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Original Investigation

Cadmium-Induced Adverse Alteration of Reproductive Parameters and Testicular Histoarchitecture of Wistar Rats: Protective Role of Palmitic Acid

Adeniran O. Akinola[1*], Wahab A. Oyeyemi[2], Dayo R. Omotoso[3], Oore-Oluwapo O. Daramola[4], Victor O. Emojevwe[1], Eunice Ogunwole[1], Blessing A. Adetula[1], Oluwatobiloba D. Adeleke[1]

[1]*Department of Physiology, University of Medical Sciences, Ondo City, Ondo State, Nigeria* | [2]*Department of Physiology, Osun State University Osogbo, Osun State, Nigeria* | [3]*Department of Human Anatomy, Redeemer's University, Ede, Osun State, Nigeria* | [4]*Department of Physiology, Redeemer's University, Ede, Osun State, Nigeria*

***Corresponding author:**
Adeniran O. Akinola
aakinola@unimed.edu.ng

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ABSTRACT

Cadmium (Cd) generates reactive oxygen species (ROS), leading to oxidative stress and adversely affecting reproductive organs and functions. Conversely, palmitic acid (PA) is a free radical scavenger naturally found in palm oil and consumed in diets. This study investigated the protective role of PA against cadmium-induced adverse alteration of male Wistar rats' reproductive parameters. Twenty mature male Wistar rats were grouped into four (n=5/group): 0.2 mL of 10% Tween 80 (Control); 2 mg/kg bw-Cd; 2 mg/kg bw - Cd+200 mg/kg bw PA; and 200 mg/kg bw PA. PA was given via oral route daily for thirty days, while a single dose of Cd was intraperitoneally given. Serum testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH); testicular concentrations of magnesium, calcium, zinc, and cadmium; epididymal sperm parameters (morphology, motility, viability, and counts) were evaluated, and the testicular histoarchitecture was assessed using standard techniques. Data obtained were statistically analysed and compared using ANOVA at $p < 0.05$. In comparison to the control group, Cd exposure significantly decreased serum testosterone, FSH, and LH levels; testicular magnesium, calcium and zinc; sperm motility, viability and counts. In addition, cadmium exposure resulted in abnormal sperm morphology and distorted testicular histoarchitecture. The co-administration of Cd and PA in group 3 showed significant reversal of the adverse effect of Cd on reproductive hormones, electrolytes, sperm parameters and testicular histomorphology. Hence, palmitic acid exhibits a protective effect against cadmium-induced adverse alterations of reproductive parameters and testicular histoarchitecture in Wistar rats.

INTRODUCTION

One of the most common environmental contaminants is Cadmium (Cd), which is characteristically harmful to different organs of the body (Gunnarsson et al., 2003). The substantial sources of Cd contact include occupation (production of batteries, mining), seafood or water, industrial activities (melting, purifying of metals), burning of metropolitan waste materials, as well as smoking of tobacco (Marini et al., 2022). Exposure to Cd could lead to a sequence of damaging effects like dysfunction of the liver, osteomalacia, oedema of the lungs, and testicular and renal damage as a result of its accumulation in different organs of the body in animals and humans (Arroyo et al., 2012).

Cd toxicity results in impairment of reproductive activity, which is characterised by testicular degeneration and necrosis induced by oxidative stress (Fouad et al., 2009). Akinola et al. (2020) reported that Cd exposure in rats caused significant degeneration of reproductive functions through oxidative stress, causing testicular histological alteration and impaired sperm parameters.

Cd toxicity in male reproduction has been reported to occur by its interference with metals like zinc, calcium, and magnesium, which are key elements in the functioning of sperm cells (Martelli et al., 2006; Bertelsmann et al., 2007). The study by Akinola et al. (2021) further reported that Cd caused the diminution of hydroxysteroid dehydrogenase, which is a major enzyme that plays a crucial role in regulating testicular androgenesis.

Palmitic acid (PA; hexadecanoic acid) is a saturated fatty acid that forms a major component of palm oil, which is a widely produced edible oil with well-documented nutritional and medicinal values (Sutapa and Analava, 2009). Numerous saturated and unsaturated fats, including glyceryl laurate, linoleate, myristate, oleate, linoleate, stearate, and palmitate, are found in palm oil (Cottrell, 1991). It is also rich in carotenoids, which function as antioxidants that protect cells from the detrimental actions of free radicals (Imoisi et al., 2015; Elham et al., 2016). Our previous study demonstrated that PA is one of the active compounds present in medicinal plants such as *Carpolobia lutea* (Akinola et al., 2022). The possible medicinal value of PA could characterise it as a choice target for the development of drugs for various pathological conditions. Thus, the present study was designed to assess the protective role of PA against Cd-induced alteration of reproductive parameters and testicular histoarchitecture in Wistar rats.

