Lead Acetate-Induced Oxidative Stress in Male Wistar Rats

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Abstract

The male gonads (testes) are the primary male reproductive organs that are very sensitive to environmental toxicants such as lead. This study investigated the influence of chromium (III) chloride (CrCl_3) on lead acetate (lead) induced testicular oxidative stress and inflammation in male Wistar rats. The study design involved twenty male Wistar rats randomly assigned to four groups ($n=5$) and treated orally for 45 days. The groupings are the control, CrCl_3 (0.3mg/kg), lead (15mg/kg), and CrCl_3 plus lead acetate. The treatments for all the groups were done orally for 45 days. The testicular antioxidant capacity and oxidative stress status were assayed spectrophotometrically, while testicular pro-inflammatory and anti-inflammatory markers were measured with ELISA. The obtained results indicate elevation of testicular oxidative stress, marked by increased malondialdehyde, hydroxyl, and superoxide radicals with reduced superoxide dismutase, catalase activities, and thiol level in lead-treated rats. Also, lead-induced testicular inflammation is evidenced by the elevation of pro-inflammatory and reduced anti-inflammatory markers with testicular histopathology alteration. The oxidative and antioxidant results of CrCl_3 are similar to the control, except for the hydroxyl radical. Also, CrCl_3 significantly increased pro-and anti-inflammatory cytokines evaluated and improved anti-inflammatory markers in the lead acetate group. The testicular morphology alteration caused by lead acetate was averted by the administration of CrCl_3 . The chromium (III) chloride could not lessen lead acetate-induced testicular oxidative stress and produced mixed immunomodulatory effects, increasing both pro-inflammatory and anti-inflammatory cytokines. Therefore, its anti-inflammatory role in this model should be interpreted cautiously.

Keywords: Antioxidant, Chromium, Reactive oxygen species, Interleukins.

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Introduction

The male reproductive tissues, being metabolically active, are vulnerable to some environmental and lifestyle stressors. The testicular tissue is highly prone to oxidative stress because of a high rate of cellular metabolism and continuous production of

spermatozoa, which are highly sensitive to reactive oxygen species (ROS), resulting in alteration of steroidogenesis and spermatogenesis (Asadi *et al.*, 2017). The testes employ a complex antioxidant defense system to counterbalance oxidative damage, protecting the cells against damage caused by

harmful substances like free radicals (Aitken and Roman, 2009). The testicular endogenous antioxidant system, including superoxide dismutase (SOD) and catalase (CAT), which prevent the formation of harmful hydroxyl radicals.

ROS are mainly produced through the mitochondrial oxidative phosphorylation and play essential roles in cellular signaling and homeostasis (Sies *et al.*, 2022). The stability between the antioxidant system and ROS generation is critical to prevent cellular damage. The common examples of ROS are hydrogen peroxide, hydroxyl radicals, and superoxide anions. Exogenous factors such as environmental pollutants, heavy metals, and ultraviolet (UV) light, ionizing radiation, and certain xenobiotics can induce ROS production (Bi *et al.*, 2024).

Heavy metals are metalloid or naturally metallic elements with high density; they are toxic even at low concentrations, posing various health risks (Jomova *et al.*, 2025). They are persistent environmental pollutants with known toxicological effects on biological systems. The various noxious health effects of contact with heavy metals are a matter of serious apprehension and a worldwide issue. They cause some health challenges due to their non-degradable nature and bioaccumulation in some soft tissues (Jomova *et al.*, 2025). Lead is a common example of heavy metals that is present in the environment via natural processes and human activities (Jomova *et al.*, 2025).

Lead is a persistent environmental pollutant owing to its widespread presence in industrial emissions, contaminated water, soil, and occupational settings (Raj and Das, 2023). It poses a significant threat to human health (Raj and Das, 2023). Despite global efforts to reduce its use, such as banning leaded gasoline and regulating lead-based paints, the exposure to lead is still high, predominantly in developing regions and industrialized areas with legacy contamination (Yu, 2025). It is used in a variety of products, including paints, pesticides, batteries, and ammunition (Yu, 2025).

Lead's toxicity arises primarily due to overwhelming endogenous antioxidant

defenses by the ROS production and oxidative stress (Lopes *et al.*, 2016). The male reproductive system, especially the testes, is vulnerable to lead toxicity, with well-documented effects on steroidogenesis, spermatogenesis, and testicular inflammation (Mukherjee *et al.*, 2022; Oyeyemi *et al.*, 2024). These effects compromise fertility, posing challenges for affected populations, particularly in high-risk occupational or environmental settings. Lead-induced ROS has been reported to affect sperm membrane fluidity, integrity, sperm DNA damage, impairing fertilization, protein oxidation, which leads to enzyme inactivation and altered signaling pathways leading to weakened sperm functions (Oyeyemi *et al.*, 2024; Wang *et al.*, 2025). Additionally, lead is known to cause testicular inflammation and damage through upregulation of pro-inflammatory markers (Zhao *et al.*, 2023). Concurrently, anti-inflammatory cytokines are suppressed, hindering the resolution of inflammation and prolonging testicular injury (Zhao *et al.*, 2023).

