

THE EFFECTS OF CADMIUM CHLORIDE (CdCl_2) ON THE FUNCTIONS OF REPRODUCTIVE SYSTEM OF THE MALE MICE

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Abstract

This study was designed to evaluate the effects of cadmium chloride (CdCl_2) on male reproductive system of Swiss albino mice. Forty male Swiss albino mice were divided randomly in two groups (treatment & control groups) . All animals were subcutaneous injected every four days for four weeks. The treatment group was injected with 2.5 mg/kg body weight from cadmium chloride solution, while the control group injected by normal saline solution. All animals of the control and treatment groups were weighted and sacrificed after the end of the experimental period, blood samples were collected and the testes, epididymes and seminal vesicles were dissected out and weighted. Hematoxylin-Eosin stained sections were prepared from these organs for light microscopic investigation. Body weight means of CdCl_2 -treated mice was showed significant reduction compared with control group ($p > 0.001$). However, combined testicular-epididymal and seminal vesicular weight means of CdCl_2 -treated mice were significant decreased compared by control group ($p > 0.001$, $p > 0.001$ respectively) and there were a significant decrease in levels testosterone compared with control ($p > 0.001$). Stained histological sections of testes and epididymis of CdCl_2 -treated group reveal a degradation of seminiferous tubules and deformity in spermatogenesis as well as absence of spermatozoa, decrease in the seminal vesicular secretion was noted in CdCl_2 -treated mice.

Introduction

Cadmium is a heavy metal that occurs widely in nature as a contaminant of zinc. As trace amount of cadmium are naturally present in water as well as in plants and animals. Human are inevitably exposed to it through the food chain. In addition, it is commercially produced and used as a paint pigment, in electroplating and in electrical batteries. Because cadmium is non-biodegradable and has a biological half-life of more than ten years, it is considered an accumulative toxicant. Cadmium produces apoptosis and necrosis in the liver (Min *et al.*, 2002), edema in the lungs and hemorrhagic necrosis in the testis (Harbison , 1998; Liu *et al.* ,2001). Chronic exposure to cadmium cause damage primarily the kidney but is also toxic to the liver and bone. Less well described are the neurotoxin effects of cadmium (White *et al.* , 1990).

Key words: cadmium; reproductive; male mice.