MATERIALS AND METHODS

Chemicals/Reagents

Cadmium chloride and palmitic acid were obtained from Loba Chemie, PVT. Limited, Mumbai, India and Santa Cruz laboratories, Dallas, Texas, USA, respectively.

Experimental Animals

Twenty male Wistar rats weighing 150 - 170 g were sourced and housed at the Animal House of the University of Medical Science, Ondo State, throughout the study. They were exposed to natural light/dark cycles and granted free access to pellet feed and water. The ethical approval for the study was granted by the Research Ethical Committee for Laboratory Animals Research, University of Medical Sciences (NHREC/TR/UNIMED-HREC-Ondo St/22/06/21).

Experimental Design

The animals were divided randomly into four groups (n=5) and were treated as follows: Group 1 (Control) was given 0.2 mL of 10% Tween 80; Group 2 received Cd (2 mg/kg) only; Group 3 received Cd (2 mg/kg) + PA (200 mg/kg); Group 4 received PA (200 mg/kg) only. The administered dosages of Cd and PA were based on our previous study (Akinola et al., 2021). A single intraperitoneal injection of Cd was given at the beginning of the study, while PA was orally given daily for thirty days. Blood samples and testicular tissue were obtained from the animals following anaesthesia using 50 mg/kg sodium thiopental for further assays.

Hormonal assay

The serum obtained was used for the analysis of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) using the Enzyme-Linked Immunosorbent Assay kit according to the manufacturer's manual (Oyeyemi et al., 2019; Akinola et al., 2021).

Sperm analysis

A drop of Caudal epididymis sperm and saline solution were placed on a microscope slide, and a microscopic examination was conducted to assess sperm motility according to the method used by Oyeyemi et al. (2015). The sperm viability and morphology were assessed following the methods previously adopted by Akinola et al. (2020). Sperm count was estimated microscopically using the improved Neubauer hemocytometer (Oyeyemi et al., 2015).

Determination of testicular magnesium, calcium, zinc and cadmium ions
The testicular levels of magnesium, calcium, zinc, and Cd ions were determined using the Perkin Elmer Absorption Atomic Spectrophotometer (AAS) with Winlab 32 software following the method previously reported by Oyeyemi et al., 2015.

Histological examination of testicular tissue
The harvested testis was fixed in Bouin's fluid for a day and then embedded in paraffin. The tissue section was done at 5 µ and stained with haematoxylin and eosin (H & E) technique. Stained sections were mounted with Canada balsam and cover-slipped. A photomicrograph of stained tissue sections was taken after microscopic examination.

Statistical analysis
Data were analysed using analysis of variance (ANOVA), followed by the least significant difference (Post Hoc test), and results were expressed as mean ± SEM. Differences between means were considered significant at $p < 0.05$.

RESULTS

Effects of PA on serum levels of testosterone, LH, and FSH in experimental animals
According to the results of this study (Table 1), the serum testosterone, LH, and FSH levels were significantly reduced in Cd-treated animals when compared to control. In comparison to the control, FSH was significantly higher in the Cd+PA group, while LH and FSH were significantly increased in the PA group. Moreover, testosterone, LH, and FSH were significantly increased in the Cd+PA groups when compared to the Cd group.

Effects of PA on Sperm Parameters in Experimental Animals
Table 2 shows that sperm motility, viability, and count showed a significant reduction in the Cd-treated group relative to the control group, while sperm abnormality was significantly increased in Cd-treated and Cd+PA groups when compared to the control group. Also, sperm count showed a significant increase in the PA-treated group relative to the control group. Moreover, sperm motility, viability, and count were significantly increased in the Cd+PA group compared to the Cd-treated group.

Effects of PA on testicular levels of magnesium, calcium, zinc, and cadmium ions in experimental animals
Table 3 shows that magnesium, calcium, and zinc ions were significantly reduced while the level of Cd ions was significantly elevated in the Cd-treated group in comparison with the control. More so, the magnesium and calcium ions levels were significantly reduced in the Cd+PA group relative to the control group.

