British Journal of Medicine & Medical Research
3(4): 2031-2041, 2013

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Changes in Some Testicular Biometric Parameters and Testicular Function in Cadmium Chloride Administered Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EIE, EKN and CPA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author EIE managed the analyses of the study. Author EKN managed the literature searches. All authors read and approved the final manuscript.

Research Article

Received 18th December 2012
Accepted 23rd May 2013
Published 4th July 2013

ABSTRACT

Introduction: Recently there has been an increased association between environmental factors and male infertility.
Aims: In the present study, the effect of changes in testicular biometric parameters (weight and volume) and testicular function (Sperm count, morphology, testosterone level) in Cadmium chloride administered Wistar rats was studied.
Methodology: Twenty male albino Wistar rats were randomized into four groups (n=5). Group A (control) received rat chow and water, while Group B, C and D received 15mg/L, 20mg/L and 25mg/L of Cadmium chloride respectively for 6 weeks.
Result: There was a significant (*P*=.05) and dose dependent decrease in testicular function parameters in the rats and a significant (*P*=.05) and positive correlation between the biometric parameters and testicular function.
Conclusion: The findings showed that Cadmium chloride has a deleterious effect on testicular function and biometric parameters of the testes may be important in the assessment of testicular function.

Keywords: Cadmium chloride; male infertility; testicular weight; testicular volume.

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1. INTRODUCTION

Over the years, infertility has been on the increase in both males and females. The increase in male infertility has become a source of global concern [1]. According to the World Health Organization, 'infertility is the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year [2].

Currently, the etiology of suboptimal semen quality and male reproductive hormone is poorly understood, and many physiological and environmental factors have been implicated [3,4], factors such as smoking, use of restricted drugs, stress, and lack of exercise specifically have been implicated [1].

Cadmium is one of environmental pollutants arising from electroplating, fertilizers, pigment and plastic manufactures. Therefore it easily contaminates the soil, plants, air and water [5]. Cadmium in tobacco represents a contributory source to the total body burden of the smoker [6,7]. Humans and animals can easily be exposed to cadmium toxicity by consuming plants, water and air. Cadmium is absorbed and accumulates in various tissues [8,9] even red blood cells [10], the heart [11] and the skeletal muscle of rats [12].

Most animals with scrotal testes are susceptible to cadmium–induced testicular toxicity [13]. Although, only about 1–2 % of acute cadmium dose is usually taken up by the testes, testicular toxicity is almost invariably evident. It has been reported that as low as 1-2 mg Cd/kg body weight can cause testicular damage without pathological changes to other organs [14]. Exposure to cadmium has been reported to reduce male fertility in both humans and rodents [15], but the mechanism is still unknown.

Exposure to cadmium can negatively affect the male reproductive system via degenerative changes in testes, epididymis, and seminal vesicles [16]. Recently, Azoospermic persons were found to have higher serum and seminal plasma cadmium level compared with oligospermic ones [17]. Also positive relationship was found between cadmium exposure and asthenozoospermia in a rat model [15].

The aim of the study is to establish an association between changes in some testicular biometric parameters and testicular function in Wistar rats induced with Cadmium chloride.

2. MATERIALS AND METHODS

2.1 Animals and Housing

Twenty male albino Wistar rats weighing between 160- 180g and aged 12-14 weeks were used in this study. Animals were raised in the Animal House Unit in Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria. They were maintained in wooden cages with stainless steel wire lids (bedded with wood shavings). Rat chow and water were supplied *ad libitum.* Rats were housed at a temperature of 28 ± 1ºC, 60 % humidity and under a 12 -hour - light: 12- hour- dark schedule.

2.2 Administration of Cadmium

Cadmium Chloride ($CdCl_2$) in crystalline form was obtained from the Petroleum Training Institute (PTI), Warri, Nigeria, and was dissolved in distilled water. The concentrations were

15mg/L, 20mg/L and 25mg/L of the solution [18]. Male rats were randomly assigned to four groups of 5 animals each (control and experimental groups). Experimental male rats were provided access to drinking water containing $CdCl_2$ for 6 weeks. The control group received tap water only.

2.3 Experimental Design

A total of twenty (20) male albino Wistar rats were randomly divided into four groups of five rats each.