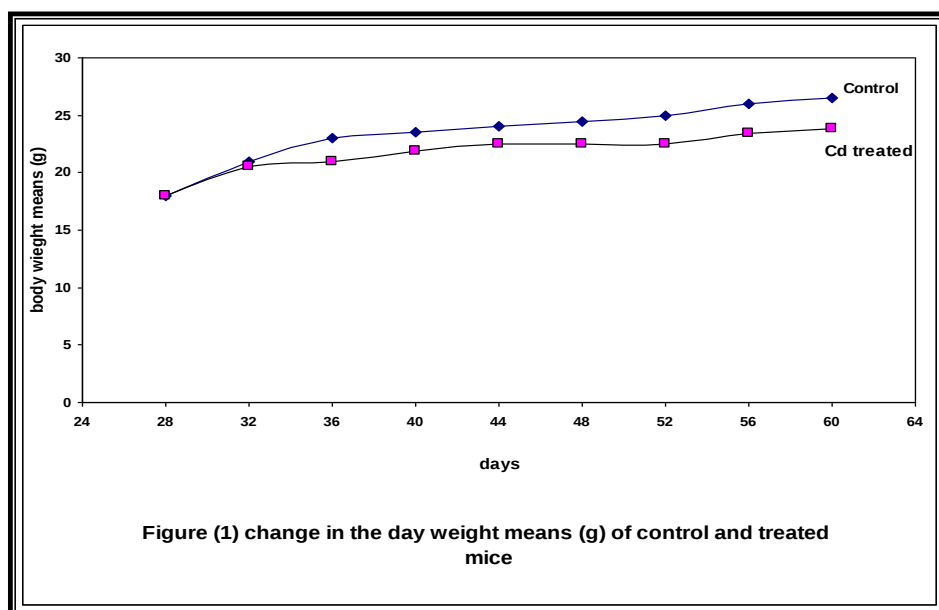
Material and Methods

Forty male mice with ages of four weeks were randomly divided into two groups. Control and treatment groups. Each groups contained twenty animals, all animals were kept under the laboratory conditions (temperature, Relative humidity and light) and supplied with the rearing diet and water *ad libitum*. Animals of the different groups were weighted and subcutaneously (Sc) injected after every four days for 32 days. The treatment group was injected with 2.5 mg cadmium chloride per kg/ body weight (B.W) , while control animals were injected with normal saline through the same route. Chemical reagent which used in the study were obtained from Merck Chemical Company – Germany. After the end of each period the animals were weighted and were further exposed to ether until anesthesiation. The

testes, epididymes and seminal vesicle from all animals were dissected out, washed with normal physiological saline solution, dried with filter paper and weighted (mg organ /100g body weight) with (GMBH) sensitive balance. After washed the tissue samples were transferred into formalin-alcohol-acetic acid (F.A.A) fixative solution and kept for histological study. Right after killing the mouse with ether the animal was held in the head down position and by a sharp scissors, head was completely cut off, where the seeping blood was collected in tubes without anticoagulant in order to get serum to determine the testosterone hormone. The serum were obtained by leaving the blood to clot at room temperature, then centrifuged in laboratory centrifuge model 800,XIAHE at 3500rpm for 15 minutes, than the serum was analyzed to determined the testosterone hormone using Elecsys Testosterone Reagent kit, Cat. No 11776061-100test,B;Roche Diagnostics GmbH.D-68298 Mannheim, Germany. For use on the Roche Elecsys 2010 TMMUNDASSAY ANALYZERS. The histological sections were carefully examined under a light microscope using different magnification powers. Microscop camera unit (Carl Zeiss, Germany) was employed to microphotograph selected field of the stained tissues. Data were presented when appropriate as mean $\pm$ standard deviation and differences between two groups by t test. The accepted level of significance was $P < 0.05$. All analysis were done by statistical package for social science (SPSS).

Results and Discussion

Control mice continued gaining body weight (means $\pm$ S.D) at consistent increments during the period from post-delivery day 28 to day 60 (table 1 and figure 1). The result showed that before starting the injection period on age day28, body weight of Cd-treated animals (19.045 ± 1.015), did not differ from that of the control mice (19.1 ± 0.844). On the following days, body weight means were significantly reduced in treated groups animals as compared with the corresponding means of the control animals (table 1and figure1). The final body weight means,96hours after the last injection for Cd, (22.14 ± 1.504) were significant reduced comparing with control (26.175 ± 0.788) with $p=0.000$ this decrease correspond with (Lafuente *et al.*, 2000). On the other hand, Cd cause hyperglycemia and anemia (Harbison, 1998), withal Cd draw on change in metabolise so it inhibits both oxidative phosphorlation and the activities of glucose-6-phosphate dehydrogenase (Shuka *et al.*, 1988). Because Cd interference with the essential metals in the enzyme and this may decrease of the activity of various enzymes (Casalino *et al.*, 1997; Patra *et al.*, 1999). Result in (Table 2; figure 2) show that Cd caused significant reduction in testicular-epididymal (0.309 ± 0.133) and seminal vesicle weights (0.153 ± 0.0814) compared with control (0.710 ± 0.099 ; 0.3414 ± 0.0731 respectively), these results agree with the finding of (Kojim *et al.*, 1992). The decrease in testicular-epididymal weight could be attributed to the above mentioned effects of Cd on metabolism, or that Cd caused vascular changes in testes could lead to hemorrhagic, ischemic, hypoxia then necrosis followed finally by the testicular atrophy (Steinberger and Klinefelter, 1993; Thomas, 1995). The decrease in seminal vesicle weight (figure3) as (0.3414 ± 0.0731) for control compared with (0.153 ± 0.0814) for Cd-treated animals may be because of Cd reduction in body weight as observed in this study. In addition to that decreasing levels of the follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone hormone (Lafuente *et al.*, 1997, 1999, 2000) after exposure to Cd and because of theses hormone are necessary for normal development of the male reproductive organs structural- functional aspects (Guyton and Hall, 1996; Ganong, 1997).