Table 1: Evaluation of serum levels of testosterone, LH, and FSH in experimental animals

Parameters	Control group	Cd-treated group	Cd + PA group	PA-treated group
Testosterone (ng/mL)	0.91 ± 0.080	0.63 ± 0.050*	0.99 ± 0.140#	1.10 ± 0.120
LH (mIU/mL)	0.30 ± 0.0012	0.20 ± 0.009*	0.32 ± 0.032#	0.36 ± 0.071*
FSH (mIU/mL)	0.45 ± 0.011	0.39 ± 0.008*	0.53 ± 0.017*,#	0.57 ± 0.058*

*, # indicates significant differences between control and Cd-treated groups, respectively, at $p < 0.05$.

Table 2: Evaluation of sperm parameters in experimental animals

Parameters	Control group	Cd-treated group	Cd + PA group	PA-treated group
Sperm motility (%)	82.0 ± 6.04	50.0 ± 5.30*	69.0 ± 3.40#	76.0 ± 5.90
Sperm viability (%)	77.0 ± 8.00	44.0 ± 4.70*	62.0 ± 6.60#	73.0 ± 6.30
Sperm count (million/mL)	12.8 ± 3.09	7.2 ± 1.54*	10.6 ± 0.98#	17.4 ± 2.03*
Abnormality (%)	12.1 ± 0.39	13.7 ± 0.33*	13.6 ± 0.15*	12.9 ± 0.49

*, # indicates significant differences when compared to the control and Cd-treated groups, respectively, at $p < 0.05$.

However, in comparison to the Cd-treated group, the magnesium, calcium, and zinc ion levels were significantly increased, while the level of cadmium ion was significantly reduced in the Cd+PA group.

Assessment of the testicular histoarchitecture of experimental animals

The findings of this study (Figure 1) show a characteristic normal testicular histoarchitecture in the control group with features like regular seminiferous tubules which contain germ cells - spermatogonia, Sertoli cells, regular maturation phases with spermatozoa within their lumen, interstitial spaces comprising Leydig cells (red arrow). In the Cd-treated group, the observable features include several atrophied seminiferous tubules with thickening of the tunica propria, cessation of maturation and moderate congestion within the interstitial spaces. The Cd+PA group shows the prominence of typical seminiferous tubules having usual germ cells and regular maturation phases with the presence of spermatozoa within their lumen and few seminiferous tubules with maturation arrest causing vacuolation, while the interstitial spaces show mild congestion. The PA-treated group shows relatively regular seminiferous tubules comprising normal germ cells, Sertoli cells, and regular development phases with spermatozoa within the lumen and interstitial spaces with Leydig cells.

DISCUSSION

Cadmium as a hazardous environmental, industrial, and occupational contaminant has been reported in numerous studies. Reactive oxygen species (ROS) and its resultant impairment of the antioxidants' defence structure in tissues is the main mechanism by which cadmium mediates its noxious effect (Akinola et al., 2020).

Table 3: Evaluation of testicular levels of magnesium, calcium, zinc and cadmium ions in experimental animals

Parameters	Control group	Cd-treated group	Cd + PA group	PA-treated group
Magnesium (mg/L)	4.95 ± 0.030	3.96 ± 0.050*	4.60 ± 0.070*,#	4.99 ± 0.160
Calcium (mg/L)	4.30 ± 0.470	3.00 ± 0.410*	3.60 ± 0.450*,#	3.90 ± 0.940
Zinc (mg/L)	0.44 ± 0.027	0.30 ± 0.039*	0.41 ± 0.025#	0.43 ± 0.023
Cadmium (mg/L)	0.010 ± 0.002	0.018 ± 0.001*	0.014 ± 0.002#	0.009 ± 0.003

*, # indicates significant differences when compared to the control and Cd-treated groups, respectively, at $p < 0.05$.

A reduction in testosterone, LH, and FSH was observed in Cd-treated animals. This observation conforms to the findings of our earlier study, whereby the downregulation of testosterone, FSH, and LH by Cd was linked to the disruption of the hypothalamic and pituitary axis (Akinola et al., 2021). In the present study, exposure to Cd led to a marked reduction in serum levels of testosterone, LH, and FSH in treated animals. This finding aligns with our previous research that indicated the downregulation of these hormones attributed to a disruption in the HPG axis (Akinola et al., 2021). The decrease in testosterone levels can also be linked to diminished LH levels, which are essential for activating steroidogenic functions in the Leydig cells and, therefore, very important in testosterone biosynthesis. This impairment may stem from Cd-induced disruption in the synthesis of substrates necessary for steroid production (Akinola et al., 2021). Conversely, treatment with PA revealed a notable increase in testosterone, LH, and FSH levels post-Cd exposure. These results suggest that PA may mitigate the adverse effects of Cd through its potential antioxidant properties, contributing to the re-establishment of normal endocrine function within the HPG axis, as supported by previous findings (Imoisi et al., 2015; Elham et al., 2016).

