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RESEARCH ARTICLE

Evaluation of toxic effect of cadmium on sperm count, sperm motility and sperm abnormality in albino mice

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Abstract

Cadmium (Cd) is an extremely toxic environmental pollutant and is one of the biggest threats to the current and future world. The present work was designed to evaluate the detrimental effects of cadmium on male fertility by concerning the sperm parameters after acute and chronic exposure to albino mice. For this, experimental mice were divided into three groups. Group I were kept as control. Group II animals were injected with a single dose (acute dose) of $2mgkg^{-1}$ body weight of cadmium chloride ($CdCl_2$) intraperitoneally`(i.p.) and sacrificed at the intervals of 1, 7, 15 and 30 days post treatment. Group III mice were administered with $CdCl_2$ at a dose of $0.1mgkg^{-1}$ body weight, i.p. daily (chronic dose) and autopsies were done on 15 and 30 days post treatment. In the present study, mice exposed to different doses of cadmium (groups 2 and 3) showed a significant decrease in the sperm count, sperm motility and increase in the fraction of morphologically abnormal sperms as compared to control mice. The testicular weight was also found to be significantly reduced in cadmium treated groups. However, these alterations were found to be more devastating in the chronic dose treated group.

INTRODUCTION

Cadmium is considered as one of the most toxic heavy metals and a potent industrial pollutant that adversely affects various organs in human and animals (De SousaViana et al., 2011). The Agency for Toxic Substances and Disease Registry (ATSDR) and International Agency for Research on Cancer (IARC) has classified cadmium as a "Group I"carcinogen (IARC, 2004). It has widely distributed in the environment due to extensive uses of cadmium and cadmium-based products such as nickel-cadmium batteries, television phosphors, stabilizer for polyvinyl chloride etc. Moreover, owing to its brilliant orange colour, it is extensively used as a pigment in paints, plasters and plastics (Singh et al., 2012).

Exposure to cadmium can occur from food, water, smoking cigarette and at work place by inhalation of fumes and dusts (Ashraf, 2012). Its concentration in food samples vary widely but the highest average concentrations are detected in molluscs, kidneys, liver, cereals, cocoa and leafy vegetables (Ogungbe and Lawal, 2008). The estimates of cadmium dietary intake derived from the world health organization regional diets based on food balance sheets, ranged from 0.35 to 9.63µg kg^{-1} body weight per day (Bellinger, 2004)

Cadmium is known to be the most harmful heavy metal as it causes various forms of diseases such as osteomalacia, osteoporosis, hypertension, arteriosclerosis, anaemia and cancer (Bernard, 2008 and Obianime and Roberts, 2009). The possible mechanism of cadmium induced toxicity is oxidative stress, generation of reactive oxygen species (ROS) resulting in oxidative deterioration of lipids, proteins and DNA and initiating various pathological conditions in humans and animals (Cuypers et al., 2010). Once absorbed, cadmium is rapidly cleared

from blood and concentrates in various tissues (Ognjanovic et al., 2003). The risk of cadmium toxicity is further increased because of its biological half life (17-30 years), resulting in accumulating and damaging effects, especially in the liver and kidneys (Shukla, and Kumar, 2009) where it causes many metabolic and histopathological changes, membrane damage, altered gene expression and apoptosis (Obianime and Roberts, 2009).

Cadmium exposure is strongly associated with reproductive toxicity in both animals and human populations culminating in infertility and cancer of the reproductive tissues (Rekha et al., 2011). According to the previous studies, at least half of the cases of human male infertility may attribute to various environmental and occupational exposures to toxic metals including lead, nickel, arsenic, cadmium etc. (Al-Azemi et al., 2010 and Chandel and Jain, 2014). Nowadays, fertility interference, regulation and control have become a matter of global concern in order to maintain adequate sustainable and healthy populations. Inspite of some reports regarding the effects of cadmium on the male reproductive organs (testes), not much is known about its spermiotoxic effects. It is therefore, in this study we seek to evaluate the effects of different doses of cadmium on sperm parameters which include sperm count, sperm motility and sperm abnormalities.

Material and methods

Chemical

Cadmium chloride ($CdCl_2$; S.D. Fine –Chemical Limited, Mumbai, India) was dissolved in double glass distilled water and injected intraperitoneally to each group.