Group A : Control, fed with rat chow and water daily (n=5)
Group B : 15mg/L Cadmium chloride solution treated rats (n=5)
Group C : 20mg/L Cadmium chloride solution treated rats (n=5)
Group D : 25mg/L Cadmium chloride solution treated rats (n=5)

2.4 Male Fertility Assessment

2.4.1 Sex organ weight and volume

The rats were sacrificed from each group by decapitation at the end of experiment. The testes were dissected and weighed using a digital electronic weighing balance (EW, Germany) and recorded. The volume of the right testes was measured by first drying the surface of the right testes (using a filter paper), and then immersing it into a 10 ± 0.2 ml measuring cylinder containing 5ml physiological saline, and the volume of displaced fluid in the cylinder recorded.

2.4.2 Semen quality analysis

Seminal content of epididymis was obtained by cutting of the cauda epididymis using surgical blades and squeezed in a sterile clean watch glass. This content was diluted 10 times with 2.9% sodium citrate dihydrate solution and thoroughly mixed to estimate the progressive motility and sperm count [19]. One drop of the suspension was smeared on a glass slide and stained by Eosin – nigrosin stain to determine the percentage of sperm cell morphology [20].

2.4.3 Testosterone assay

Blood samples were collected directly from the heart, by cardiac puncture, in plain non-heparinized bottles and the serum was separated and frozen until hormonal assay was done. Testosterone was estimated in the serum as described by Ismail [21].

2.4.4 Histological section

The portion of testis was fixed in Bouins fluid, dehydrated in ascending grades of alcohol, Embedded in paraffin wax and sectioned at 5μ and stained with haematoxylene and eosin. The micrometric measurements such as diameter of seminiferous tubule and diameter of the Leydig cell were measured from randomly selected twenty round sections from each group.

2.5 Statistical Analysis

Values were expressed in mean ± SEM. SPSS version 17 was used for statistical analysis. Differences between groups were assessed by Student *t*-test and Pearson's product moment correlation. P <0.05 was taken as significant.

3. RESULTS

Result showed the effect of cadmium chloride on testicular function and some testicular biometric parameters, it showed a dose dependent significant (*P*=.05) reduction in the semen quality, serum testosterone, testicular weight/volume.

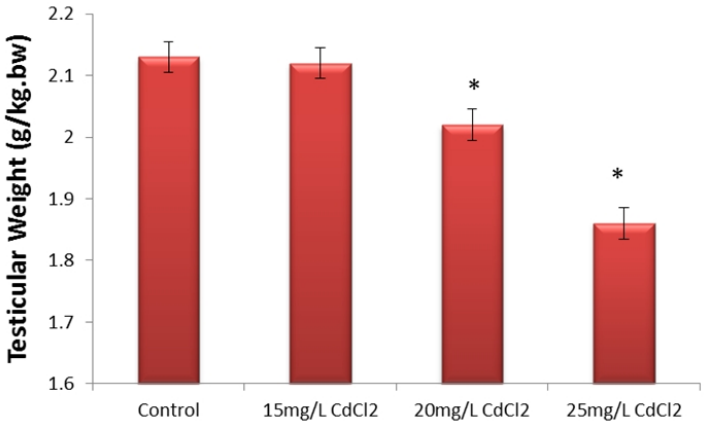

Fig. 1. Effect of cadmium chloride on testicular weight
** p<0.05 compared with the Group 1(control); (n=5)*

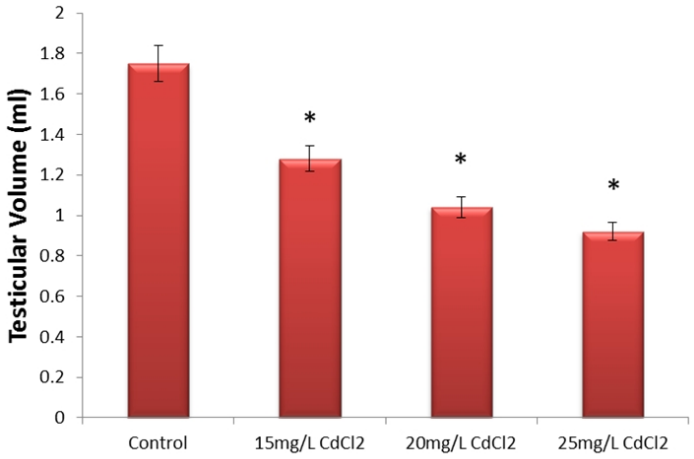

Fig. 2. Effect of cadmium chloride on testicular volume
** p<0.05 compared with the Group 1(control); (n=5)*

