Exposure of Adult Male Rats to Cadmium: Assessment of Sexual Behaviour, Fertility, Aggression as well as Anxiety like Behaviour with Special Reference to Biochemical and Pathological Alterations.

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Abstract: Because Cadmium is widely used in industry and in our daily life, it's likely that many people are exposed to it . The main aim of this study is to further investigate the effects of cadmium on sexual and aggressive behaviour as well as anxiety – like behaviour in adult male rats. Forty five male Wistar rats weighing (140-160 g) were administered $CdCl_2$ in drinking water at one of three concentrations: 0, 5 and 5o mg / L for a period of 12 weeks .Sex organs tissues (testes, prostate glands & seminal vesicles), representing all treatments were taken for biochemical , histopathological examination and male fertility assessment (semen quality analysis). Results revealed marked impairment in sexual activity with noticed influence on both territorial aggressive behaviour and anxiety – like behaviour in males exposed to $CdCl_2$. These behavioural alterations were paralleled by biochemical changes, showing that $CdCl_2$ at concentrations (5mg) and (50 mg) induced a serious decrease in the level of testosterone and a significant elevation in serotonin. Additionally, increased oxidative stress in testicular tissue. Poor semen quality (sperm count, sperm motility, sperm viability) was observed in the treated male rats. furthermore, histopathological alterations were observed in the testes, prostate gland and seminal vesicles of the cadmium treated rats . Our results strongly suggest that Cd intoxication produces adverse effects on sexual behaviour , aggression , fertility and anxiety – like behaviour , with biochemical and pathological alterations in adult male rats.

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1. Introduction

Cadmium is one of environmental pollutants arising from electroplating, fertilizers, pigment and plastic manufactures. Therefore it is easily contaminate the soil, plants, air and water (Ognjanovic *et al.*, 2008). Humans and animals can easily expose to cadmium toxicity by consuming plants, water and air. Cadmium absorbed and accumulates in various tissues (Casalino *et al.*, 2002, Waisberg *et al.*, 2003) even red blood cells (Kostic *et al.*, 1993), the heart (Zikic *et al*., 1998) and the skeletal muscle of rats (Pavlovic *et al*., 2001). Cadmium is considered as ubiquitous toxic metal that induce oxidative damage by disturbing the peroxidant – antioxidant balance in the tissue (Ognjanovic *et al*., 2008). Moreover, Cadmium is a recognized reproductive toxicant and has been reported to reduce male fertility and altered sexual behaviour in both humans and rodents (Thomas and Brogan, 1983). Most animals with scrotal testes are susceptible to cadmium – induced testicular toxicity (King *et al*., 1999). Testes are included among the most target organs for cadmium intoxication (Stajn *et al*., 1997). Rodent testes are more susceptible to cadmium toxicity than liver, as manifested in testicular damage without pathological changes to other organs (Adaikpoh and Obi 2009). Exposure to cadmium can negatively affect the male reproductive system via degenerative changes in testes, epididymis, and seminal vesicles (Ibrahim and Sameh 2002). Recently, Azoospermic persons were found to have higher serum and seminal plasma cadmium level compared with oligospermic ones (Oluyemi Akinloye *et al*., 2006). Also positive relationship was found between cadmium exposure and asthenozoospermia in a rat model (Benoff *et al*., 2008).

Since aggressive behaviour are controlled by androgenic hormones (Clark and Henderson, 2003). It was hence concluded that the different parameters of aggression and violent are likely to be involved in this study. Exposure to toxic metals has been reported to affect aggressive, antisocial violent behaviour (Melvyn, 1995). Moreover, oral exposure to cadmium can cause anxiety and fear as well as alterations in the biochemical activity of the brain (Bull, 2010).

To the best of our knowledge, only a few studies have addressed the effect of orally administered cadmium chloride on both sexual and aggressive behaviour in male rats. Furthermore, the involvement of various arrays of measurement to evaluate anxiety and emotionality in rats treated with cadmium is not well implemented.

Thus, the objectives of the current study were to evaluate the impact of elevated levels of cadmium chloride in drinking water on sexual and aggressive behaviour in adult male rats. Moreover, biochemical and histopathological evaluation were carried out to detect the degenerative changes and oxidative damage in male reproductive system. Additionally, long – term changes in anxiety – like behaviour were monitored.

2. Material and Methods:

2.1. Animals and housing:

Forty five Wistar male albino rats weighing 140- 160 g.were used in this study. Animals were raised in the Animal House Unit in Faculty of Veterinary Medicine, Cairo University. They were maintained in plastic cages with stainless steel wire lids (bedded with wood shavings). Food and water were supplied ad libitum. Rats were housed at a controlled temperature of 21 ± 1ºC, 60 % humidity and under a 12 -hr - light: 12- hr- dark schedule. Animal care was in compliance with the applicable guidelines from Cairo University Policy on Animal Care and Use.

2.2. Administration of Cadmium:

Cadmium Chloride ($CdCl_2$) in crystalline form was obtained from Sigma Chemical Company (Sigma, Aldrich). $CdCl_2$ was dissolved in tap drinking water at different concentrations, namely ; 5 mg / L (low dose) and 50 mg / L (high dose) (Waalkes *et al*., 1999). Male rats were randomly assigned to three groups of 15 animals each (control or experimental groups). Experimental male rats were provided access to drinking water containing $CdCl_2$ for 12 weeks .The control group received tap water only.