Animals

Albino mice weighing 22-25g were procured from the small animal house, Central Research Institute, Kasuali, India. The animals were acclimatized to laboratory conditions for 15 days (temperature 25±3ºC, 12hours light and dark cycle) and fed standard mice feed (purchased from Ashirwad Limited, Chandigarh) and water *ad libitum*. The animals were handled in accordance with the guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animals, India. Institutional animal ethical committee (Reg No. 107/1999/CPCSEA/2009-03) approved the study.

Experimental protocol

Forty two mice were divided into following groups and treatments were done accordingly. The doses administered to animals were decided on the basis of experiments conducted in our laboratory and other published reports (Ogungbe and Lawal, 2008 and Yusuf et al., 2008).

Group 1: Animals were kept as control.

Group 2: Albino mice were administered a single dose of $2mgkg^{-1}$ body weight of $CdCl_2$ intraperitoneally (i.p.). Then, animals were further divided into 4 subgroups of 1, 7, 15 and 30 days post treatment and were sacrificed on the respective days.

Group 3: Mice were divided into 2 subgroups of 15 and 30 days post treatment and were injected with $CdCl_2$ once daily at a dose of $0.1mgkg^{-1}$ body weight, i.p. All subgroups had 6 mice each. On the day of autopsy, the right testis from each mouse was removed, washed with saline (0.9% NaCl) solution and weighed. Then, the relative weight of testis was calculated according to the equation of Bearden and Fuquay (1980).

$$\textbf{Relative weight} = \frac{\text{Testicular weight}}{\text{Body weight}} \times 100$$

Sperm analysis

Immediately after autopsies, the right cauda epididymis of each mouse was minced and thoroughly mixed in 0.5ml of luke warm 0.9% NaCl and was processed for:

Sperm motility

20µl of above mixture was immediately put on dry, clean and warm slide and examined (×400) under the light microscope (Kyowa Getner, N-800M, Tokyo). The motile and non motile sperms were counted in 6 fields in each slide and percentage of motile spermatozoa was determined (Salisbury et al., 1978).

Sperm count

The sperm count was conducted as per standard procedure in Neubauer chamber (Bearden and Fuquay, 1980).

The whole epididymis was thoroughly minced and then diluted with 100µl of 1% eosin aqueous solution. The diluted stained content was sucked up to the mark 0.5 on the erythrocyte pipette; the pipette was then filled up to the mark 101 by 1% eosin aqueous solution. The content in the pipette bulb was mixed and the sperms were counted in five large squares. The sperm cell concentration per ml epididymal fluid was calculated using the following equations:

Sperm concentration/ml = (400 ×200 ×100 ×10) × N/ 80

Where:

N = Counted sperm number

80 = Number of small squares in five large squares

400 = Total number of small squares in chamber

10 = Depth of haemocytometer chamber

200 = Dilution factor on pipette

100 = Dilution factor of epididymal content.

Sperm abnormalities

The smear was drawn from the diluted epididymal fluid. After drying, slides were fixed in methanol and stained in 1% eosin (Y). The prepared slides were evaluated for morphological abnormalities of head, mid piece and tail regions of spermatozoa under light microscope (Kyowa Getner, N-800M, Tokyo). For each mouse, 300 spermatozoa were assessed from three different slides by adopting the method of Wyrobek and Bruce (1975).

Statistical analysis

Data was expressed as arithmetic mean± standard error and analyzed statistically by using Student's t test and One-way analysis of variance ANOVA (SPSS 16 version). Statistical significance was accepted at $P<0.05$.

Results

The present study revealed that a single dose of cadmium exhibited a gradual reduction in the testicular weight from day 1 to 30 days post treatment as compared to control (Fig. 1). However, chronic dose treated groups showed a highly significant ($P<0.001$) reduction in the testicular weight on 15 and 30 days post treatment (Fig. 2).

The data (Table 1 and 2) displays the mean ± standard error values of sperm parameters of control and treated mice at different intervals of the experiment and defines that acute (single dose) dose of cadmium induces significant decrease in sperm count ($P<0.05$) and sperm motility ($P<0.01$) immediately after 1 day post treatment in comparison to control mice, which further reduced highly significantly ($P<0.001$) at 15 and 30 days post treatment in both the cadmium treated groups 2 and 3 (Table 1 and 2). However, most devastating results were seen in chronic dose treated group 2 (Table 2).

When the mean percentage of abnormal sperm was compared in control and day 1 post treated group of mice, it was found to be significantly increased ($P<0.01$, Table 1, