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Research Article

Oxidative Stress in Testis of Rats Exposed to Cadmium

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ABSTRACT

The effect of acute oral cadmium (Cd) exposure on testicular oxidative and antioxidant status of adult Wistar rats was investigated. Twenty male rats of comparable weight (130-160g) were assigned to 4 groups (three tests and a control; n = 5). The Cd-exposed groups consumed drinking water contaminated with $CdCl_2$ in concentrations of 10ppm, 100ppm and 1000ppm for 7 days. At the end of exposure, blood samples and the testes were obtained for assessment of blood Cd level, serum testosterone, testicular tissue oxidation and antioxidant levels as well as histology. Significant increase in blood Cd level was noticed in the 100ppm (0.0153±0.0085ppm) and 1000ppm (0.0298±0.0204ppm) exposed groups compared to control (0.0008±0.0005ppm). Acute Cd-exposure causes a dose-dependent significant decrease in percentage body weight gain, serum testosterone but a non-significant increase in testicular weight. There was a significant dose-dependent increase in testicular malondialdehyde (6.52±1.06 vs 10.78±0.43 vs 16.74±1.76 vs 24.06±0.98) u/mg protein but a decrease in superoxide dismutase (76.74±5.49 vs 62.39±1.58 vs 40.02±5.48 vs 25.92±3.06) u/mg protein and catalase (245.66±23.85 vs 216.75±12.61 vs 117.11±13.05 vs 68.61±4.36) u/mg protein activities. These may indicate that acute exposure can adversely affect the testes through stimulation of testicular oxidation and inhibition of the antioxidant system to cause a decrease in testosterone production.

Keywords: *Cadmium, Testis, oxidative stress, antioxidant*

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INTRODUCTION

Following the abundance and accessibility to chemical materials over a few decades ago, the rate of intoxication from their derivatives have risen tremendously (Rafati and Moghadamnia, 2010; Rafati *et al*., 2015). It is now well recognized that these chemicals have increased exponentially mainly due to the rapid growth of the human population and demand for several household materials (Samarghandian*et al*., 2013). These chemicals discharged into the ecological systems find their way into the food chain and subsequently into man through the digestive system (Syers and Gochfeld, 2000) and thus humans are at risk for heavy metal poisoning (Osifo, 2018).

Cadmium (Cd), one of the most important environmental and occupational toxic metals, is widely dispersed in the environment (Samarghandian *et al*., 2015) and forms a variety of complexes and soluble salts. Depending on the dose, route and duration of exposure, Cd can damage various organs including the lung, liver, kidney, bones, testes and placenta (Pari and Murugavel, 2007; Osifo, 2018).

Recent studies have demonstrated that Cd stimulated free radical production, resulting in oxidative deterioration of macromolecules (Almasiova *et al*., 2012). Cadmium affects cell proliferation, differentiation, and apoptosis and these activities interact with DNA repair mechanism, the generation of reactive oxygen species (ROS) and the induction of apoptosis (Rani *et al*., 2014). Cadmium causes mutations and chromosomal deletions potentially (Joseph, 2009). Its toxicity involves depletion of reduced glutathione (GSH), binds sulfhydryl groups with protein, and causes to enhance the production of reactive oxygen species (ROS) such as superoxide ion, hydrogen peroxide, and hydroxyl radicals. Cadmium also inhibits the activity of antioxidant enzymes, such as catalase, manganese-superoxide dismutase, and copper/zinc-dismutase (Filipic, 2012). In this study, we report the effects of acute oral cadmium exposure on the testes of rats with emphasis on levels of MDA, SOD and CAT.

MATERIALS AND METHODS

Materials: Cadmium chloride ($CdCl_2$) was obtained from a Chemical store in Nigeria. All other chemicals were of

analytical grade. The rat chow was obtained from a poultry store in Ekpoma, Nigeria. Except otherwise stated, the drinking water used was clean tap water.