Chromium is a transition metal seen in multiple oxidation states. The trivalent chromium is the most stable and biologically relevant form, documented as an important trace element for human health (Vincent 2023). It is widely used as a supplement for humans and animals because of its importance in the metabolic process and involvement in various biochemical processes (Vincent, 2023). It is recognized as an essential micronutrient involved in lipid and glucose metabolism (Monga *et al.*, 2022). While trivalent chromium is essential at trace levels, it also poses a risk to male reproductive tissue at higher exposure (Pereira *et al.*, 2021).

Chromium (III) chloride (CrCl_3) is a trivalent chromium compound, which has antioxidant and anti-inflammatory properties that have been highlighted by recent studies to mitigate oxidative stress and inflammation across various tissues (Sahin *et al.*, 2018; Morvaridzadeh *et al.*, 2022). Studies have suggested that chromium supplements can reduce inflammatory markers and enhance antioxidant enzyme activity (Sheikhhossein *et al.*, 2020; Zhang *et al.*, 2021). However, its

potential to counteract lead-induced testicular inflammation remains largely unexplored. We hypothesized that chromium (III) chloride would attenuate lead acetate-induced testicular oxidative stress, inflammatory imbalance, and histological disruption in male Wistar rats.

Hence, this study investigated the effects of chromium (III) chloride and lead acetate on the testicular oxidative stress and inflammation in Wistar rats.

Materials and Methods

Chemicals

The CrCl_3 and lead acetate used are the products of Loba Chemie Pvt. LTD Mumbai, India, and Molychem India LLP, respectively.

Experimental animals

Twenty male Wistar rats with a weight between 150 and 170 g were obtained and acclimatized under normal laboratory conditions in the animal house, Osun State University, Osogbo, Nigeria. The animals were acclimatized over a period of two weeks. The animals had free access to feed and clean water throughout the study. They were handled in accordance with the standard guidelines for Experimental Animal care and handling (NIH, 1985).

Experiment design

The animals were randomly assigned to four groups, and each group contained five rats. The grouping and treatment are as follows:

- i). Control: The animals were given the vehicle (0.2 mL of water).
- ii). CrCl_3 : The animals were administered 0.3 mg/kg of CrCl_3 .
- iii). Lead acetate (Lead): The animals were exposed to 15 mg/kg of lead.
- iv). CrCl_3 +Lead: The group was administered with 0.3 mg/kg of CrCl_3 and 15 mg/kg of lead sequentially.

The vehicle, CrCl_3 , and lead acetate were administered once daily by oral gavage for 45 days. The dose of CrCl_3 represented a nutritional recommendation, while lead was based on the subacute toxicity dose in accordance with previous studies (Lebedev *et al.*, 2018; Oyeyemi *et al.*, 2025).

The animals were sacrificed, and the testes were harvested after exposure to CrCl_3 and lead. The left testes were homogenized in

phosphate-buffered saline with pH 7.4 in a ratio of 1:5 (w/v) and centrifuged at 10,000 revolutions per minute (RPM) for 20 minutes with a cold centrifuge at -20°C . The testicular supernatants were used for the determination of oxidative/antioxidant and inflammatory markers. The right testes were fixed in Bouin's fluid for testicular histology. The biochemical analyses and histological assessment were blinded to treatment groups.

Malondialdehyde evaluation

The testicular malondialdehyde level was determined as thiobarbituric acid reactive substances (TBARS) as previously reported (Dera and Abushouk, 2015).

Hydroxyl radical assay

Testicular hydroxyl radical was evaluated spectrophotometrically according to an earlier study (Halliwell and Gutteridge, 2018).

Superoxide radical assay

Testicular superoxide radical level was measured using the cytochrome c method as previously reported (Anitha *et al.*, 2013).

Superoxide dismutase activity assay

The principle of pyrogallol autoxidation was used for the determination of the testicular superoxide dismutase activity (Gao *et al.*, 1998).

Determination of testicular catalase activity

A spectrophotometric method was used for the catalase activity determination (Aebi, 1984).

Thiol assay

The level of thiol in testes was measured according to Ellman's method as reported (Simpson 2008).

Anti-and pro-inflammatory markers evaluation

The anti-inflammatory cytokines (interleukins 4 and 10) and pro-inflammatory (interleukins 6 and 8) were measured using an enzyme-linked immunosorbent assay. Elabscience ELISA kits with catalog numbers Rat IL-4 (E-EL-R0014), IL-6 (E-EL-R0015), and IL-10 (E-EL-R0016), and human IL-8 (E-EL-H6008) were used. The manufacturer's manual procedure was strictly followed. Cytokine concentrations were normalized to total protein and expressed as pg/mg protein.

Testicular histology

The testicular sections were fixed in Bouin's fluid. The fixed tissues were dehydrated in the

graded ethanol solution and rinsed with water. The tissues were embedded with paraffin. The testicular sections were dewaxed in xylene for fifteen minutes. Each section was placed on a slide, and all the slides were hydrated in graded ethanol and Carnoy's solution. The fixed slides were rinsed with deionized water. The testicular tissue was stained with hematoxylin and eosin. The stained slides were carefully rinsed with tap water. The coverslips were mounted onto the stained slides with DPX and examined under a microscope at 200 magnification, and a digital camera (Omax 18.0MP) was used for the photomicrograph (Oyeyemi *et al.*, 2024).