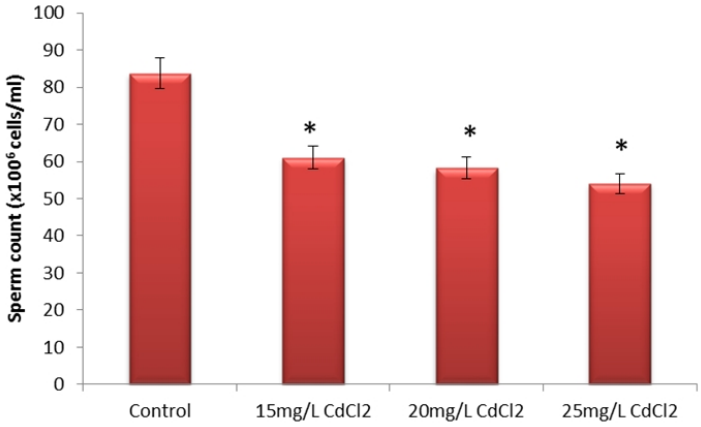

Fig. 3. Effect of cadmium chloride on sperm count
** p<0.05 compared with the Group 1(control); (n=5)*

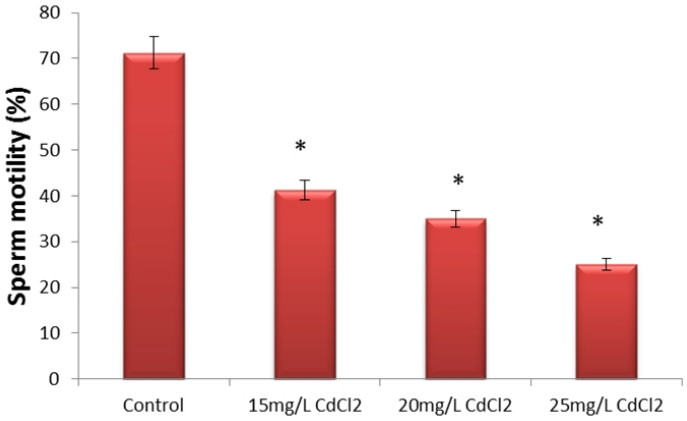

Fig. 4. Effect of cadmium chloride on sperm motility
** p<0.05 compared with the Group 1(control); (n=5)*

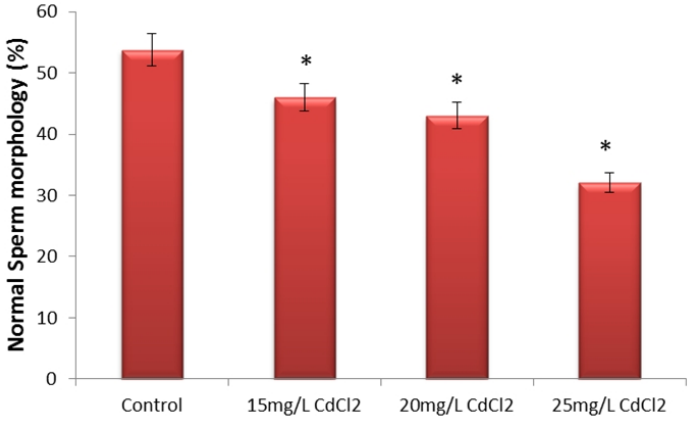

Fig. 5. Effect of Cadmium Chloride on Sperm morphology
** p<0.05 compared with the Group 1(control); (n=5)*

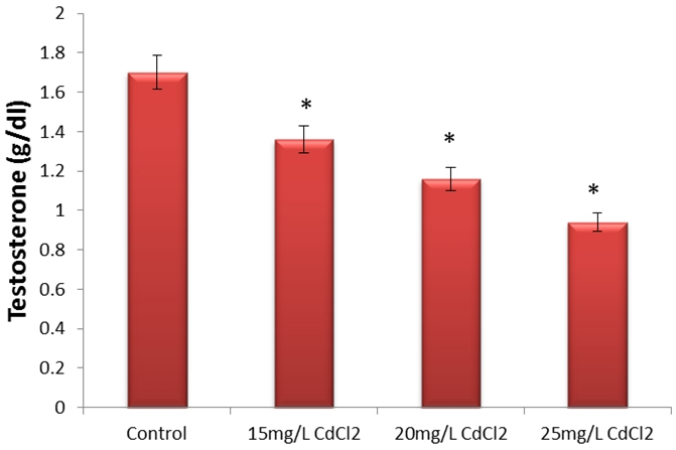

Fig. 6. Effect of Cadmium Chloride on Testosterone
** p<0.05 compared with the Group 1(control); (n=5)*

3.1 Histological Sections

The histology of the control rats (Plate 1) showed normal morphology of the testes. Severity in testicular morphological derangement was dose dependent as the effect of 15mg/L cadmium chloride on the histology of rat testes (Plate 2) caused a mild interstitial fibrosis, 20mg/L cadmium chloride solution on the testes (Plate 3) induced interstitial fibrosis and degeneration of the seminiferous tubules; and 25mg/L of cadmium chloride (Plate 4**)** caused severe interstitial edema, calcification of the tunica albuginea and hypertrophy of the leydig cells.