2.3. Behavioural assessment:

2.3.1. Sexual behaviour testing:-

Each male rat was tested for sexual behaviour with a stimulus female (induced estrus).Female rats of the same strain were used in this experiment receiving subcutaneous treatment of estradiol benzoate 5.00mg/rat and progesterone 0.5mg/rat dissolved in 0.2ml sesame oil 52 and 4 h before the test sessions, respectively. Male rat was observed alone for 5min, an estrus female was then introduced in the centre of the arena and the behaviour of the male was then recorded. The time to the first mount and the number of mounts. Also ejaculatory latency, number of ejaculation and post-ejaculatory interval (latency period) were observed (Cagiano *et al.*, 1998; Nabil and Zeyad 2005).

2.3.2. Aggressive behaviour testing:

A rectangular observation cage (45×27× 40 cm) was used for aggression assessment. A stud male rat was placed in the testing arena for 10 days. A second male (control or CdCl treated) was then placed in the test arena with the stud male for 5 min. and the following parameters were recorded:lateralization, boxing bouts ,fights with stud male ,tooth chattering and leaping (Batainch *et al.*, 1998 and Nabil and Zeyad 2005).Animals were observed between 09:00 and 15:00 hr. and all behavioural measures were monitored by a single observer unfamiliar with the cadmium treated males.

2.3.3 .Fear and anxiety measurements:

The elevated plus-maze was used for testing of anxiety and emotionality. The degree of avoidance of the open arms of the maze considered a measure of the strength of a fear drive (Trullas and Skolnick,1993).The apparatus consists of 4 crossed arms, two open arms (50×10× 0 cm)and two closed arms (50×10×30 cm).The maze was elevated 0.65m above the floor. The rat was placed in the centre of the maze and the number of entries in open and closed arms, respectively, as well as the time the animal spent in the open and enclosed arms during a period of 5 minutes was recorded. All testing took place during the first 6 h of the dark phase of the L: D cycle. After each trial the maze was wiped with a cloth dipped in 70% ethyl alcohol and allowed to dry (Kierstin, 2003).

2.4. Male fertility assessment:

2.4.1. Sex organs weight:

Five rats from each group were sacrificed at the end of experiment. The testes, prostate gland and seminal vesicles were dissected and weighed in relative to body weight.

2.4.2. Semen quality analysis:

Seminal content of epididymis was obtained by cutting of the cuda 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 concentration (Bearden and Fluquary, 1980). One drop of the suspension was smeared on a glass slide and stained by Eosin – nigrosin stain to determine the percentage of sperm cell viability and morphological abnormalities (Miller and Pass 1952). Abnormal head

and tails were evaluated by using the criteria of Okamura *et al*. (2005).

2.5. Biochemical assessment:

2.5.1. Testosterone and Serotonin assay:

On completion of all behavioural assessments, five male rats per treatment were randomly selected to obtain blood samples (orbital plexus of rats) .Blood was then centrifuged at 2000 rpm for 15 min and the serum was stored at -20 °c. Testosterone was estimated in the serum as described by Ismail (1986).Also , serotonin was assessed by an improved Miller's fluorophotometry method (Zhang *et al*., 1994) .

Briefly, 1.8 ml acidified *n*-butanol (adding 0.85 ml 12 M HCl per liter *n*-butanol saturated with NaCl) was added to 0.2 ml serum, vortexed for 5 min, centrifuged at 3000 rpm for 10 min. 1.5 ml supernatant was collected, added with 1.5 ml *n*-heptane and 0.5 ml 0.1 M HCl, vortexed for 5 min, centrifuged at 3000 rpm for 5 min. After the supernatant phase (mainly *n*-heptane) was discarded, 0.25 ml aqueous phase, drawn from the bottom, was mixed with 0.05 ml 82.4 mM (10 g/l) l-cystine and 0.75 ml 60 mg/l *o*-phthaldialdehyde (OPA, prepared with 10 M HCl), and kept in boiling water for 10 min, then put into icy water to stop reactions. Fluorescence was measured in a spectrophotofluorometer (1420 Mutilabel HTS, PerkinElmer, USA) using 96-well plate. Excitation and emission wavelengths were 355 and 460 nm, respectively. The standard line was made using serotonin creatinine sulfate complex dissolved in 0.01 M HCl following the same procedure above.

2.5.2. Oxidative stress assessment:

Testicular tissue specimens were homogenized in 9 fold volumes phosphate buffered solution (PH 7.4). The homogenate was then centrifuged at 4000 rpm for 15 min at 4 °C and the supernatant was kept at -80°c until used in the studied enzymatic assay.

2.5.2.1. Determination of super oxide dismutase (SOD) activity:

Superoxide dismutase (SOD) activity was measured according to Giannopolitis and Ries (1977) by means of SOD assay kit (Cayman, MI, USA) according to manufacturer's instructions. The kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD was defined as the amount of enzyme needed to produce 50% dismutation of superoxide radical. The SOD assay measures all the three types of SOD (Cu/Zn, Mn, and FeSOD).Enzyme activity was determined as the amount of the enzyme required to induce 50% inhibition of nitro – blue tetrazolium (NBT) reduction rate.

2.5.2.2. Testicular lipid peroxidation :

The level of lipid peroxidation in terms of TBARS formation (nmoles / min / mg protein) was determined (Esterbauer and Cheeseman , 1990).