Statistical Analysis

Data collected were analyzed using GraphPad Prism 8.0.1 (244). The data were subjected to a normality test using the Shapiro-Wilk test, and the data were normally distributed ($p > 0.05$). The data were analyzed using one-way ANOVA followed by the Tukey-Kramer test for multiple comparisons. Results were expressed as mean $\pm$ SEM (standard error of mean) in graphs, and the level of significance was less than 0.05.

Results

Testicular malondialdehyde, hydroxy radical, and superoxide radical levels

The results for testicular malondialdehyde level, hydroxyl radical, and superoxide radical are presented in Figure 1. Figure 1A shows that testicular malondialdehyde levels (μM) in the control (3.9 ± 0.57) and CrCl_3 group (4.1 ± 0.41) are similar, while a significant increase was observed in the lead group (7.4 ± 0.60) and CrCl_3 +lead group (7.2 ± 0.32) relative to the control group. Also, the level of malondialdehyde increased ($p < 0.05$) in the lead group compared to CrCl_3 -exposed rats.

The testicular hydroxyl radical level (nmol/L) increased greatly ($p < 0.05$) in the CrCl_3 group (1.6 ± 0.11), lead group (0.8 ± 0.04), and CrCl_3 +lead group (0.9 ± 0.03) compared to the control group (0.3 ± 0.03). A significant reduction ($p < 0.05$) was observed in the lead group when compared with the CrCl_3 group (Figure 1B).

Figure 1C shows the result for the effect of chromium (III) chloride and lead acetate on testicular superoxide radical level in male Wistar rats. The testicular superoxide radical levels (μL) in the control (13.3 ± 0.88) and CrCl_3 group (15.4 ± 0.18) are comparable, whereas significantly higher level of superoxide radical was observed in the lead group (21.9 ± 0.53) and CrCl_3 +lead group (19.3 ± 2.08) relative to the control group. Also, the level of superoxide radical was amplified ($p < 0.05$) in the lead group compared to CrCl_3 -treated rats.

Testicular superoxide dismutase, catalase, activities, and thiol level

Figure 2 demonstrated the effects of Chromium (III) chloride and lead acetate on testicular superoxide dismutase activity, catalase activity, and thiol level in male Wistar rats. The testicular superoxide dismutase activities (Figure 2A) in the animals exposed to lead ($5.0 \pm 0.30 \text{ U/mL}$) and CrCl_3 +lead ($5.6 \pm 0.34 \text{ U/mL}$) are comparable and significantly lower compared to the control group ($8.5 \pm 0.41 \text{ U/mL}$). Also, the superoxide dismutase activity was reduced in the lead group compared to the CrCl_3 group ($7.1 \pm 0.39 \text{ U/mL}$).

Figure 2B illustrates that the testicular catalase activities ($\mu\text{mol/mL/min}$) in the control animals (12.1 ± 0.69) and the CrCl_3 group (12.6 ± 0.40) are similar. A great reduction ($p < 0.05$) in the testicular catalase activity was observed in the lead group (4.9 ± 0.23) and the CrCl_3 +lead group (6.1 ± 0.16) compared to the control group. Also, a significant reduction ($p < 0.05$) was observed in the lead group when compared with the CrCl_3 group.

The thiol levels ($\mu\text{mol/L}$) in the control group (15.7 ± 0.81) and CrCl_3 group (15.9 ± 0.63) are insignificantly different ($p > 0.05$). However, the thiol level significantly reduced in the lead group (10.1 ± 0.62) and CrCl_3 +lead group (11.8 ± 0.40) relative to the control group. Also, the level of thiol was reduced ($p < 0.05$) in the lead group compared to the CrCl_3 group (Figure 2C).