The testosterone hormone concentration (ng/ml) in mice treated with CdCL₂ (0.0403+₋ 0.0263) was reduced a compare to control (4.6906+₋1.302) group (p=0.000) (table 3, figure 4), the finding similar to (Laskey *et al.* , 1984 and Lafuente *et al.* , 2000) in rats. Reduction in Testosterone hormone was manifested by lowered FSH and LH plasma levels (Davidson *et al.* ,1993 ; Paksy *et al.* , 1996 ; Lafuente *et al.* ,1997,1999,2000), as well as, Cd decrease the total testosterone hydroxylase activity (Clark *et al.* , 1994) whose caused a dramatic decreasing in testosterone hormone. In addition this downturn may be response to significant decrease in the h CG – stimulated serum testosterone (Laskey *et al.* ,1984), interfere of Cd with c AMP in testis and depression of protein kinase (Singhal *et al.* , 1976). Further study found that Cd caused necrosis in Leydig cells (Laskey *et al.* , 1984), these cells count primary synthesis site to testosterone hormone (Guyton and Hall ,1996).

Table 1 . Body weight means (gm) of control and treated mice .

DAYS	CONTROL	CD- TREATED
* 28	19.1 ± 0.844	19.045 ± 1.015 #(p > 0.05)
32	20.89 ± 0.828	20.5 ± 1.794
36	22.62 ± 0.868	21.09 ± .289
40	23.125 ± 0.853	21.485 ± 1.918
44	23.715 ± 0.913	22.55 ± 1.652
48	24.37 ± 0.9002	22.88 ± 1.67
52	24.95 ± 0.9577	22.72 ± 1.67
56	25.5 ± 0.769	22.74 ± 1.143
** 60	26.175 ± 0.788	22.14 ± 1.504 #(p > 0.05)

Mean + S.D. n=20.

* Body weight was recorded immediately before the first injection .

** Body weight was recorded 96 hours after the last injection .

As compared with control , (p > 0.05) .

Table 2. Testicular-epididymal and Seminal vesicular weight means (mg/100g body weight) of control and treated mice

Organ	CONTROL	Cd-TREATED
Testicular-epididymal	0.710±0.099	0.309±0.133 (P=0.000)*
Seminal vesicular	0.3414±0.0731	0.153±0.0814 (P=0.000)*

Mean + S.D. n=20

- * As compared with control.
P< 0.05

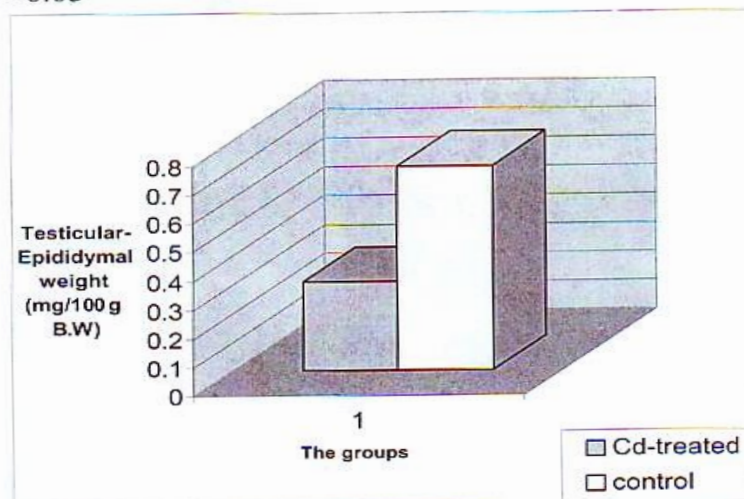


Figure 2: comparison of Testicular-Epididymal weight (mg/100g body weight) means of control and treated mice.