One volume of testicular homogenate was mixed with 2 volumes of cold 10% (w/v) trichloroacetic acid to precipitate protein. The precipitate was pelleted by centrifugation, and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm.

The absorbance of the sample was measured at 535 nm using a blank containing all the reagents except the sample. Since 99% TBARS was malondialdehyde (MDA), so TBARS concentrations of the samples were calculated using the extinction co-efficient of MDA, which is 1.56×10^5 M^{-1} cm^{-1}.

2.6. Histopathological studies:

Specimens from testis, prostate gland and seminal vesicle 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).

2.7. Statistical analysis:

Statistical analyses were performed by using SPSS statistical software package (SPSS, 2006). 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.

3. Results:

3.1. Effect of $CdCl_2$ on sexual behaviour in male rats:

The results presented in table (1) shows the effect of $CdCl_2$ on the parameters related to male rats sex-behaviour .Group of rats administrated high $CdCl_2$ concentrations had a significantly prolonged time to the first mount and a significant decrease in the number of mount ($p<0.05$). In addition, Cd cl - treated rats have shown a significant increase ($p<0.05$) in ejaculatory latency compared to their counterparts in the control group .Decrease number of ejaculations and increase post-ejaculatory interval was significantly ($p<0.05$) seen in male rats exposed to high concentrations of $CdCl_2$ compared to those exposed to low doses of $CdCl_2$ and rats in control group.

3.2. Effect of $CdCl_2$ on aggressive behaviour

The parameters of territorial aggression in adult male rats are demonstrated in table (2) .High $CdCl_2$ group displayed lateralization and boxing bouts ($p<0.05$) fewer compared to control group .A significant reduction ($p<0.05$) in the number of tooth chattering and leaping was observed in treated rats (high and low doses) when compared with the control group. Although high $CdCl_2$ seemed to reduce fighting significantly by male rats with the stud male rat, this effect was not significant in low $CdCl_2$ group.

3.3. Effect of $CdCl_2$ on fear and anxiety:

The effect of $CdCl_2$ treatment on the number of entries in closed and open arm in the elevated plus-maze has been summarized in table (3).There was a significant ($p<0.05$) increase occupancy in closed arms by exposed rats to different $CdCl_2$ concentrations as compared to the control rats .Furthermore, the treated rats spent more time in closed arms while no significant differences in the number of entries of both closed and open arms among the control rats.

3.4. Effect of $CdCl_2$ on male fertility:

3.4.1. Sex organs weight:

A significant decrease in the relative body weights of testes, seminal vesicles and prostate glands of $CdCl_2$ treated male rats as compared with those in the control group (Table 4).

3.4.2. Semen quality analysis:

Semen characteristics are shown in table (5). There was a significant decrease in sperm cell concentration, percentage of sperm motility and viability in $Cdcl_2$ treated rats compared to the control group. Sperm cell abnormalities in the form of detached head and coiled tail were increased significantly ($P < 0.05$) in male rats administered $CdCl_2$.

3.4. 3.Testosterone and serotonin assay:

Testosterone serum level was significantly decreased in $CdCl_2$ treated male rats as compared with those in the control group ($p< 0.05$). While serotonin was significantly increased when $CdCl_2$ was administered to male rats (Table, 6).

3.5. Oxidative stress parameters:

SOD activity and TBARs formation in testicular tissue of rats are presented in table,7. The level of TBARs formation was significantly higher in case of $CdCl_2$ treated rats. SOD activity was significantly lowered in $CdCl_2$ treated rats compared to $CdCl_2$ free rats.

3.6. Pathological examination:

3.6.1. Gross Pathological examination:

There were no obvious gross pathological alterations were observed in testes, prostate glands and seminal vesicles of rats in all groups.

3.6.2.Histopathological examination :

3.6. 2.1.Testes:

Microscopically, examined testes of rat treated with low dose of Cd revealed degeneration of spermatogonial cells lining seminiferous tubules and the tubules lumen were filled with degenerated germ cells (Fig. 1). Vacuolization of seminiferous epithelium and complete absence of germ cells associated with intestinal oedema were also noticed (Figs. 2 & 3). Also multinucleated spermatid giant cells (symplasts) were observed in the lumen of seminiferous tubules (Fig. 4). Testes of rats treated with high dose of $CdCl_2$ revealed more or less similar histopathological changes to the previous group. Those alterations described as marked degeneration and vacuolization of seminiferous epithelium (Fig. 5), necrosis of germ cells with complete absence of spermatozoa as well as interstitial oedema (Fig. 6), interstitial haemorrhage and necrosis of leydig cells (Fig. 7). Meanwhile, testes of $CdCl_2$ free rats revealed no histopathological changes (Fig. 8).

3.6. 2.2. Prostate glands:

Examined sections of rat treated with low dose of $CdCl_2$ showed slight hyperplasia of epithelial lining prostatic acini (Fig. 9) associated with interstitial edema. Meanwhile, prostate gland of rats treated with high dose revealed hyperplasia of prostatic epithelium, interstitial edema and hemorrhage (Fig. 10) associated with intestinal fibrous connective tissue proliferation (Fig. 11). However, prostate gland of control, untreated rat revealed no histopathological alterations. (Fig. 12).