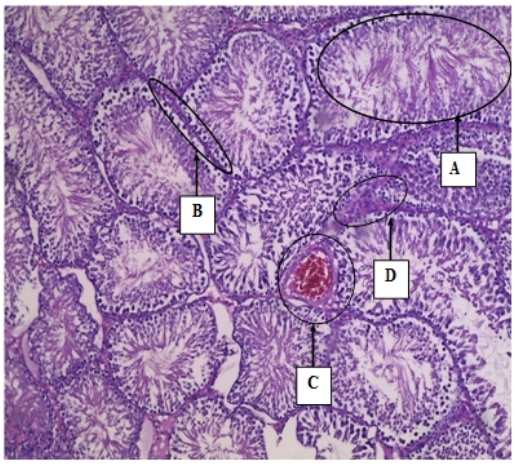

Plate 1. Normal Testis Showing Seminiferous tubules (A), tubules that are lined by stratified layers of cells those are of different stages of maturation (B). Blood vessels (C) and leydig cells (D). (H & E x100)

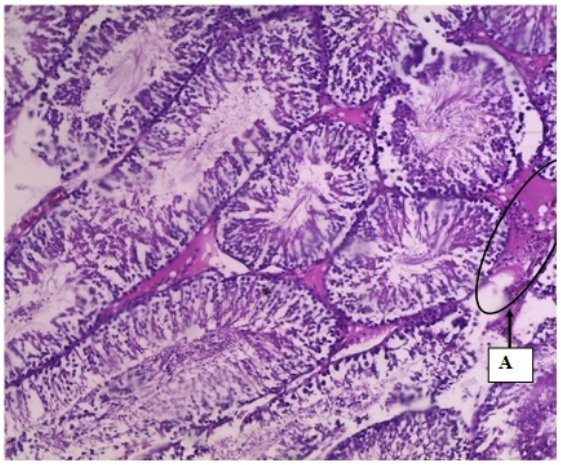

Plate 2. Testis of Wistar rats treated with 15mg/L of Cadmium chloride treated rats showing mild interstitial fibrosis (A). (H & E, x100)

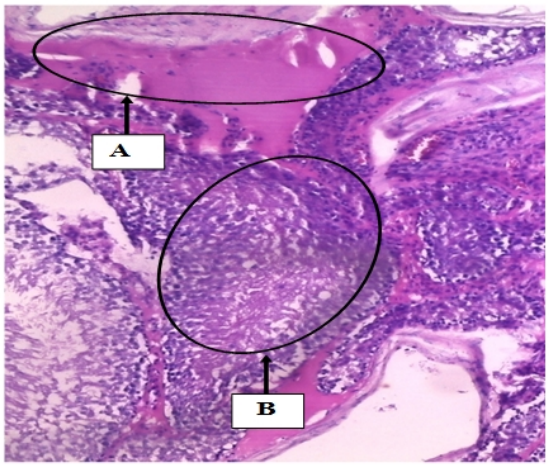

Plate 3. Testis of Wistar rats treated with 20mg/L of cadmium chloride, showing interstitial fibrosis (A), degeneration of seminiferous tubules (B). (H & E, x100).

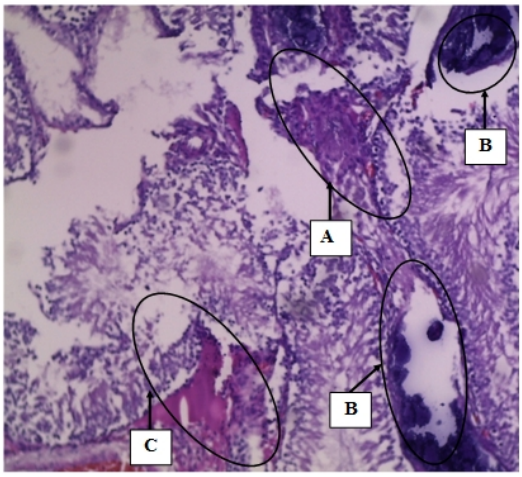

Plate 4. Testis of Wistar rat treated with 25mg/L of cadmium chloride, showing severe Interstitial edema (A) and Focal area of calcification at the tunica albuginea (B). Also present was Leydig cell hypertrophy (C) (H & E, x100).