Animals: Twenty male Wistar rats, weighing 140g to160g were obtained from the Animal House, College of Medicine, Ambrose Alli University, Ekpoma. The rats were transported and kept in their cages in physiology laboratory II and maintained in a room with 12 hours light/dark cycle. They were fed standard rat chow and water *ad libitum* for two weeks of acclimatization.

Study design: After acclimatization, the rats were randomly assigned to four experimental groups, (n = 5) as follows: Group 1; the control and was supplied clean tap water throughout the experiment. Group 2, 3 and 4 served as test groups and were served drinking water contaminated with cadmium chloride in the concentration of 10ppm, 100ppm, and 1000ppm respectively for 7 days as previously documented by Osifo and Iyawe (2018). All animals had access to normal meals and drinking water throughout the study period.

Sample collection: At the end of exposure, the animals were cervically decapitated and a blood sample was obtained for blood Cd level (1ml) and testosterone assessments (2ml). The testes were harvested, weighed and the right testis was put into a container containing ice-cold phosphate buffer (10% organ weight) while the left was submitted for histological processing. The collected blood sample for blood Cd levels was digested with 2ml of HNO_3 and left overnight. The processes of preparation were as documented in Gonçalves *et al*. (2012) and blood Cd level determined by atomic absorption spectrophotometer. The 2ml blood samples were centrifuged at 3500 rpm for 10 minutes and the serum was obtained for testosterone estimation using a testosterone ELISA kit (Accu Bind Elisa microwells by Monnnobind Inc., USA) and the procedures were as presented by the kit manufacturer.

The right testes were homogenized and the homogenate was centrifuged to obtain the supernatant to assay for tissue malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT). The protein concentrations of the tissue samples were determined by the Biuret method as described by Gornal *et al*., (1949). MDA was determined by measuring the thiobarbituric acid reactive substances produced as described by Varshney and Kale (1990). The level of SOD was determined as previously described by Misra and Fridovich (1972) and CAT levels by Sinha(1971). The left testes were submitted for histological processing following standard histological procedure and were processed into the slide and viewed under the microscope for histological observations and interpretation by a histopathologist, "blind" of the treatment and grouping.

Statistical analysis

Statistical analyses were performed using the SPSS version 20.0 programme. The data were subjected to one-way analysis of variance (ANOVA) and the LSD *post hoc* test was used for multiple comparisons and expressed as mean ± standard deviation, $p< 0.05$ was considered significant.

RESULTS

Figure 1 shows the effect of exposure to varying concentrations of Cd in the drinking water on blood Cd-level. There was a dose-dependent increase in blood Cd levels and was significantly different in the 100ppm (0.0153±0.0085ppm) and 1000ppm (0.0298±0.0204ppm) groups compared to the control (0.0008±0.0005ppm).

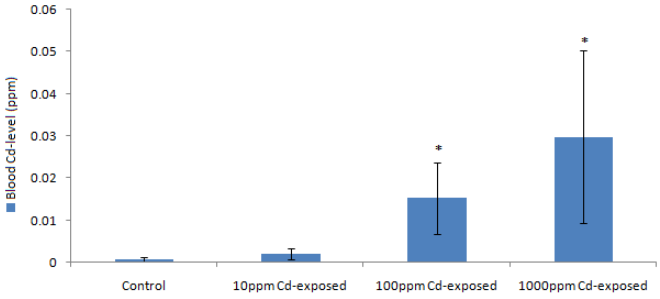

Figure 1.
Effect of varying concentrations of Cd exposure on blood Cd level in adult Wistar rats (n = 5; * p<0.05 compared with control).

Table 1 shows the effect of exposure to Cd on body and testicular weight and serum testosterone. Cd exposure resulted in a dose-dependent significant decrease in percentage body weight gain in the 1000ppm dose (6.81±2.58% vs 0.80±1.46%) compare to the control but a non-significant increase in testicular weight in the exposed groups. Serum testosterone was significantly decreased in the exposed groups in a dose-dependent manner compared with the control.