3.6. 2.3.Seminal vesicles:

Microscopically, examined sections of rats treated with low dose of $CdCl_2$ revealed congestion of all blood vessels (Fig. 13), hyperplasia of epithelium lining (Fig. 14) as well as interstitial edema and hemorrhage (Fig. 15). Moreover, sections of rats treated with high dose of $CdCl_2$ showed marked hyperplasia of epithelial lining, interstitial edema and hemorrhage (Fig. 16) accompanied with leucocytic cells infiltration (Fig. 17). No histopathological changes were noticed in examined sections of $CdCl_2$ free rats (Fig. 18).

Table (1) : Effect of $CdCl_2$ on sexual behaviour in adult male rats .

Parameters	Control	Low dose	High dose
-Latency to 1st mount(s).	113.60±19.125^{ab}	104.71±16.867^{abc}	241.25±41.486^{bc}
- No. of mount.	14.83±3.400^{ab}	13.50±2.217^{ab}	10.00±2.047^{c}
-Ejaculatory latency(s).	242.00±30.550^{a}	314.67±55.247^{bc}	375.29±30.552^{bc}
- No. of ejaculation.	10.50±3.085^{ab}	7.50±1.190^{ab}	2.71±0.474^{c}
- post ejaculatory Intervals (s).	69.83±9.005^{ab}	67.46±10.240^{ab}	153.24±35.986^{c}

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA .
Values represent mean ± SEM.

Table (2) : Effect of $CdCl_2$ on aggressive behaviour in adult male rats .

Parameters	Control	Low dose	High dose
-No. of lateralization.	3.55±0.78^{ab}	1.7±00.68^{ab}	0.78±0.32^{c}
-No. of Boxing bouts.	3.36±1.00^{ab}	1.88±0.61^{ab}	0.80±0.35^{c}
-No. of fights with Stud male.	1.80±0.66^{ab}	1.50±0.59^{ab}	0.02±0.02^{c}
-No. of tooth chattering.	0.73±0.23^{a}	0.10±0.01^{bc}	0.20±0.01^{bc}
-No. of leaping.	0.36±0.15^{a}	0.02±0.01^{bc}	0.10±0.10^{bc}

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA .
Values represent mean ± SEM.

Table (3) : Effect of $CdCl_2$ on fear and anxiety measurements in adult male rats .

Parameters	Control	Low dose	High dose
-No . of entries in open arms.	3.56±0.37	2.64±0.47	3.53±0.40
-Time spent in open arms(s).	96.67±9.67^{a}	49.46±12.37^{bc}	55.80±10.60^{bc}
- No . of entries in closed arms.	3.67±0.52^{a}	5.82±0.66^{bc}	5.53±0.57^{bc}
- Time spent in Closed arms .	103.78±14.35^{a}	125.53±15.72^{bc}	150.46±18.35^{bc}

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA .
Values represent mean ± SEM.

Table (4): Effect of $CdCl_2$ on sex organs weight in adult male rats. (gm/100g. b.wt.)

Organs	Control	Low dose	High dose
Testes.	1.566±0.068^{ab}	1.268±0.745^{abc}	1.164±0.171^{bc}
Seminal vesicles.	0.789±0.046^{a}	0.580±0.336^{bc}	0.584±0.036^{bc}
Prostate gland.	0.372±0.027^{a}	0.226±0.0132^{bc}	0.189±0.0123^{bc}

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA .
Values represent mean ± SEM.

Table (5) : Effect of $Cdcl_2$ on semen quality in adult male rats .

Organs	Control	Low dose	High dose
- Sperm count (10^6/ ml)	69.60±2.49 [ab]	63.60±2.80 [abc]	56.40±2.29[bc]
-Motility (%).	85.20±1.77 [a]	64.20±3.54 [b]	48.60±2.73[c]
-Viability (%).	91.60±2.34 [a]	66.40±2.73.[bc]	55.20±2.06[bc]
-Total sperm Abnormalities (%)	5.20±0.37 [a]	11.40±0.68[bc]	19.60±0.93[bc]

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA . Values represent mean ± SEM.

Table (6): Effect of $CdCl_2$ on serum testosterone and serotonin in adult male rats.

Parameter	Control	Low dose	High dose
-Testosterone (ng/ml)	2.49±0.892 [a]	1.89±0.095 [b]	1.48±0.017 [c]
-Serotonin(ng/ ml)	58.92±2.77 [a]	70.30±2.17 [b]	93.78±2.71 [c]

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA . Values represent mean ± SEM.

Table (7) : Effect of $CdCl_2$ on testicular lipid peroxidation and oxidative states in adult male rats.

Parameter	Control	Low dose	High dose
-SOD (µmol/min/mg protein)	0.09±0.002[a]	0.08±0.002 [b]	0.07±0.002[c]
-TBARS (nmol/ mg protein)	0.11±0.004[a]	0.13±0.003 [b]	0.16±0.005[c]

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA . Values represent mean ± SEM.