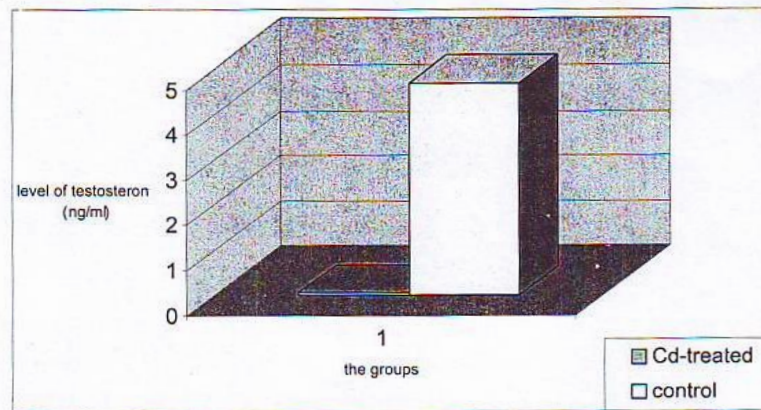


figure 4: comparison of means of testosterone

level ng/ml of control
and treated animals.

The histological results in Cd-treated group showed damage and necrosis of seminiferous tubules in testes (plate 2). These results agree with the findings of (Hew *et al.* , 1993; Rikans and Yamano, 2000) and might be accounted that Cd due to primary vascular injury. Damage to the testicular vascular endothelium causes increased permeability across capillaries and venules, elevated intratesticular pressure, and decreased blood flow within the testes, resulting in necrosis (Wong and Klaasse, 1980; Nolar and Shaihk, 1986a). On the other hand testicular damage caused by Cd is attributed to compete of Cd with Zn in Zn-containing enzymes and decreased activity of Testis-specific enzymes (Verbast *et al.* , 1988; Caslino *et al.* , 1997). The testicular section of Cd-treated mice investigated impair of spermatogenesis. This result similar to the observation of (Xu *et al.* , 1993 and Chia *et al.* , 1994) could be traced to obliterated hypothalamus-pituitary-testicular axis by Cd (Massanyi *et al.* , 1995 ; Telisman *et al.* , 2000) and the above mentioned effects of Cd on plasma levels of FSH, LH and testosterone hormone. Low active epithelium with lumens free of spermatozoa of epididymal sections (head, body and tail) of Cd-treated animals (plate 4, plate 6, plate 8 respectively) compared to plate 3, 5 and 7 to control animals respectively which indicated decrease plasma level of testosterone hormone, whereas reduced testicular testosterone output impairs normal features of the accessory reproductive organs that are depending on androgens to maintain normal structure and function. Less active epithelium with reduced amount of secretion are the characteristic features of the seminal vesicles from mice of Cd-treated group (plate 10) in contrast with control sections (plate 9). These results might be associated with a degree of reduction in the weight of seminal vesicles observed in this study and the study of Laskey *et al.* , (1984) on the other hand, Significant reduction in testosterone hormone concentration might help in interpret the changes in the seminal vesicles.