Table 1.
The effect of varying concentrations of Cd exposure on body weight gain, testicular weight and serum testosterone level in adult male Wistar rats.

	Control	10ppm Cd-exposed	100ppm Cd-exposed	1000ppm Cd-exposed
Percentage body weight gain (%)	6.81 ±2.58	4.94 ±4.82	3.08 ±0.66	0.80 ±1.46*
Testicular weight (g)	1.24 ±0.42	1.26 ±0.26	1.28 ±0.34	1.52 ±0.42
Serum testosterone (ng/ml)	4.62 ±0.49	2.87 ±0.47*	2.10 ±0.18*	1.57 ±0.32*

*n = 5; * p<0.05*

Figures 2, 3 and 4 show the effect of exposure to Cd on testicular MDA, SOD and CAT levels. Testicular MDA increased significantly compared to the control (6.52±1.06 u/mg protein vs 10.78±0.43u/mg protein vs 16.74±1.76u/mg protein vs 24.06±0.98u/mg protein in control, 10ppm, 100ppm and 1000ppm respectively). On the other hand, testicular SOD (62.39±1.58u/ng protein vs 40.02±5.48u/ng protein vs 25.92±3.06u/ng protein) and CAT (216.75±12.61u/ng protein vs 117.11±13.05u/ng protein vs 68.61±4.36u/ng protein) decreased significantly in the exposed groups compared to the control (SOD = 76.74±5.49u/ng protein and CAT =245.66±23.85u/ng protein vs).

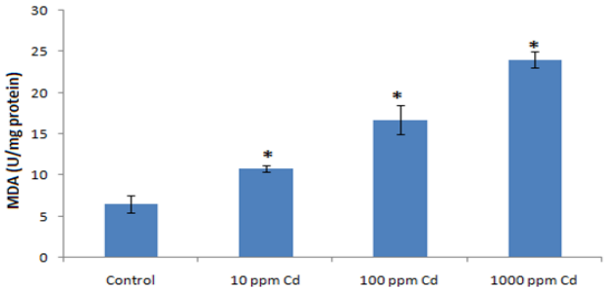

Figure 1.
The effect of varying concentrations of Cd exposure on testicular MDA in adult male Wistar rats (* $p<0.05$).

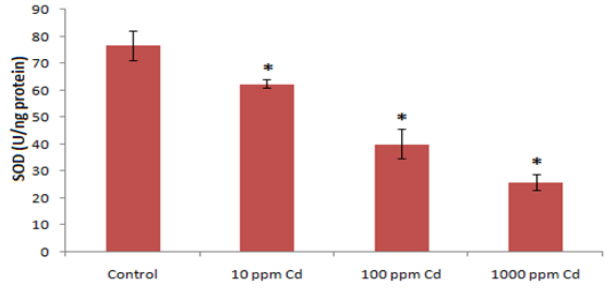

Figure 2.
The effect of varying concentrations of Cd exposure on testicular SOD in adult Male Wistar rats (* $p<0.05$).

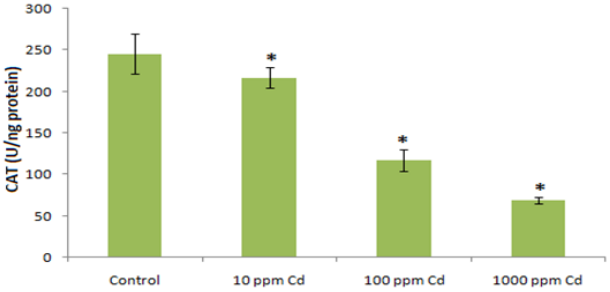

Figure 3.
The effect of varying concentrations of Cd exposure on testicular CAT in adult Wistar rats (* $p<0.05$).