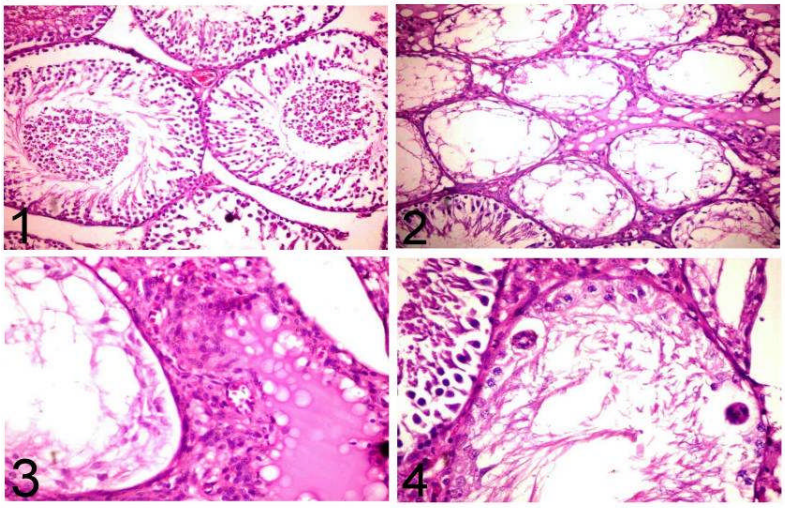

Fig. (1): Testis of rat treated with low dose of Cd showing degeneration of spermatogonial cells lining seminiferous tubules and the tubules lumen filled with degenerated germ cells. (H & E X 200).

Fig. (2): Testis of rat treated with low dose of Cd showing Vacuolization of seminiferous epithelium and complete absence of germ cells associated with intestinal oedema. (H & E X 100).

Fig. (3): Testis of rat treated with low dose of Cd showing Vacuolization of seminiferous epithelium and complete absence of germ cells associated with intestinal oedema. (H & E X 200).

Fig. (4): Testis of rat treated with low dose of Cd showing multinucleated spermatid giant cells (symplasts) in the lumen of seminiferous tubules. (H & E X 200).

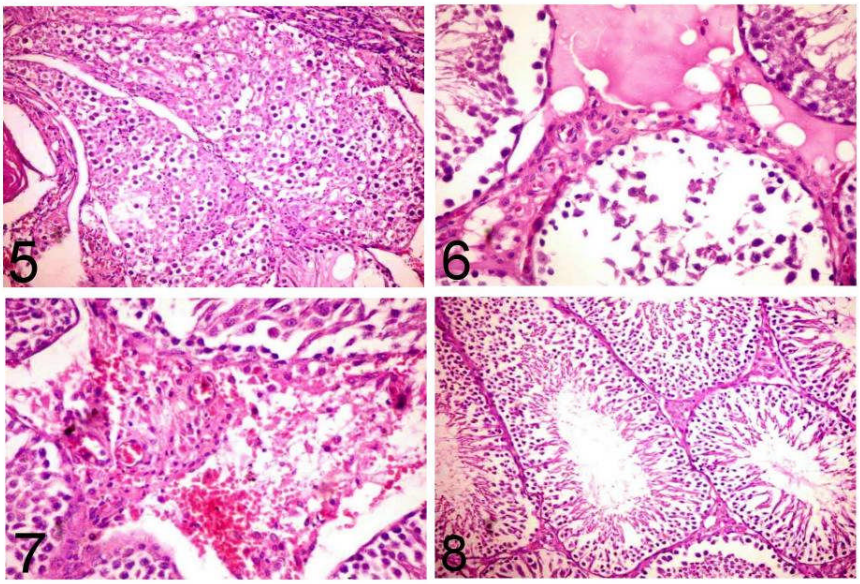

Fig. (5): Testis of rat treated with high dose of Cd showing marked degeneration and vacuolization of seminiferous epithelium with pyknosis of their nuclei. (H & E X 200).
Fig. (6): Testis of rat treated with high dose of Cd showing necrosis of germ cells with complete absence of spermatozoa as well as interstitial oedema. (H & E X 200).
Fig. (7): Testis of rat treated with high dose of Cd showing interstitial haemorrhage and necrosis of leydig cells. (H & E X 200).
Fig. (8): Testis of control, untreated rat showing no histopathological changes. (H & E X 200).

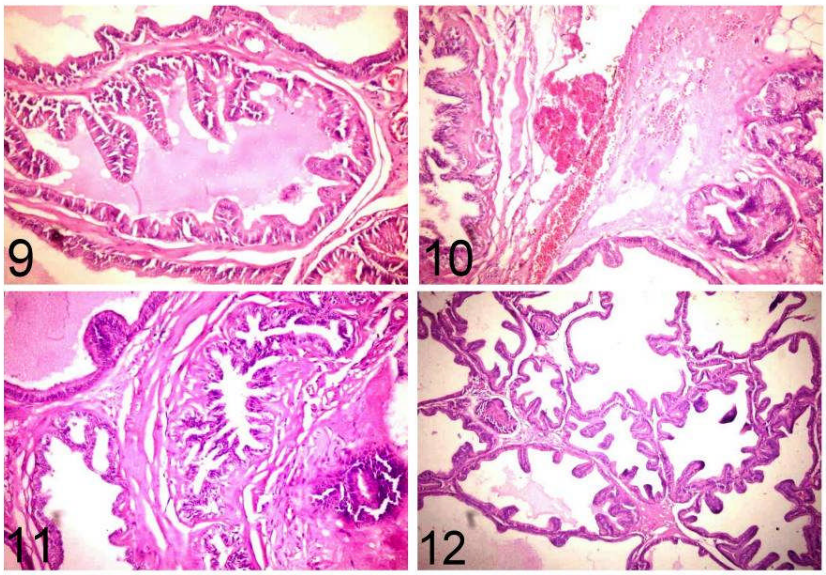

Fig. (9): Prostate gland of rat treated with low dose of Cd showing slight hyperplasia of epithelial lining prostatic acini. (H & E X 200).
Fig. (10): Prostate gland of rat treated with high dose of Cd showing interstitial oedema and haemorrhage. (H & E X 100).
Fig. (11): Prostate gland of rat treated with high dose of Cd showing interstitial fibrous connective tissue proliferation. (H & E X 100).
Fig. (12): Prostate gland of control, untreated rat showing no histopathological changes. (H & E X 100).