The relationship between the testicular weight and/or volume with the semen quality and serum testosterone level, following cadmium chloride treatment was ascertained on the basis of correlation coefficient. This was done to establish a possible relationship between the testicular weight or volume with semen quality and serum testosterone level.

The values in the table below indicate the correlation coefficient, a positive value signify a positive correlation.

In the above table (Table 1), the testicular volume was positively and significantly correlated with the sperm count, sperm motility, percentage of sperm with normal morphology and serum testosterone levels. Similarly, the testicular weights of the rats were positively and significantly correlated with the sperm count, progressive sperm motility, percentage of sperm with normal morphology and serum testosterone level.

Table 1. Relationship between the testicular weight and volume with semen quality and serum testosterone level after cadmium chloride treatment

	Correlation	
	Testicular weight (g/kg.bw)	**Testicular volume (ml)**
Sperm count (x10^6 cells/ml)	0.67*	0.60*
Sperm motility (%)	0.56*	0.69*
Sperm morphology (%)	0.75*	0.68*
Testosterone (ng/ml)	0.58*	0.61*

** Correlation is significant at the p<0.05 level (2-tailed); (n=20)*

4. DISCUSSION

In this study, effects of administration of $CdCl_2$ in the drinking water of adult male rat on semen quality, testosterone and some testicular biometric parameters were investigated. Cadmium has been found to produce wide range of biochemical and physiological dysfunctions in humans and laboratory animals [22] and such target organs as the testes have been affected [23].

From the study there are evidences that exposure to cadmium chloride can interfere with male fertility. At the various dose of administration, Cadmium Chloride adversely affected (P=.05) the semen quality parameters and testosterone, to the extent that it may impair fertility in the male Wistar rat used for the study. The histological results in $CdCl_2$ treated groups showed damage and degeneration of seminiferous tubules in testes (Plates 2, 3 and 4). Cadmium, is very dangerous to testicular function [24]. According to Waisberg et al. [25], through increase in oxidative stress, cadmium chloride causes testicular damage, and impairs male fertility. Testicular damage caused by $CdCl_2$ is attributed to compete of $CdCl_2$ with Zinc in Zinc-containing enzymes and decreased activity of Testis-specific enzymes [26,27]. In line with the above, Neeven et al. [28], Benoff et al. [15], Bench et al. [29] and Babara et al. [30] have also shown that cadmium chloride administration would interfere with male fertility, especially by its detrimental effect on testicular function (Figs. 3, 4 and 5). The decrease in testicular function from this study due to $CdCl_2$ treatment proves that cadmium impairs spermatogenesis. This result is similar to the observation of [31,32]. The decrease in the testicular function could also be ascribed to the effect of cadmium chloride at the hypothalamic-pituitary-gonadal axis as suggested by Lafuente et al. [33] whose study revealed that cadmium affects the hypothalamic- pituitary-testicular axis function by acting at the three levels.

Different studies have shown that cadmium affects plasma gonadotropin levels [34]. Reduction in Testosterone hormone as shown in this study (Fig. 6) was manifested by lowered FSH and LH plasma levels [35,36], as well as, $CdCl_2$ decrease the total testosterone hydroxylase activity [37] which caused a dramatic decreasing in testosterone hormone. In this study the reduced testosterone level can also be attributed to interstitial fibrosis (Plate 3) and interstitial edema (Plate 4) caused by cadmium chloride which was in line with Laskey et al [38], who found that $CdCl_2$ caused necrosis in Leydig cells, these cells count primary synthesis site to testosterone hormone [39].

Cadmium chloride also significantly (P=.05) decreased the testicular weight (Fig. 1) and volume (Fig. 2), this could be understandable after reports from Rekha et al. [40] show derangement in some testicular structures such as seminiferous tubules and leydig cells that consist 70% to 80% of testicular mass [41].

According to Bailey et al. [42], testicular volume and weight are highly correlated. Also, Arai et al. [43] have suggested a possible correlation between testicular volume and testicular function which was also observed in this present study.

5. CONCLUSION

The exposure of rats to cadmium chloride induces biochemical effects in the testes. Cadmium chloride decreased some testicular biometric parameters, semen quality and

testosterone and these changes in the testicular function and testicular biometric parameters are positively correlated.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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