Plate 1 represents a photomicrograph of testicular sections stained by haematoxylin and eosin (X100) of rats exposed to varying concentrations of Cd. Histologically, there were normal seminiferous tubules epithelium, spermatogonia cells, spermatocytes, spermatids and full development of germ cells seen in the control and 10ppm exposed. These were also seen in the 100ppm and 1000ppm exposed but coupled with mild to moderate interstitial spaces congestion in the 100ppm and maturation arrest in the seminiferous tubules and mild interstitial spaces congestion in the 1000ppm.

DISCUSSION

Cadmium is a well-recognized environmental pollutant with numerous adverse health effects following prolongs exposures (Patra *et al*., 2011). Direct and indirect evidence exists on Cd-induced deleterious health effects through reactive oxygen species following acute overload and chronic toxicity in animals (Patra *et al*., 2011) and early catabolic damage within 7 days has been documented by Osifo and Iyawe (2018). Blood Cd level is a measure of acute exposure (Adams and Newcomb, 2014) and in the present study, exposure to 100ppm and 1000ppm Cd for 7 days significantly increase blood Cd level. Other studies have also documented increase blood Cd levels following oral exposure. In this instance, Adegoke *et al*. (2017) reported increased blood Cd levels higher than those reported in this study following 28 days of exposure to 100ppm.

The increased blood Cd level in the present study showed adequate exposure in the test groups. This assertion is based on the documentation that increase blood Cd level is an indication of sufficient exposure (Taylor, 1988; Jin *et al*., 2002).

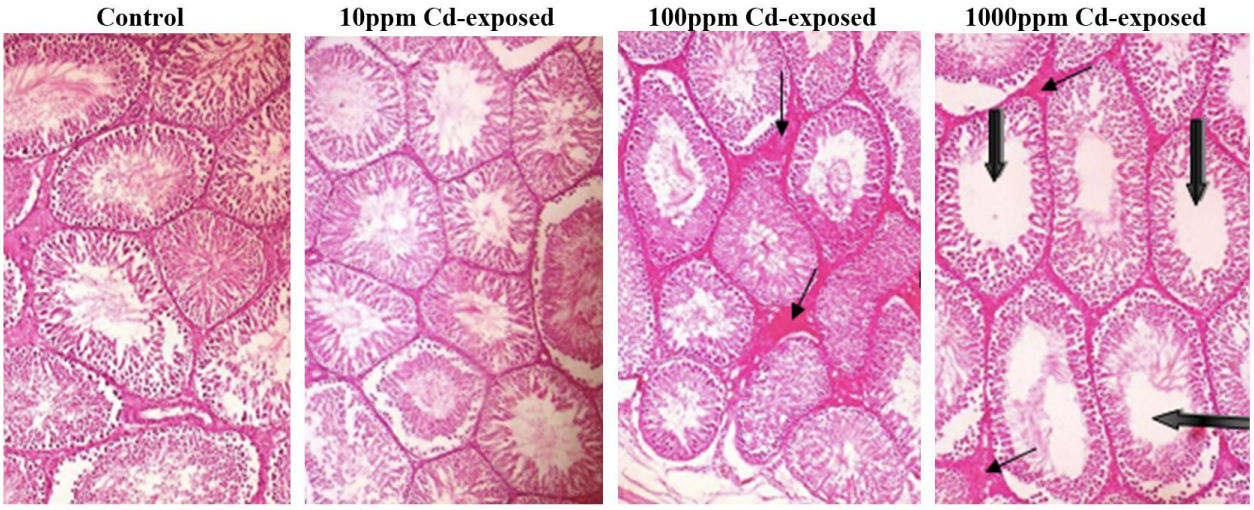

Plate 1.
Photomicrograph of testicular sections stained by haematoxylin and eosin (X100)
The control showed normal seminiferous tubules epithelium, spermatogonia cells, spermatocytes, spermatids and full development of germ cells. 10ppm Cd-exposed presented similar findings with control. There were mild to moderated interstitial spaces congestion (slender arrow) in the 100ppm Cd-exposed and maturation arrest of the seminiferous tubules are (black arrow) and mild interstitial spaces congestion (slender arrow) in the 1000ppm Cd-exposed