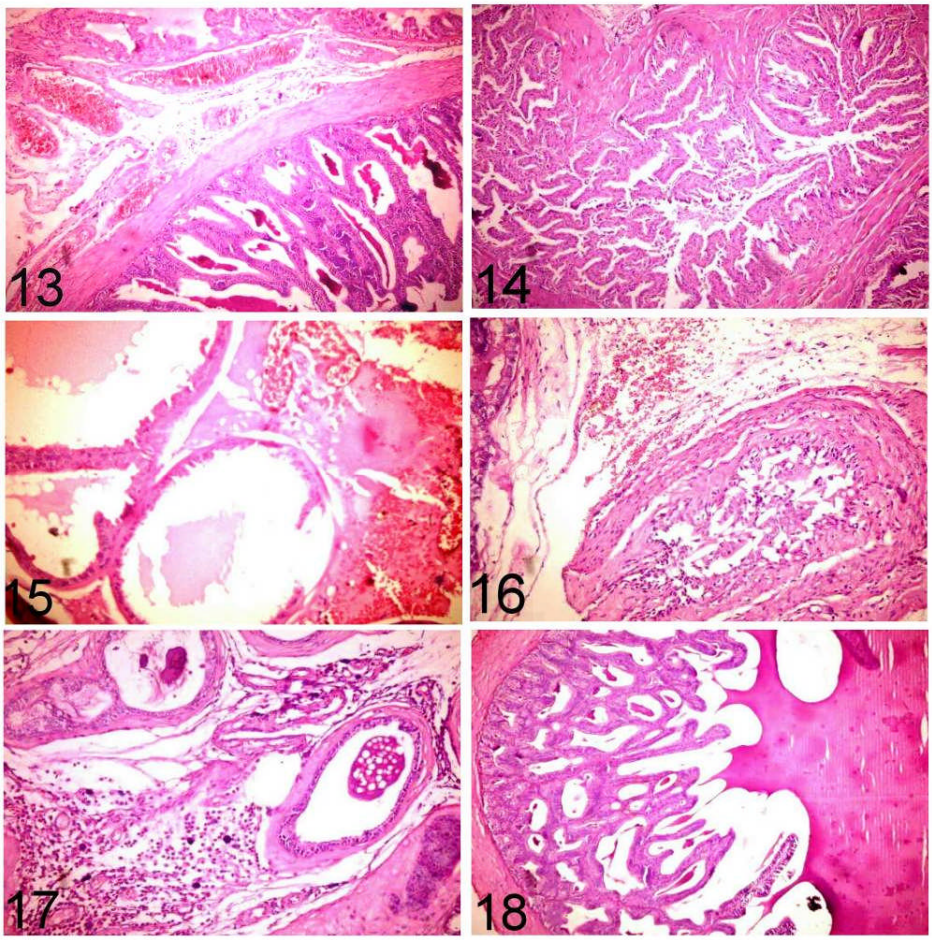

Fig. (13): Seminal vesicle of rat treated with low dose of Cd showing congestion of all blood vessels. (H & E X 100).
Fig. (14): Seminal vesicle of rat treated with low dose of Cd showing hyperplasia of epithelium lining. (H & E X 100).
Fig. (15): Seminal vesicle of rat treated with low dose of Cd showing interstitial oedema and haemorrhage. (H & E X 100).
Fig. (16): Seminal vesicle of rat treated with high dose of Cd showing interstitial oedema and haemorrhage. (H & E X 100).
Fig. (17): Seminal vesicle of rat treated with high dose of Cd showing interstitial leucocytic cells infiltration. (H & E X 100).
Fig. (18): Seminal vesicle of control, untreated rat showing no histopathological changes. (H & E X 100).

4- Discussion:

In this study, effects of exposure of adult male rat to $CdCl_2$ administered in drinking water on parameters of sexual behaviour, aggressive behaviour as well as anxiety and fear were investigated. Our results revealed that, male rat sexual behaviour was suppressed after the ingestion of $CdCl_2$ solution as evidenced by prolongation of the latency to first mount and reduce the number of mounts and ejaculations. Moreover marked increase in the latency to first ejaculation and post – ejaculatory interval in the Cd treated groups. These data were in agreement with John *et al* (1994) who suggested that exposure of male rats to cadmium is associated with alterations in sexual functions (copulatory and erectile dysfunction) .

The other main finding, is that oral administration of CdCl2 markedly abolished territorial aggressive behaviour in adult male rats namely, a suppression in boxing bouts, fight with stud and lateralization.