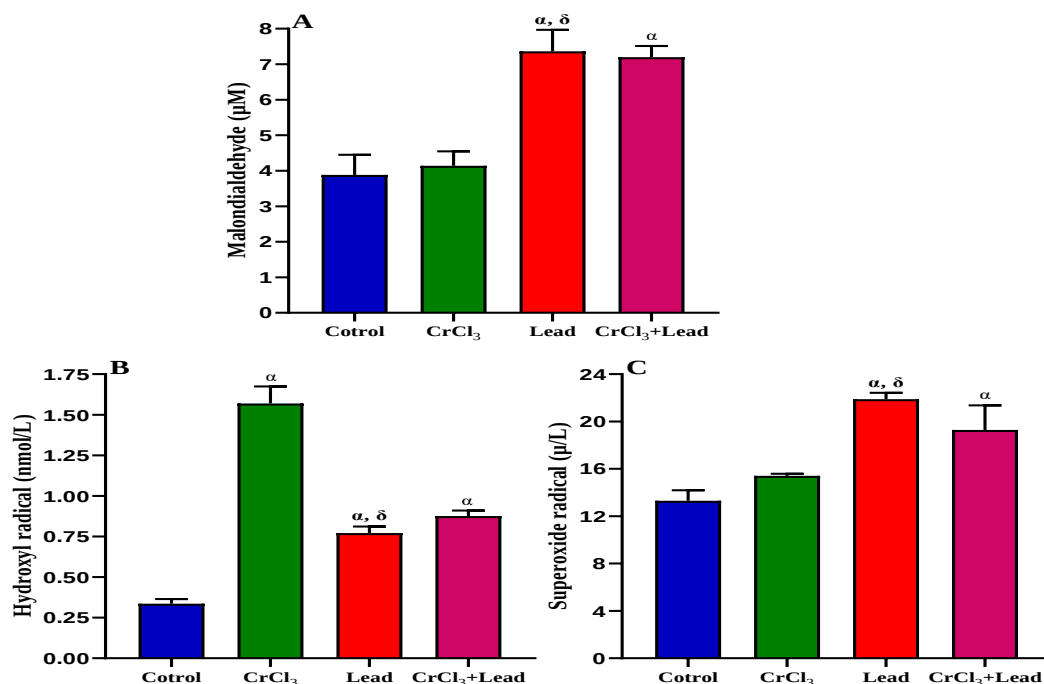


Figure 1: Effects of chromium (III) chloride and lead acetate on testicular oxidative markers (A) malondialdehyde level, (B) hydroxyl radical, and (C) superoxide radical level in male Wistar rats.

Bars represent mean ± SEM, n=5 in each group. ^ap<0.05 was significant compared with control, ^δp<0.05 considered significant relative to CrCl₃.

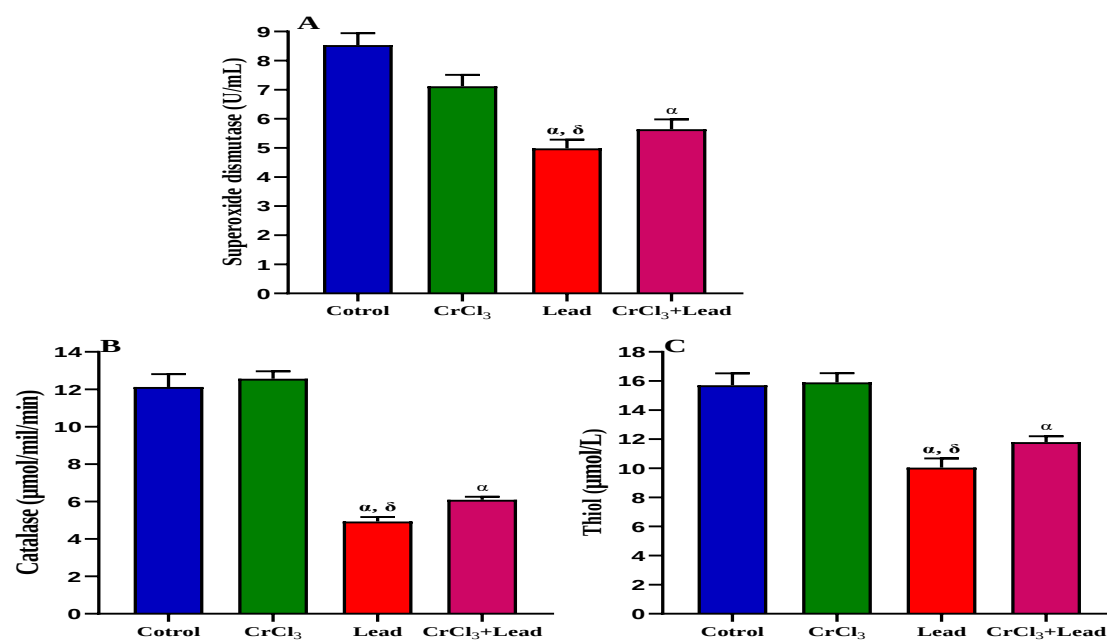


Figure 2: Effects of Chromium (III) chloride and lead acetate on testicular (A) superoxide dismutase activity, (B) catalase activity, and (C) thiol level in male Wistar rats.

Bars represent mean ± SEM, n=5 in each group. ^ap<0.05 was significant compared with control, ^δp<0.05 considered significant relative to CrCl₃.

Testicular interleukin-6 and interleukin-8 levels

Figure 3 depicts the effects of chromium (III) chloride and lead acetate on testicular interleukin-6 and interleukin-8 levels in male Wistar rats. The testicular interleukin-6 level (Figure 3A) increased significantly in the CrCl₃ group (214.3 ± 12.93 pg/mL) and the CrCl₃+lead group (185.3 ± 16.15 pg/mL) compared with the control (105.3 ± 3.23 pg/mL). The level of interleukin-6 in the lead

group (134.6 ± 7.62 pg/mL) is lower ($p < 0.05$) compared to the CrCl₃ group, while a significant increase was recorded in the CrCl₃+lead group relative to the lead group. Figure 3B demonstrates that the testicular interleukin-8 (pg/mL) level is significantly higher in the animals exposed to CrCl₃ (28.5 ± 0.97), lead (20.1 ± 0.86), and CrCl₃+lead (19.6 ± 1.02) versus the control group (6.6 ± 0.71). The level of interleukin-8 in the lead group is lower ($p < 0.05$) than in the CrCl₃ group.