The effect of Cd exposure on percentage body and testicular weights were also investigated. Cd resulted in a significant reduction in body weight in the 1000ppm exposed indication the highest dose to have a significant impact on body weight while there was no significant difference in the testicular weight. A similar decrease in body weight following Cd-exposure has been documented in other studies (Zeng *et al*., 2003; Amara *et al*., 2008; Layachi and Kechrid, 2012; Adegoke *et al*., 2017; Osifo, 2018) suggesting a decrease in body weight as the primary manifestation of Cd exposure in rats. Also in line with the observed non-significant increase in testicular weight following Cd-exposure, Osifo (2018) had reported a similar increase in testicular weight after acute exposure to Cd. The observed increase in testicular weight maybe because the testis is an organ where Cd is known to be deposited. Biswas *et al*. (2001) and Amara *et al*. (2008) have previously reported the testes as one of the major targets organs of Cd toxicity in rats, rabbits and dogs. The result of serum testosterone in this study may lay credence to this. Specifically, there was a significant dose-dependent decrease in serum testosterone following acute Cd exposure. A similar result has been reported by Osifo and Iyawe (2018). Lafuente *et al*. (2000) has documented Cd accumulation in the hypothalamus, pituitary, and testis of rats coupled with decreased plasma follicle-stimulating hormone levels. This may be the cause of the observed decrease in serum testosterone noticed in the present study considering the stimulating relationship between the follicle-stimulating hormone and testosterone level. However, Siu *et al*. (2009) have reported Cd-induced testicular toxicity to be the probable result of an inter-digitating complex interaction that involves the disruption of the blood-testis barrier.

In the present study, the effect of acute oral cadmium exposure in the drinking water on testicular oxidative and antioxidant status was investigated in adult Wistar rats. Acute Cd exposure was observed to stimulate testicular tissue MDA level and inhibit endogenous activities of SOD and CAT in the testes. The observed induction of testicular MDA and weakening antioxidant system was dose depended and was significant even at the lowest dose. In support of these, several studies have documented oxidative stress as an important mechanism underlying Cd-induced testicular damage. Exposure of Swiss mice to $CdCl_2$ (1 mg/kg body weight) for 5–8 weeks, increased testicular lipid peroxidation, thereby impairing intracellular defences leading to altered spermatogenesis (Acharya *et al*., 2008). The high membrane lipid content of testicular Leydig cell mitochondria and microsomes makes these cells more susceptible to Cd-induced lipid peroxidation (Georgiou *et al*., 1987). Testicular Leydig cells are also the target cell population for Cd carcinogenesis as a single carcinogenic dose of $CdCl_2$ (30 μmol/kg body weight) caused severe hemorrhagic damage in rat testis within the first 12 h after exposure together with increased iron content, H_2O_2 production and lipid peroxidation in a study by Koizumi and Li (1992). Also, studies have documented a significant reduction in enzymatic activities of SOD, GPx as well as CAT and a decline in ascorbic acid content in testicular cells exposed to Cd (Acharya *et al*., 2008). Present results appeared consistent with these studies.

Histologically, Cd exposure in the doses of 100ppm and 1000ppm was observed to cause interstitial spaces congestion and maturation arrest of seminiferous tubules. The observed maturation arrest of seminiferous tubules may be linked to the decreased serum testosterone which may have been caused by the observed altered testicular oxidative and endogenous antioxidant system in the study.

Findings from this study showed that Cd exposure induces testicular reactive oxygen species indicated by increased MDA level and weakens the endogenous antioxidants system indicated by the reduced testicular tissue SOD and CAT levels. It was also noticed that Cd exposure caused a dose-dependent on alterations in testicular oxidative and endogenous antioxidant system. This suggests therefore that the mechanism of testicular damage by Cd may be via activation of ROS and deteriorating tissue antioxidant causing the arrest of seminiferous tubules maturation and inturn decreased production of testosterone.

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