The results suggest that sexual and aggressive behaviour are very susceptible to the toxicity produced by $CdCl_2$ and could be explained by the direct or indirect effect of cadmium on the testes and the influence on androgen biosynthesis. As testosterone plays a key role in sexual arousability as well as cause of aggressive behaviour in males and this in turn is equated with violence (Kathrin, 2001). Our data presented in this work strongly indicates a serious decrease in the level of testosterone in the exposed groups of male rats. This might be regarded to the degenerative testicular changes exerts in the testicular tissue as showed in histopathological examination in the present study, suppression of testicular enzymes and/or decrease in leutenizing hormone (LH) secretion by the pituitary gland. In this respect, 17-α-hydrozylase and 17-20 lyase enzymes are responsible for normal testicular steroidogenesis .Their function is controlled by cytochrome P-450 which is represented in high concentration in the leydig cells and is highly affected by Cd toxicity **(**Maines 1984**).**

Concerning, LH and steroidogenesis Ellis and Desjardins (1982) and Fatma *et al*. (2009) reported that LH acts upon the leydig cells of the testis and is responsible for the production of testosterone, an androgen that exerts both endocrine activity and intratesticular activity on spermatogenesis. Cd administration significantly increased nitric oxide (NO) production (Waisberg *et al.*, 2003) leading to decrease in testosterone synthesis in the leydig cells through acting centrally on the pituitary gland and inhibiting LH secretion (Dobashi *et al*., 2001) . Our findings are in accordance with previous reports from Piasek and Laskey, (1994) that have demonstrated lowering in steroidogenesis in Cd treated female rats. Further evidence derived from a study for Murugesan *et al*., (2007) where poor pituitary LH secretion with reduced leydig cell steroidogenesis was reported in highly contaminated environment. Here, the marked inhibition of sexual behaviour in Cd treated male rats, might be attributable to the reduction of testosterone level .Corresponding to the results of Gunn *et al*. (1970) , where testosterone treatment can prevent the inhibitory effect of cadmium on mating behaviour six to nine days after cadmium injection .

As testosterone is important in sexual arousability, also serotonin (5-HT) is involved in copulatory behaviour and ejaculation. In the study of Rastogi *et al*., (1977) and Antonio *et al.*, (1988) , significant increase in serotonin(5-HT) levels in pup brains after cadmium exposure was reported. Fernandez *et al*. (1992) noted impairment of sexual behaviour by 5- HT microinjection into the Medial Preoptic Area (MPOA) by a large dose. Additionally, Mas *et al.* (1995) reported that 5- HT is released more laterally in the preoptic area (POA) , after ejaculation , that these high levels of 5- HT may lead to the sexual quiescence that follows ejaculation .Similar results reported by Rosen *et al*., (1999) where serotonin(5-HT) was regarded as inhibitory to male sexual behaviour.

Contrary to our findings, the reduced level of serotonin (5-HT) was detected by high cadmium intake in pups exposed to cadmium via the milk from exposed dams (Kierstin, 2003). This contradiction in $CdCl_2$ effect on serotonin might be due to the level of exposure of cadmium, timing in age at exposure and individual sensitivity to the chemicals.

Testosterone and serotonin have been the two most researched chemical messengers with regards to aggression (Marco *et al*., 2005). Here, marked impairment in aggression was a significant remark for $CdCl_2$ group and this could be attributed to the decline in testosterone level. There are numerous reports about testosterone and aggressive behaviour .Bermond *et al.* (1982) showed that the intraspecific aggressiveness in male rats in the same cage is influenced by testosterone and is reduced by castration. Additionally, Marco *et al*. (2005) reported that inter- male aggression in rats, mice, monkey and man is controlled by testosterone. Testosterone induced suppression of DA (dopamine) turnover in the medial preoptic nuclei and anterior hypothalamic nuclei may well be involved in androgen- dependent aggression and copulatory behaviour (Simpkins *et al.*, 1983).

The brain chemical serotonin has long been known to play an important role in regulating anger and aggression. In this study, rats exposed to $CdCl_2$ showed a significant elevation in serotonin and a concomitant impairment in territorial aggression. According to the serotonin (5-HT) deficiency hypothesis of aggression, highly aggressive individuals are characterized by low serotonin (Caramaschi *et al*., 2007). Further studies revealed that deficit in serotonin activity correlates with impulsive and aggressive behaviour (Moeller *et al.*, 1996 and Pier *et al*., 2005).

The results of this study, show a significant decrease in the relative sex organs weight (testes, seminal vesicles, and prostate gland) in $CdCl_2$ treated rats. Barbara *et al* .,(2008) recoded that rats treated with higher doses of cadmium for 12- 15 months showed a marked reduction in absolute weight of testes and impairment of seminiferous tubules . Moreover , the wide array of abnormalities observed when histopathological sections of the testes were examined , provides further evidence for the reduced

sexual activity and reduced fertility of treated male rats . Our histopathlogical examination revealed that the examined testes of the treated rats showed marked degeneration & vacuolization of seminiferous epithelium, necrosis of germ cells, interstitial hemorrhage and necrosis of leydig cells. Moreover, prostate gland of rats treated with high dose declared hyperplasia of prostatic epithelium, interstitial edema and hemorrhage .In addition to congestion of all blood vessels & hyperplasia of epithelial lining, interstitial edema & edema of seminal vesicles. Among the proposed mechanisms of cd toxicity on the testes are circulatory failure due to vascular damage and decreased utilization of Zn by spermatogenic cells due to competitive action of cadmium **(**Amara *et al*., 2008). There is paucity of information on the effect of cadmium on the lipids of the testes. Consequently, there is a lack of information also on the role of cadmium-induced lipid changes in testicular function. Adaikpoh and Obi (2009) concluded that the mechanism of cadmium toxicity of the testes and prostate may involve elevation of cholesterol levels in these organs.