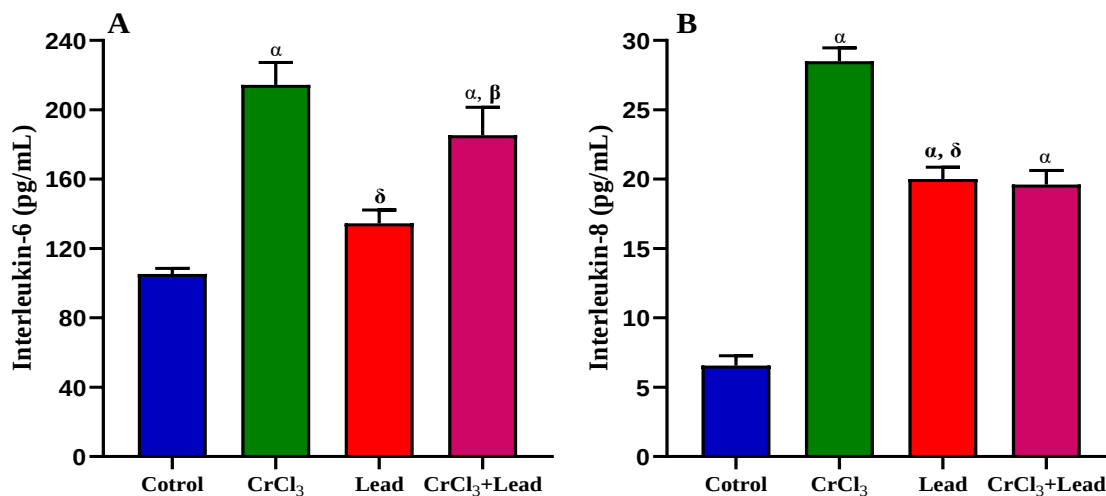


Figure 3: Effects of chromium (III) chloride and lead acetate on testicular (A) interleukin-6 and (B) interleukin-8 levels in male Wistar rats.

Bars represent mean $\pm$ SEM, $n=5$ in each group. $^{\alpha}p < 0.05$ was significant compared with control, $^{\delta}p < 0.05$ considered significant relative to CrCl₃, $^{\beta}p < 0.05$ considered significant compared to lead.

Testicular interleukin-4 and interleukin-10 levels

Figure 4 shows the effects of chromium (III) chloride and lead acetate on testicular interleukin-4 and interleukin-10 levels in male Wistar rats. The testicular interleukin-4 level (Figure 4A) is higher ($p < 0.05$) in CrCl₃ (225.2 ± 20.30 pg/mL) compared to the control group (102.8 ± 10.21 pg/mL). Also, testicular interleukin-4 level decreased ($p < 0.05$) in the animals treated with lead (64.7 ± 3.66 pg/mL) versus the CrCl₃ group, while elevation

($p < 0.05$) was seen in the CrCl₃+lead (116.8 ± 3.84 pg/mL) group compared to the lead group.

Figure 4B indicates elevation ($p < 0.05$) in testicular interleukin-10 (pg/mL) level of CrCl₃ group (7.5 ± 0.51) compared to the control group (5.4 ± 0.40), while a significant reduction was recorded in the lead group (3.4 ± 0.51) versus the control. The level of interleukin-10 (pg/mL) increased significantly in the CrCl₃+lead group (5.8 ± 0.29) compared with the lead group.

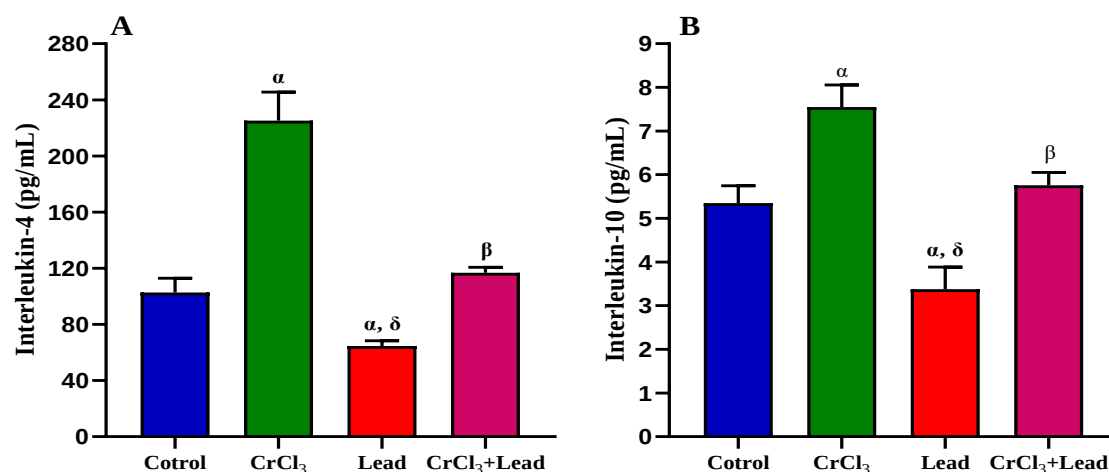


Figure 4: Effects of Chromium (III) chloride and lead acetate on testicular anti-inflammation (A) interleukin-4 and (B) interleukin-10 level in male Wistar rats.

