

Original Research Paper

Effect of Long Term Cadmium Chloride Exposure on Testicular Functions in Male Albino Rats

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Abstract: This study was carried out to investigate the adverse effects of different doses of Cadmium Chloride ($CdCl_2$) on reproductive functions in male rats. Forty male albino rats weighted 100-130 g were equally divided into 4 groups. The control group received distilled water throughout the experimental period, while the three treated groups received 5, 50 and 100 ppm of $CdCl_2$ in drinking water for consecutive 100 days. At Day 100, all rats were sacrificed and immediately the reproductive organs were dissected and the relative weight of each organ was estimated. The epididymis was treated for estimation of sperm concentration and sperm abnormalities. Section of the testis was kept in 10% formalin saline for histopathology. The relative weight of the testis of treated rats was reduced compared to that in control rats but not reach to a significant difference. The weights of epididymis, seminal and prostate glands were significantly ($p<0.001$) decreased particularly in rats received 100 ppm of $CdCl_2$. Moreover, the sperm concentration was significantly ($p<0.001$) declined in treated rats in dose dependent manner, while the number of abnormal sperms was significantly ($p<0.01$) increased in rats treated with 50 and 100 ppm of $CdCl_2$. In rats received 100 ppm of $CdCl_2$, the diameter of the seminiferous tubules was markedly reduced compared to that in control rats. Additionally, multinucleated giant cells as well as sloughing of the germinal epithelium of the seminiferous tubules were observed in testes of rats received 100 ppm of $CdCl_2$. In conclusion, administration of $CdCl_2$ for 100 days in drinking water even in low doses could adversely affect the reproductive functions in male rats.

Keywords: Testes, Albino Rats, Cadmium Chloride, Infertility

Introduction

In the “Top 20 Hazardous Substances Priority List” by the Agency for Toxic Substances and Disease Registry and the U.S. Environmental Protection Agency, cadmium (Cd) is ranked the seventh substance (Fay and Mumtaz, 1996). Principal uses include nickel-cadmium batteries, pigments and plastic stabilizers. Major occupational exposures to Cd occur in nonferrous metal smelters, production and processing of Cd alloys and compounds and, increasingly, in the recycling of electronics (WHO, 1992). Cigarette smoke is by far the greatest source of Cd exposure (Zalups and Ahmad, 2003).

Morphological and biochemical changes in testes due to Cd exposure have been described in several species of mammals. The weight of testes and accessory sex organs

are the primary indicators of a possible alteration (Biswas *et al.*, 2001). It is well known that weight of the testis depends on the mass of undifferentiated spermatogenic cells. In experimental models, Cd exposure can affect testes weight and induce pathogenesis leading to reduced sperm counts and impaired sperm motility, which adversely affects the male fertility (Biswas *et al.*, 2001; Xu *et al.*, 2001; El-Demerdash *et al.*, 2004; Yang *et al.*, 2006). Moreover, Cd induced drastic changes with alteration of biochemical composition of testes. The formation of multinucleated giant cells in Cd-intoxicated rats suggests continuous degeneration of spermatogenic epithelium and appears to represent a non-specific reaction to injury (Ponnusamy and Pari, 2011). The giant cells formation

may also result from the inability of primary spermatocytes to undergo meiotic divisions to generate haploid sperm cells, which undergoes additional DNA replication, giving rise to multinucleated giant cells (Rotter *et al.*, 1993; Yang *et al.*, 2005). The long retention of Cd in tissues and increased oxidative state promoted by Cd might contribute to the pathological changes in testes. Recent studies indicate that Cd is involved in inhibition of H₂O₂ removal system, resulting in H₂O₂ accumulation and inhibition of steroidogenesis in Leydig cells (Diemer *et al.*, 2003).

Low doses are more relevant to human exposure since the general population is exposed to Cd in contaminated food and cigarette smoke at relatively low concentrations. Few studies have quantified the morphological consequences of subclinical and chronic cadmium exposures. The purpose of this article was to histomorphometrically evaluated whether a long-term exposure to low, medium and high doses of cadmium causes modifications in testicular morphology and structure.

Materials and Methods

Animals and Housing

Forty male albino rats weighing 100-130 g were used in this study. Animals were raised Faculty of Veterinary Medicine, Suez Canal University. They were maintained in stainless steel cages with wood shavings. Food and water were supplied *ad libitum*. Rats were housed at a controlled temperature of 26±1°C, 60% humidity and under a 12 hr light: 12 hr dark schedule.

Administration of Cadmium

Cadmium Chloride (CdCl₂) in crystalline form was obtained from Sigma Chemical Company (Sigma, Aldrich). CdCl₂ was dissolved in distilled water at different concentrations; 5, 50 and 100 mg L⁻¹ (Waalkes *et al.*, 1999). Male rats were randomly assigned to four groups of 10 animals each (control or experimental groups). Experimental male rats were provided access to drinking water containing CdCl₂ for 100 days. The control group received dis. water only.

The Relative Weights of Reproductive Organs

Rats were killed at 100 days of the experiment. Testicular weights were recorded immediately after killing and the gonado-somatic index (testis weight X 100/body weight) was calculated. Moreover, the relative weights of epididymis, seminal and prostate glands were also measured.

Sperm Concentration and Morphology Assay

The rats were scarified at 100 days of the experiment and the 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 5 times with 2.9% sodium citrate dihydrate solution and thoroughly mixed to estimate the sperm concentration (Bearden and Fluquary, 1980). One drop of the suspension was smeared on a glass slide and stained by alkaline methyl violet stain to determine the percentage of sperm abnormalities by using the criteria of Okamura *et al.* (2005).

Histopathology and Seminiferous Tubules Diameter

Specimens from testis were collected from all experimental groups and fixed in 10% neutral buffered formalin, dehydrated in ascending concentrations of ethyl alcohol (70-100%) and then prepared using standard procedures for Hematoxylin and Eosin staining as described by Bancroft *et al.* (1996). In each testis the diameter of 30 randomly selected seminiferous tubules was measured using Image Analysis software (Visual measure 32 for Windows, Version 1.7, Rise Corporation, Japan). Only tubules with visible lumen were measured.

Statistical Analyses

Statistical analyses were performed by using GraphPad Prism Version 5.01 (GraphPad Software, San Diego, CA, USA). Data are presented as means with their standard error. Normality and homogeneity of the data were confirmed before ANOVA, differences among the experimental groups were assessed by one-way ANOVA followed by Duncan's test.

Results

Weight of Reproductive Organs

There were no difference in testicular weight among control and treated rats with CdCl₂, while a significant decrease ($p<0.01$) were observed in the weights of epididymis, seminal glands and prostate specifically in rats treated with 100 ppm of CdCl₂ compared with that in control rats (Fig. 1).

Sperm Cell Concentration and Sperm Abnormalities

There were remarkable reductions ($p<0.001$) in the sperm cell concentration in rats treated with 5, 50 and 100 ppm compared with that in control rats (Fig. 2). The number of normal spermatozoa was reduced significantly ($p<0.01$) in treated rats compared with that in control rats. Moreover, the numbers of secondary and total abnormalities were increased significantly ($p<0.01$) in treated rats with CdCl₂ compared with that in control rats (Fig. 3).

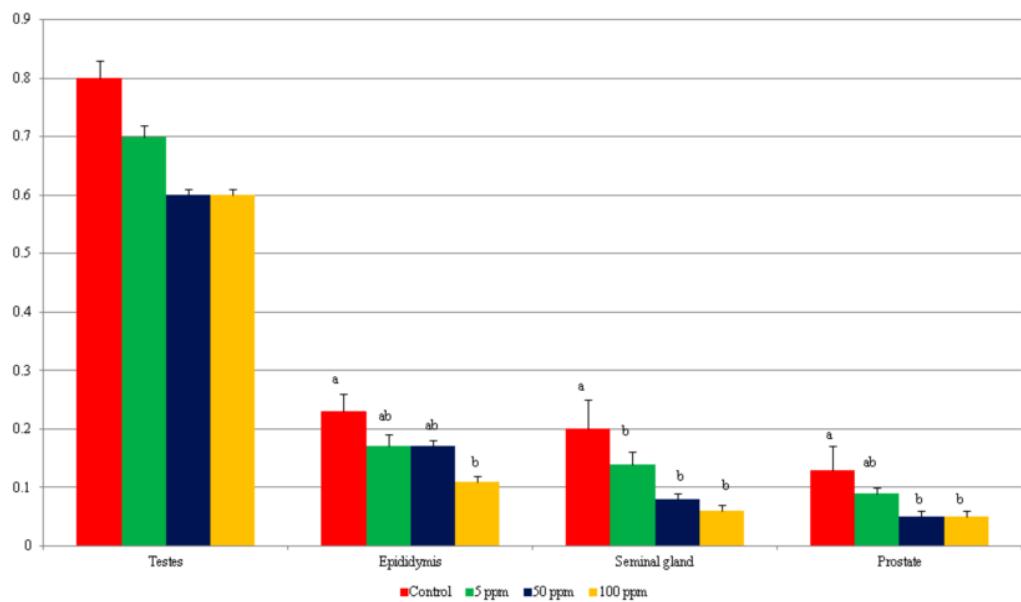


Fig. 1. Weight of reproductive organs (gm) in control and treated rats with different doses of cadmium chloride

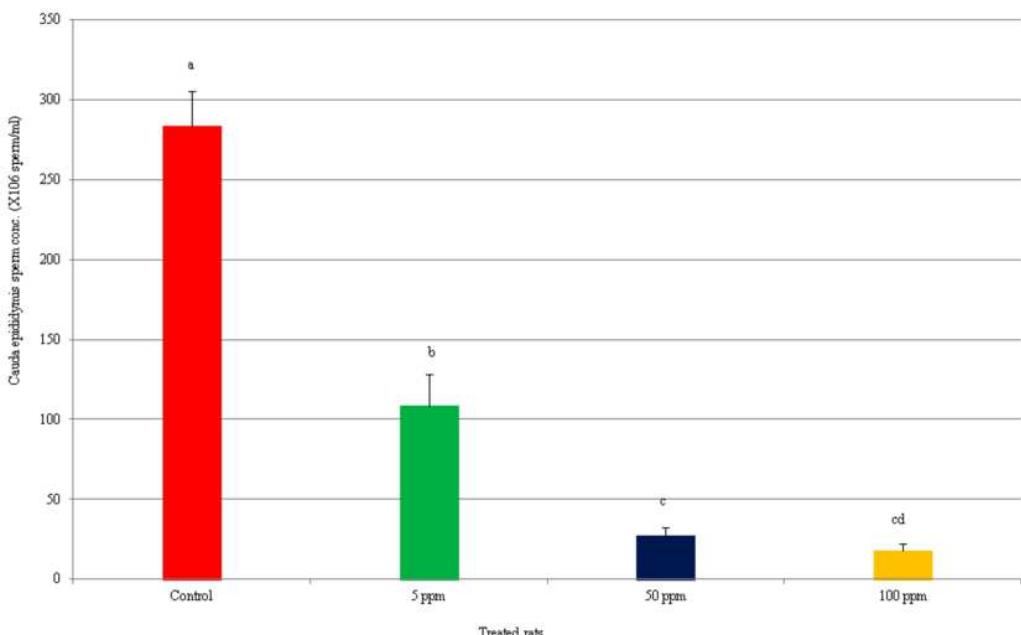


Fig. 2. Cauda epididymis sperm cell concentration in control and treated rats with different doses of cadmium chloride

Testicular Histopathology

Control rat showed normal structure and well preserved seminiferous tubules. Rats treated with cadmium exhibited degenerated and reduced the diameter of seminiferous tubules. Moreover, treated rats with 100 ppm cadmium chloride showed multinucleated cells inside the tubules. Shrunken, buckled, disorganized seminiferous tubules,

vacuolation and sloughing of the germinal epithelium of seminiferous tubules were also observed (Fig. 4).

Seminiferous Tubules Diameter

Rats treated with different doses of CdCl₂ (5, 50 and 100 ppm) showed a significant reduction ($p<0.001$) in the diameter of seminiferous tubules compared with that in control rats (Fig. 5).

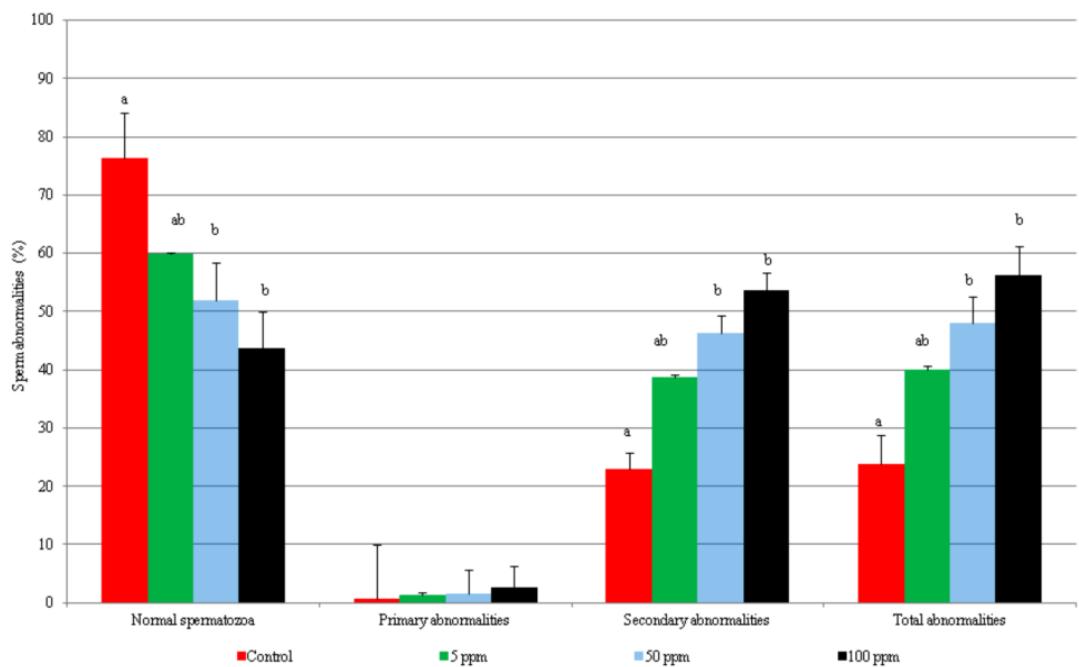


Fig. 3. Sperm abnormalities in control and treated rats with different doses of cadmium chloride