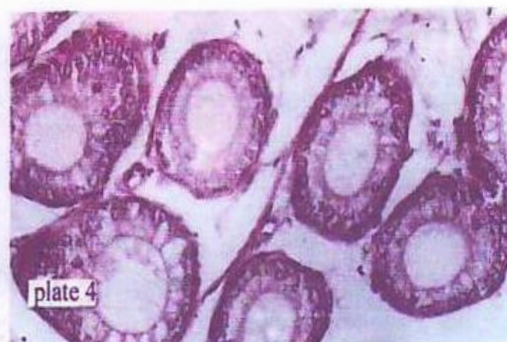
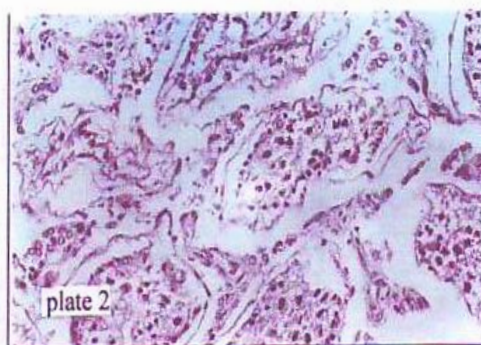
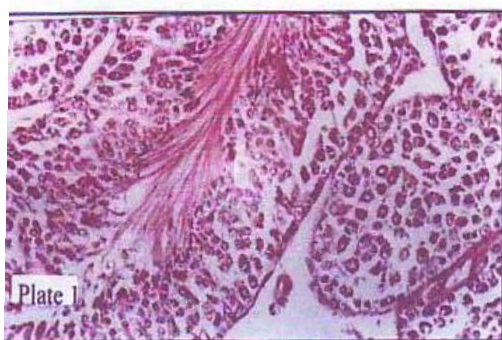


Plate 1 : Histological section of mouse control testis with clear spermatogenesis and spermatozoa in tubular lumens(H.&E. x 317).

Plate 2 : Section of Cd-treated mice with extensive tubular degeneration, necrosis and absence of spermatogenesis(H.&E. x 312).

Plate 3 : Histological section of head of control epididymis , the epididymal tubules show active epithelium with long Stereocilia.(H.&E. x 500).

Plate 4 : Cd-treated head of epididymis , the tubules have low active epithelium with individual cell necrosis and few Stereocilia could be observed. (H.&E. x 317).

Plate 5 : The body of epididymal tubules of control mouse have active epithelium and lumens filled with sperms. (H.&E. x 317).

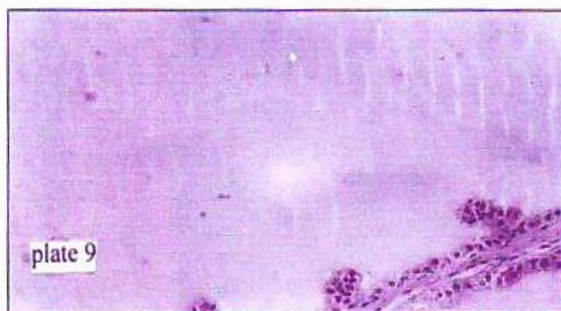
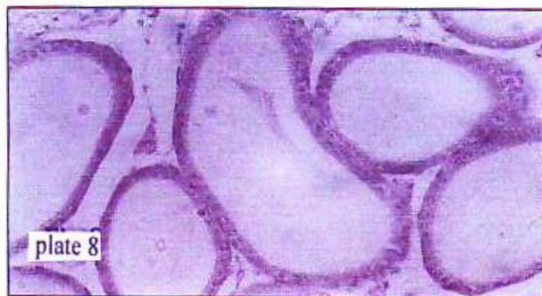
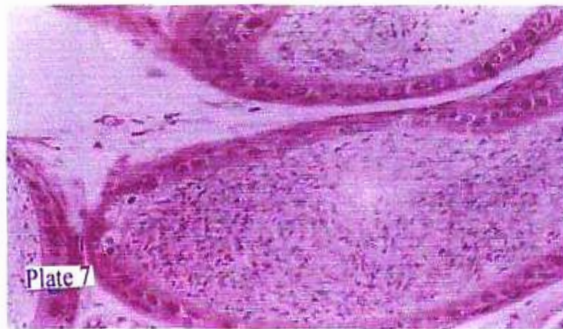
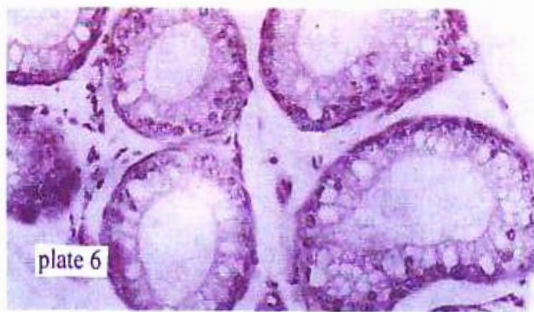


Plate 6 : Histological sections of the body of epididymis treated with Cd, showed no spermatozoa in the lumen with damaged epithelial cells. (H.&E. x 317).