Bars represent mean $\pm$ SEM, $n=5$ in each group. ^α $p<0.05$ was significant compared with control, ^δ $p<0.05$ considered significant relative to CrCl₃, ^β $p<0.05$ considered significant compared to lead.

Testicular histology

The control group showed normal seminiferous tubules with normal interstitial spaces, Leydig cells (White arrow), spermatogonia cells (SG), germ cells layer (black thin spanned) with normal maturation stages, the lumen appeared normal with the presence of spermatozoa (SP). There are a few distortions in the germ cell layer.

The Chromium (III) chloride (0.3 mg/kg) group shows an interstitial space, Leydig cells (White arrow), seminiferous tubules with normal spermatogonia cells (SG), germ cell layer (black spanned) with normal maturation

stages, the lumen appears normal with the presence of spermatozoa (SP).

The lead (15 mg/kg) group showed some abnormal seminiferous tubules with distorted spermatogonia (SG); there are some tubules with a lack of germ cells (black spanned), maturation arrest, and their lumen appeared wide with a lack of spermatozoa (SP).

Chromium III chloride + Lead group showed normal interstitial space, seminiferous tubules with normal spermatogonia cells (SG), normal Sertoli cells, and normal germ cell layer (black spanned) with normal maturation stages; the lumen appeared normal with the presence of sperm cells (SP).

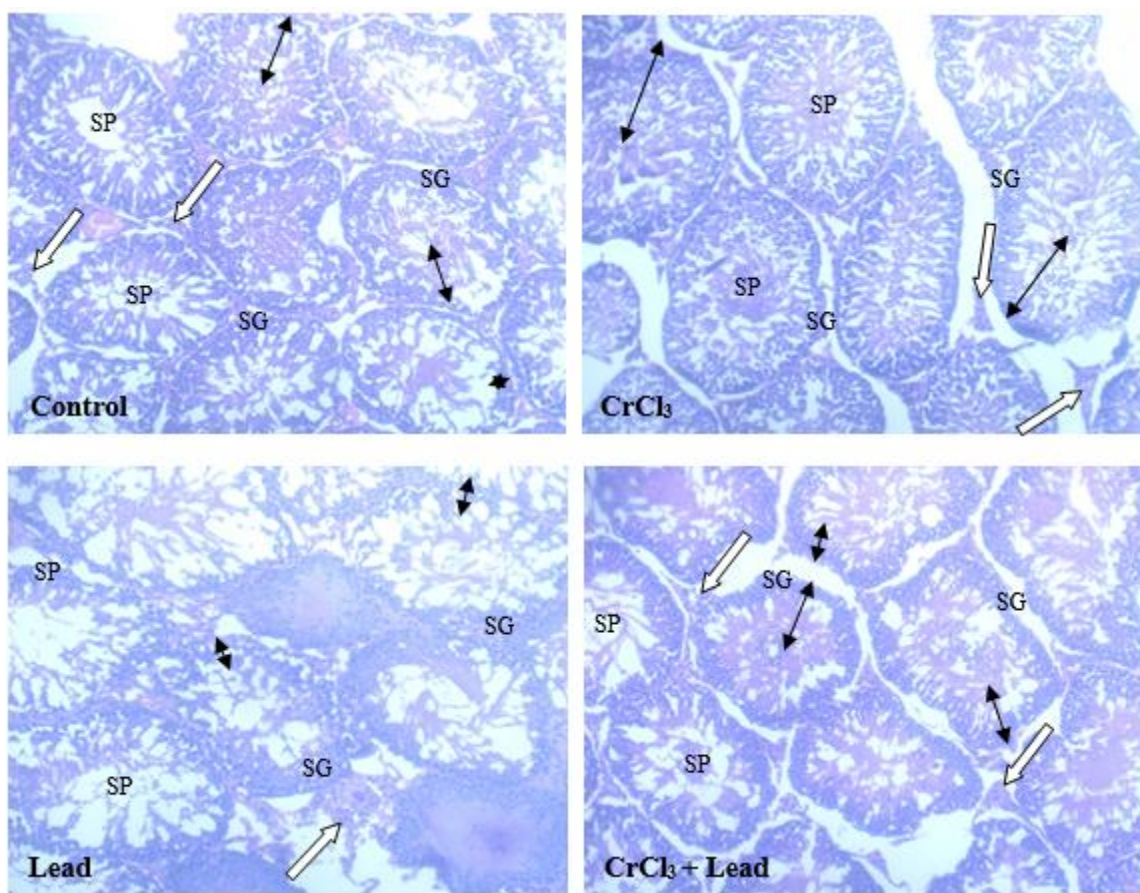


Figure 5: Effects of chromium (III) chloride and lead acetate on testicular histology of male Wistar rats. (H&E stained) $\times 200$

Discussion

Lead acetate is well documented to cause testicular damage via aggravated oxidative stress and reduced endogenous antioxidant enzyme activities (Mukherjee *et al.*, 2022; Oyeyemi *et al.*, 2024). Studies have reported that hexavalent chromium induced testicular toxicity through destruction of the testicular antioxidant defense system (Navin and Aruldas, 2021; Li *et al.*, 2023). While studies on trivalent chromium effects on testicular functions are limited, trivalent chromium has been reported to possess modest antioxidant effects, especially at very low quantities (Monga *et al.*, 2022). Hence, this study focused on the effects of chromium chloride and lead acetate on the testicular antioxidant system and inflammation.