The present study shows decrease in sperm count, sperm motility, sperm viability and increase in sperm abnormality (detached head and / or coiled tail) in male rats treated with high dose of $CdCl_2$ .These detected reduction and abnormalities in sperm could be the result of reduced spermatogenesis and the histopathlogical alterations observed in the testes of the treated rats. Similar results reported by Neveen *et al.* (2007) , where exposure of adult male mice to CdCl2 significantly decreased sperm counts, total number of sperms per mg of testis , daily sperm production efficiency. In addition, Bench *et al.* (1999), reported that cadmium has a detrimental effect on testicular function (stages of spermatogenesis) that could result in reduced sperm production leading to reduced male fertility. Cadmium is known as competitor of calcium, which is essential for sperm motility regulation (Beyersmann and Hechtenberg, 1997, Martelli *et al*., 2006). Further evidence derived from a study for Benoff *et al*. (2008) where aberrant sperm motility was correlated with altered expression of L–type voltage – dependent calcium channel isoforms found on the sperm tail, which regulate calcium and cadmium influx.

Also, the increased oxidative stress resulted from cd intoxication in testicular tissue might be responsible , at least in part , for poor semen quality , testicular damage and impairment of fertility. Moreover , it has been reported that the oxidative stress affect the sperm cell via interfering with the membrane fluidity which is the main factor for sperm motility and fusion with the oocyte (Kim and Parthasarathy 1998, Aitken, 1995). Toxic effect of Cd on testes is known to deplete glutathione and protein – bound sulphydryl groups, which results in enhanced production of reactive oxygen species (ROS) such as superoxide ion , hydroxyl radicals and hydrogen peroxide (waisberg *et al.*, 2003). In the current study ,rats exposed to $CdCl_2$ showed a significant reduction in the activity of antioxidant enzyme (SOD)and a concomitant enhancement in lipid peroxidation (TBARS) , in accordance with earlier reports in cadmium intoxication in rats (El- Missiry and Shalaby ,2000; Turner and Lysiak 2008, Fatma *et al.*, 2009 ; Kanbura *et al.*, 2009).

Since cadmium classified as neurotoxic substance and generally impairs enzymes involved in the synthesis of neurotransmitters (Murphy, 1997). The serotonergic system is involved in anxiety response (File *et al*., 2000). In the present work, increasing anxiety was evidenced by increased number of entries in closed arms and the time spent in cd intoxicated rats. Our results are in line with data of (Bull, 2010) who stated that sub chronic oral exposure to cadmium can cause anxiety and alterations in the biochemical activity of the brain in laboratory animals Also, Leret *et al.* (2003) recorded that, the intoxicated rats with cadmium and lead acetate, showed an increase on indices of anxiety on the elevated plus – maze. These long –term changes in anxiety – like behaviour can be related to dopaminergic and serotoninergic alterations detected in hippocampus . Most of us are probably aware that serotonin plays an important role in depression and anxiety and people with low levels of serotonin are more likely to become depressed. Recent research indicates that the reverse may actually be the true and that people with too much serotonin in certain parts of the brain may develop depression. Evidence derived from a study for lowry and Hale (2010) where there are subgroups of serotonin neurons that are overactive were observed in depressed patients , rather than underactive .Our results , showed high serotonin level in Cd treated rats in comparison with the control male rats .Similar results reported by rastogi *et al* (1977) and Antonio *et al* (1998), where a significant increase in serotonin (5 – HT) levels in pup brains after pre- and post natal cadmium exposure . The chemical hypothesis of anxiety and depression comes from rat models. As rats have up to 8 times more serotonin in brain regions associated with anxiety disorders and clinical depression – the nucleus accumbens, prefrontal cortex, hippocampus and hypothalamus – than controls (Zangen *et al.*, 1999). Edwards (2005) stated that scientists believe that dysregulation of neurotransmitters such as serotonin , dopamine and gamma aminobutyric acid or GABA , have been implicated in anxiety disorders and phobias , Also , he added that the data on fear and anxiety show dense

serotonin input to the amygdale . In contrast to our results , Kierstin (2003) stated that a marked decrease in serotonin and its metabolite 5-hydroxyindoleacetic acid was observed in cerebral cortex and hippocampus in pups exposed to cadmium via the milk from exposed dams . The contradicting results may be due to differences in experimental design e..g. cadmium dose , timing in age at exposure or measurement of outcome and brain regions investigated .

There is definitive medical and scientific evidence, that testosterone elevations were associated with reduced male anxiety (Jeremy *et al.*, 2002). Kassandra and Cheryl (2007), confirmed that sexual experience is associated with lower levels of anxiety – like behaviour and higher levels of androgen secretion. Rats exposed to $CdCl_2$ showed higher levels of anxiety – like behaviour associated with lower levels of testosterone. Gonadectomy (GDX) in male rats would increase anxiety – like behaviour, an effect which would be reversed by systemic administration of dihydrotestosterone (DHT). Testosterone reduced anxiety – like behaviour through actions of its 5 α – reduced metabolite, & DHT (dihydrotestosterone) (Kassandra and Cheryl , 2006) .

In conclusion, where testes is greatly targeted to damage by cadmium intoxication. Along with evidence derived from our study, where exposure to cadmium constitutes a great threat being associated with reproductive injurious effects. Hence, concern should be directed to limit the inadvertent incorporation of cadmium in human–consumed products.

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