Plate 7 : Histological section of the tail of the control epididymis showed normal epithelium with prominent spermatozoa concentration. (H.&E. x 500).

Plate 8 : Epididymal section of the tail region of Cd-treated mouse with no spermatozoa in the lumen. (H.&E. x 317).

Plate 9 : Seminal vesicles of a control mouse. The vesicles are filled with large quantities of secretion reflecting the secretory activity of the epithelial cells. (H.&E. x 317).

Plate 10 : Significant reduction in amount of secretion and reduction of secretory epithelium are obvious in seminal vesicular section of Cd-treated mouse. (H.&E. x 317).

Conclusion

Results of this study have revealed an insult to structural and functional of reproductive system of the male mice associated with exposure to 2.5 mg/kg B.W, sc

dose of CdCL₂ . Whereas, combined relative testicular-epididymal and seminal vesicle weights of animals have significantly reduced by Cd. Cd has revealed changes in histological study of the testis, seminal vesicle and epididymal and restraint of spermatogenesis, as well as Cd caused subsidence in testosterone hormone concentration in blood. On the other hand, the significant devaluation of body weight have pointed after Cd injection. Whereas , precession we conclude that Cd could be affect both hypothalamic-pituitary-testicular axis and the testes directly.

تأثير الكاديوم على عمل الجهاز التناسلي الذكري للفئران د . سعد جبارة كاطع فائزة بلقاسم الحرابي قسم البيئة / كلية العلوم / جامعة الكوفة

الخلاصة :

هذه الدراسة صممت لتقييم التأثيرات التي يحدثها كلوريد الكاديوم (CdCl₂) على عمل الجهاز التناسلي الذكري للفئران السويسرية البيضاء . استخدمت في هذه الدراسة (40) فأر ذكر . قسمت الحيوانات عشوائياً إلى مجموعتين هي مجموعة السيطرة و مجموعة المعالجة بمعدل (20) حيواناً لكل مجموعة وحقت الحيوانات تحت الجلد كل أربعة أيام ولمدة أربعة أسابيع . حيوانات التجربة حققت بجرعة قدرها 2.5 ملغم /كغم من وزن الجسم من محلول كلوريد الكاديوم بينما حيوانات مجموعة السيطرة حققت بالمحلول الملحي الفسيولوجي . قتل جميع الحيوانات في نهاية الدراسة . جمع الدم وزنت الحيوانات وكذلك كل من الخصى والبربخ والحوصلات المنوية بعد ازالتها . عملت شرائح نسجية من النماذج وصبغت بصبغة الهيماتوكسيلين ايوسين وفحصت تحت المجهر الضوئي . أظهرت نتائج الدراسة انخفاضاً ملموساً احصائياً في وزن حيوانات المعالجة وكذلك معدل مجموع اوزان كل من الخصى والبرابخ مع الحوصلات المنوية لحيوانات مجموعة المعالجة (p > 0.001) بالمقارنة مع مجموعة السيطرة . كما بينت الدراسة انخفاض حاد في كمية هرمون التستوستيرون (p > 0.001) في الحيوانات المعالجة بالمقارنة مع حيوانات السيطرة . الشرائح النسيجية للحيوانات أوضحت انخفاضاً في الأنبيبات المنوية في الخصى مع تغيير في عملية التخليق للحيوانات المنوية ، وكذلك فقدانها مع انخفاض في افرازات الحوصلات المنوية بالمقارنة مع انسجة حيوانات مجموعة السيطرة .

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