Malondialdehyde (MDA) is a useful indicator of oxidative injury at the molecular level (Singh *et al.*, 2014). The result of this current study indicates a surge in testicular MDA level

in the group exposed to lead and lead with CrCl_3 . The observed increase in the testicular MDA by lead is similar to previous studies (Abdel Moneim, 2012; Oyeyemi *et al.*, 2025). This is an indication that lead acetate might facilitate hydroxyl radical and other reactive oxygen species to attack testicular membrane lipids, which may result in elevating testicular MDA. The increased MDA level by lead acetate signifies possible testicular damage. The CrCl_3 treatment in this study did not exhibit an elevation in testicular MDA level, which indicates that CrCl_3 might avert testicular oxidative stress (Monga *et al.*, 2022). This observation might be due to the property of CrCl_3 in regulating glucose metabolism because impaired glucose metabolism is known to cause mitochondrial dysfunction and the generation of excess reactive oxygen species (Monga *et al.*, 2022). Although CrCl_3 could not avert the increased testicular MDA level in the lead acetate group,

this might be due to its moderate antioxidant property that has been proposed.

The hydroxyl radical can induce damage to almost all types of biomolecules, including lipids, proteins, and DNA, via oxidative stress (Chandimali *et al.*, 2025). Lead acetate has been severely reported to generate free radicals such as hydroxyl radical (Ding *et al.*, 2000; Patrick, 2006). The current study shows a significant increase in testicular hydroxyl radical levels in Wistar rats exposed to lead acetate. This observation supported the elevation of testicular MDA seen in this study and is an indication of lead acetate induced testicular oxidative damage via elevation of oxidative stress markers. Also, surprising rise in the testicular hydroxyl radical level was seen in the CrCl_3 treated animals than that of lead acetate group. Although excessive CrCl_3 exposure can cause elevation in free radical but moderate dose of CrCl_3 was used in this study.

Superoxide radical is a primary ROS generated mainly in the mitochondria during oxidative phosphorylation (Dröse and Brandt, 2012). The result of the testicular superoxide radical level in this study is similar to the MDA level result. This observation illustrates a significant increase in the testicular superoxide radical level of rats exposed to lead acetate. The increase in the superoxide radical level might be due to lead acetate inducing oxidative phosphorylation in the testicular mitochondria. The superoxide radical level in the CrCl_3 group is lower than that of the lead acetate-treated group. This is an indication that CrCl_3 might avert the elevation of superoxide radical via inhibition of oxidative phosphorylation and its antioxidant property (Monga *et al.* 2022). CrCl_3 did not significantly increase MDA or superoxide radical levels but significantly increased hydroxyl radical levels, suggesting a selective pro-oxidant signal at the dose tested.

Superoxide dismutase and catalase are important endogenous antioxidant enzymes that protect cells from ROS and oxidative damage. The current study indicated that the superoxide dismutase and catalase activities in the control and CrCl_3 -exposed animals are similar, while a significant reduction was

recorded in the lead and lead co-administered with CrCl_3 . The testicular reduction in these antioxidant enzyme activities in lead acetate-treated rats is in agreement with a previous study (Oyeyemi *et al.*, 2024; Costa *et al.*, 2025). Their reduction by lead acetate can be linked to the elevation in testicular MDA, hydroxyl, and superoxide radicals recorded in this study. Despite the modulation of testicular superoxide dismutase and catalase activities in the CrCl_3 group, the CrCl_3 could not avert the reduction in testicular superoxide dismutase activity observed in lead-exposed rats. This observation is an indication of the weak antioxidant capacity of CrCl_3 , which could not prevent lead acetate-induced testicular oxidative stress.

Thiol (-SH groups) are sulfur-containing compounds that serve as major cellular antioxidants that can maintain redox homeostasis and protect cells from oxidative damage (Poole, 2015). In this study, the testicular thiol level reduced significantly following lead acetate exposure. This result is similar to earlier reports (Dobrakowski *et al.*, 2014; Oyeyemi *et al.*, 2025). The decrease in thiol level observed in the lead acetate group can be used to support the elevation in MDA, which has an inverse relationship with the thiol (Bergsma *et al.*, 2022). In the lead acetate-treated rats, the high MDA, hydroxyl, and superoxide radicals, reduced superoxide dismutase and catalase activities may be responsible for the lower thiol level recorded. The CrCl_3 administration maintained the testicular thiol level, although the level is not beyond that of control animals. The CrCl_3 co-administration with lead acetate could not reverse the reduction in thiol level caused by lead acetate.