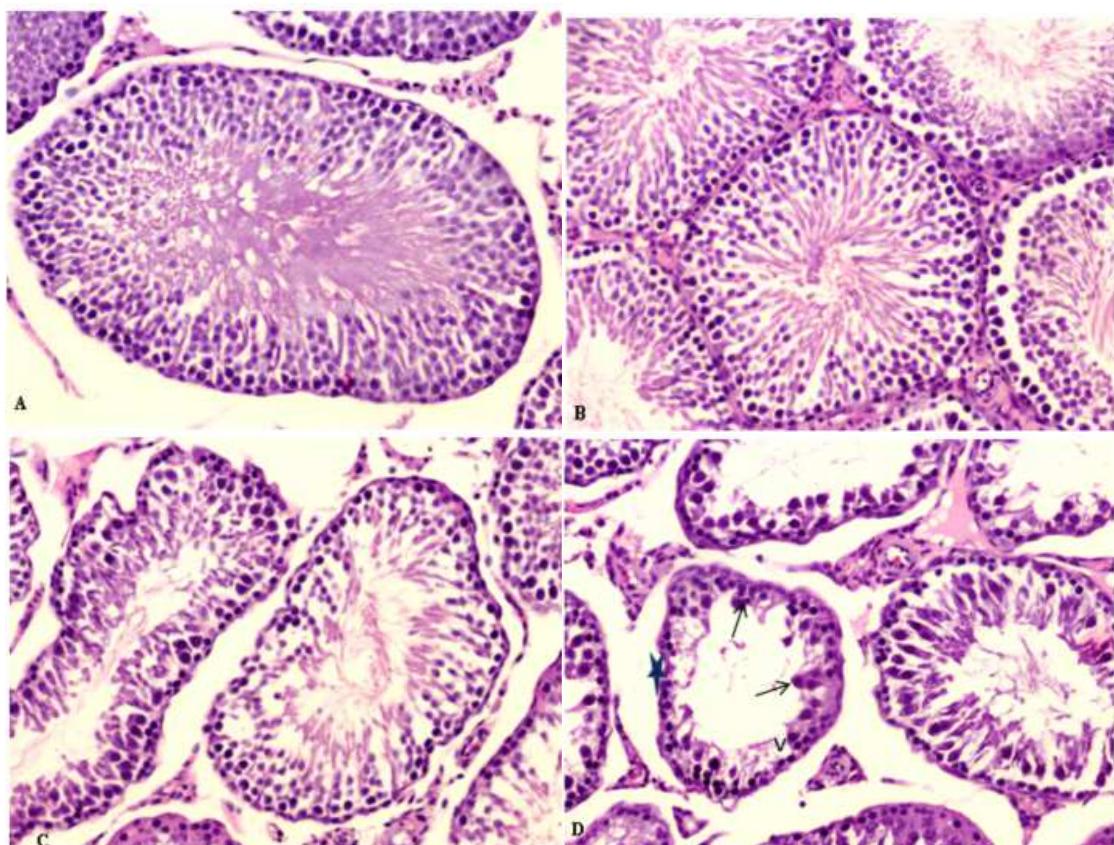


Fig. 4. Testis of control (A) and treated rats with cadmium chloride 5 ppm (B), 50 ppm (C) and 100 ppm (D), in which multinucleated cells were shown inside the tubules (arrows). Shrunken, buckled, disorganized sominiferous tubules, vacuolation (v) and sloughing (star) of the germinal epithelium of sominiferous tubules, (200X)

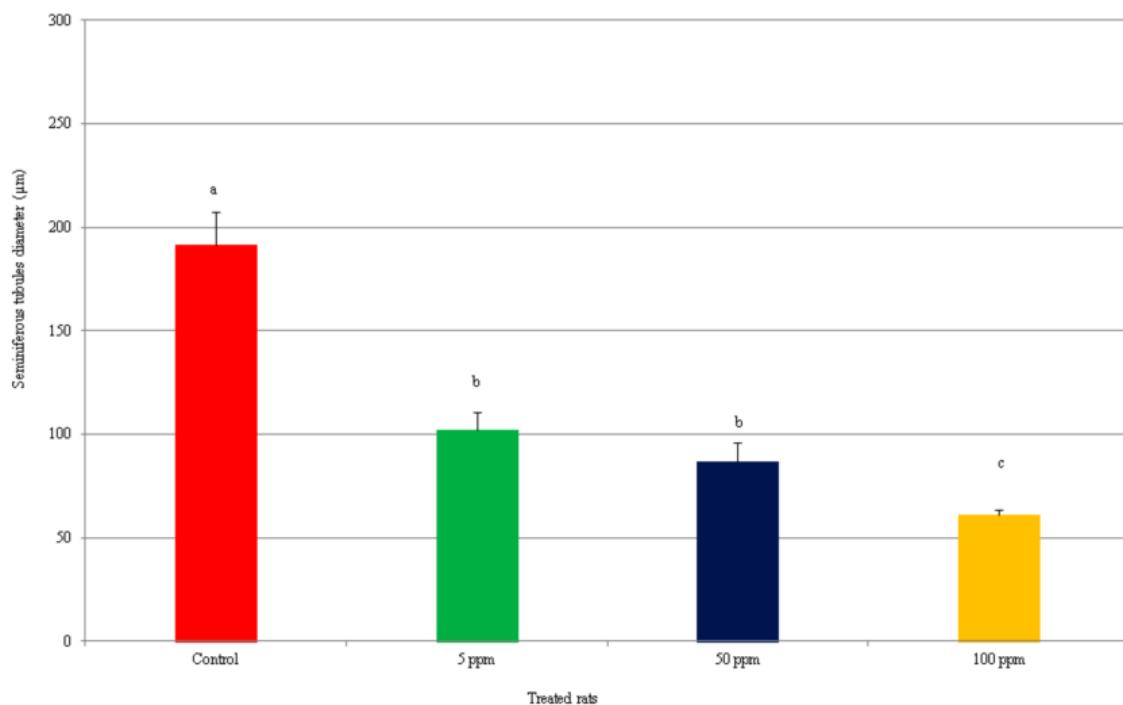


Fig. 5. The diameter of seminiferous tubules in control and treated rats with different doses of cadmium chloride