The results of this study also indicated that Cd exposure triggers a significant decline in sperm motility, count, and viability, and a significant rise in abnormal sperm morphology. These outcomes indicate spermatogenic impairment caused by Cd exposure. This can be attributed to the production of reactive oxygen species by Cd as similarly reported by an earlier study (Akinola et al., 2020). However, the treatment with PA exerted significant counter-effects against the harmful action of Cd on the sperm parameters.

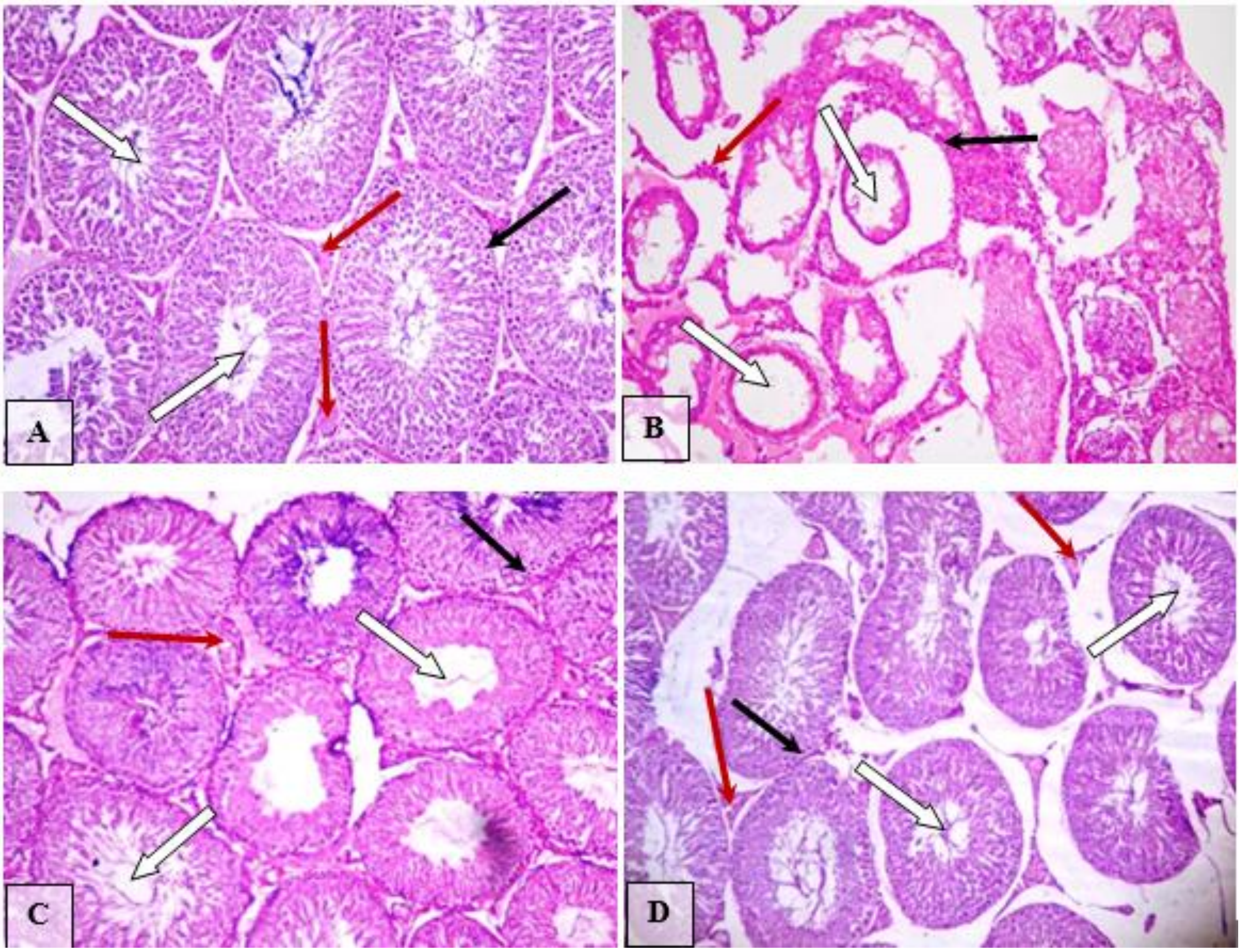

Figure 1: Photomicrographs showing testicular histoarchitecture of experimental animals in control (A), Cd-treated (B), Cd+PA (C) and PA-treated (D) groups (H & E ×100). Lumen of seminiferous tubules (white arrow), spermatogonia (black arrow), Interstitial spaces with Leydig cells (red arrow).