Pro-inflammatory markers such as interleukin-6 and -8 usually trigger and intensify the immune system's inflammatory response. Interleukin-6 and -8 play a multifaceted role in the inflammatory response to toxicant exposure by activating the NF- κ B pathway and increasing ROS production (Dutta *et al.*, 2021; Yadav and Mali, 2024). This study shows that administration of lead acetate increased both interleukin-6 and -8. The observed elevation

of the cytokines is an indication of testicular inflammation induced by lead acetate. Previous studies have reported significant elevation of pro-inflammatory cytokines in the lead-exposed Wistar rats, reflecting a robust inflammatory response driven by lead (Su *et al.*, 2021; Arab *et al.*, 2024). Unexpectedly, the testicular level of pro-inflammatory markers evaluated increased in the CrCl₃-treated group compared to the lead acetate group. Previously, it has been reported that CrCl₃ possesses anti-inflammatory potential, especially in diabetic model rats by lowering TNF- α and IL-6 via glucose metabolism (Moradi *et al.*, 2019; Georgaki *et al.*, 2024). The elevation of pro-inflammatory cytokines following CrCl₃ administration might be due to the immunostimulatory effects of CrCl₃, which can be supported by the previously reported increased inflammatory cytokines (Dworzański *et al.*, 2021). This study also recorded possible testicular inflammation and hyperimmune response due to the elevation of IL-6 and IL-8 in animals co-treated with lead acetate and CrCl₃ in this study.

Interleukin-4 and IL-10 are crucial anti-inflammatory markers. They exert anti-inflammatory effects by impeding the production of pro-inflammatory markers (Wang *et al.*, 2019). This study recorded a reduction in the testicular IL-4 and IL-10 levels of lead acetate-exposed rats. The suppression of these cytokines by lead acetate is an indication of disruption of the testicular anti-inflammatory regulatory balance, which might cause unchecked inflammation and cause testicular damage (Bidanchi *et al.*, 2022; Khafaji, 2023). The CrCl₃ has been reported to demonstrate anti-inflammatory potential via antioxidant activities, inhibition of NF- κ B, reduction in C-reactive protein and TNF- α , especially in a diabetic animal model (Zheng *et al.*, 2023). CrCl₃ produced a mixed cytokine profile, with elevation of both pro-inflammatory and anti-inflammatory cytokines. This suggests immunomodulation rather than straightforward anti-inflammatory protection.

This current study demonstrated that administration of lead acetate for six weeks caused some testicular anomalies, especially

in the seminiferous tubules with distorted germ cell layers, maturation arrest, and their lumen appearing wide with a lack of spermatozoa. The observations are consistent with the well-documented reproductive toxicity of lead acetate on testicular morphology (Besong *et al.*, 2024; Oyeyemi *et al.*, 2024). Previous studies have reported that lead acetate disruption of seminiferous tubules is categorized by atrophy, vacuolation, reduced tubular diameter, and spermatogenic cells, which impair spermatogenesis (Besong *et al.*, 2024; Oyeyemi *et al.*, 2024). The observed testicular distortion could be ascribed to lead acetate inducing oxidative stress, reduced antioxidant activities, enhancing pro-inflammatory and reducing anti-inflammatory cytokines reported in this study. Previous studies have attributed lead acetate-induced oxidative stress and inflammation to testicular damage (Besong *et al.*, 2024). The current study shows that the testicular morphology of CrCl₃-exposed Wistar rats is similar to that of the control animals. The interstitial space, Leydig cells, seminiferous tubules, and germ cell layer of the CrCl₃ and control groups are normal. These observations can be supported by the reduced oxidative stress markers, increased antioxidant activities, and anti-inflammatory markers evaluated in this study. Furthermore, the CrCl₃ prevents lead acetate-induced testicular damage, which is evidenced by normal interstitial space, seminiferous tubules with normal spermatogonia cells, normal Sertoli cells, and normal germ cell layer. The apparent histological preservation by CrCl₃ in the lead group, despite persistent oxidative stress in the group, may reflect partial structural protection.

In conclusion, this study shows that lead acetate exposure induced testicular oxidative stress, antioxidant depletion, inflammatory imbalance, and histological disruption in male Wistar rats. Chromium (III) chloride did not significantly attenuate lead acetate-induced oxidative stress, as MDA, superoxide radical, SOD, catalase, and thiol indices remained altered in the co-treated group. However, chromium (III) chloride partially restored anti-inflammatory cytokines and appeared to

preserve testicular morphology. Notably, chromium (III) chloride alone increased hydroxyl radical, IL-6, IL-8, IL-4, and IL-10, indicating a mixed redox and immunomodulatory profile. Therefore, chromium (III) chloride should not be described as fully protective in this model; rather, its effects appear partial, tissue-structural, and cytokine-dependent. Further studies with larger sample sizes, reproductive hormone assays, sperm analysis, metal bioaccumulation data, and quantitative histomorphometry are required.

Conflict of interests

The authors declare no conflict of interest in this study.

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