Discussion

In the present study, oral administration of CdCl₂ even in a low dose of 5 ppm altered the testicular functions through the reduction of reproductive organs weights, declining the sperm cell concentration, increasing the sperm abnormalities and reducing the diameter of seminiferous tubules. Moreover, the present results showed that the toxic effects of Cd in the male reproductive system were dose-dependent. The difference between the doses is small, but the morphological and morphometrical results are clearly very different. This suggests that the defenses of the testis against Cd contamination are efficient up to a very precise level. Siu *et al.* (2009) and Xu *et al.* (2005) affirmed that Cd treatment induces metallothionein production, but this appears to be limited to a certain level in the testis. Toxic effects probably result as the amount of MT becomes insufficient to bind with the Cd present, resulting in oxidative stress and disruption of spermatogenesis (Xu *et al.*, 2005). Studies of the consequences of Cd contamination have demonstrated that the testis is more sensitive to Cd and low doses (with no detectable effects on general health) can interfere with testis function (Blanco *et al.*, 2007). These observations agree with our results showing that the weight and morphology of the testis were clearly affected only by the higher dose of Cd. The previous studies of acute Cd exposure have reported diminished

testicular weight in relation to the Cd dosage. These studies attributed this effect to the necrotic and degenerative cadmium-induced changes (Blanco *et al.*, 2007). Reduction of testes-body weight ratio in Cd-intoxicated rats indicated testicular damage and impaired function (Ponnusamy and Pari, 2011). Moreover, the previous studies (Gupta *et al.*, 2003; El-Demerdash *et al.*, 2004) have reported weight reductions of accessory sex organs after Cd administration. In this study, after the higher dose of cadmium, the seminal vesicles and prostate reduced their weights.

In histological analysis, the absence of tubular lumen, germ cell loss and presence of multinuclear giant cells were observed. These findings for the higher dose of Cd were in accordance with the previous studies (Biswas *et al.*, 2001; Yang *et al.*, 2006; Blanco *et al.*, 2007). When massive cellular loss from seminiferous epithelium occurs, a sharp decline in testicular morphometric parameters can be verified (Franca and Russell, 1998). Indeed, this decline has also been observed in the current work. A positive relationship usually exists between the tubular diameter and the spermatogenic activity of the testis (Sinha-Hikim *et al.*, 1988; Franca and Russell, 1998). Our data showed a marked reduction of seminiferous tubular diameter after the higher dose of Cd. Taking into account the fact that the weight of the testis was also reduced, it can be deduced that the total length of seminiferous tubule clearly diminished as a consequence of cadmium exposure. Supporting these results, Franca and Russell (1998) stated

that the total seminiferous tubule length is related to three structural parameters: testis size, tubular diameter and seminiferous tubule volume density. Testicular changes due to Cd toxicity have been seen in a variety of animal models at different stages of growth and maturity. Cd-induced testicular pathogenicity includes severe haemorrhage, edema, necrosis and atrophy, as well as reduction in counts and motility of sperm and decreased the testosterone concentrations in plasma and testes (Santos *et al.*, 2006; Thompson *et al.*, 2008). In experimental models, Cd exposure can affect testis weight and induce pathogenesis leading to reduced sperm counts and impaired sperm motility to adversely affect male fertility (Waisberg *et al.*, 2003; Thompson *et al.*, 2008).

Conclusion

Our findings showed the progressive morphological and morphometrical alterations caused by different doses of CdCl₂ because of their direct effect on rat testis. Our results highlight the direct relationship of dose and time lapse to morphological alterations. Moreover, the difference in doses caused very different degrees of damage, apparently overcoming the natural defenses of this tissue.

Author's Contributions

All authors equally contributed in this work.

Ethics

No part of this manuscript is being considered for publication in whole or in part elsewhere. All authors have read and approved of the manuscript. There is no conflict of interest between the authors.

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