As also shown in this study, Cd exposure resulted in a significant decline in testicular concentrations of Mg, Ca and Zn ions with a concomitant significant increase in Cd ion concentration. Magnesium plays a crucial role in cellular physiology and is essential for sperm motility; reduced levels of Mg in the seminal fluid are associated with infertility across different populations and species (Wong et al., 2001; Emojevwe et al., 2022). Moreover, during the process of spermatogenesis, Calcium ion is one of the essential elements that help to regulate the division, growth, and apoptosis of spermatogonia and spermatocytes (Golpour et al., 2017). Furthermore, Zinc, which ranks as the second most prevalent trace element in the human body, plays a crucial role in male reproductive health (Vickram et al., 2021). It acts as an anti-inflammatory agent, enhances oxidative metabolism in sperm, regulates testosterone levels, and preserves the lining of reproductive tissues. It is essential for sperm capacitation, acrosome reaction, and the maintenance of sperm DNA integrity. Zinc deficiency disrupts spermatogenesis and lowers testosterone levels, potentially resulting in infertility (Yamaguchi et al., 2009; Vickram et al., 2021; Emojevwe et al., 2024).

The significant decrease of testicular Mg and Zn ions following Cd exposure could be attributed to the potency of Cd ions in displacing the Mg and Zn ions from the binding sites of enzymatic reactions, as reported by Hari et al. (2016). Similarly, the significant decline in the testicular Ca ion during Cd exposure could be attributed to its displacement by the Cd ions. However, co-treatment with PA resulted in a reversal of the adverse effect of Cd on the testicular electrolyte profile, thereby preventing the displacement of these essential elements by Cd ions. Previous studies have reported similar findings regarding the action of PA and associated the effect with its antioxidant properties, which helps to prevent the generation of free radicals due to Cd ion accumulation within the testicular tissue (Imoisi et al., 2015; Elham et al., 2016). Furthermore, these findings show conformity with findings from previous studies, which reported that compounds with antioxidant properties could trigger an increased level of the essential elements (Cheng and Li, 2012; Oyeyemi et al., 2022).

The findings of this study further reveal that Cd exposure triggered significant alterations of the testicular histoarchitecture with features including atrophied seminiferous tubule, cessation of sperm maturation, vacuolation of tubule and congestion of interstitial space. These testicular histopathological presentations showed similarity to a previous study which reported the potency of Cd to cause prominent morphological alterations in the testicular tissue of experimental animals (Herranz et al., 2010). However, co-treatment with PA exerts a potent protective effect against the Cd-induced testicular histopathology, as demonstrated by the appearance of normal seminiferous tubule with germ cells and maturation phases with spermatozoa within the lumen.

CONCLUSION

The obviation of the adverse outcome of Cd exposure on the reproductive hormones, sperm parameters, testicular electrolyte profile and testicular histoarchitecture by PA treatment enables the preservation of the physiological function of the testicular tissue. Hence, PA exhibits a protective effect against Cd-induced adverse alteration of reproductive parameters and testicular histoarchitecture in Wistar rats. Further study needs to be carried out to elucidate the mechanism of action of PA.

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Authors contribution

Adeniran O. Akinola: conceptualization, Data curation, investigation, methodology, supervision, writing the original draft.
Adekunle W. Oyeyemi: formal analysis, Software, validation, writing-review and editing.
Dayo R. Omotoso: validation, visualization, writing-review and editing.
Oore-Oluwapo O. Daramola: validation, visualization, writing-review and editing.
Victor O. Emojevwe: supervision, validation, visualization, writing-review and editing.
Eunice Ogunwole: supervision, validation, visualization, writing-review and editing.
Blessing A. Adetula: project administration, manuscript writing.
Oluwatobiloba D. Adeleke: project administration, manuscript writing.

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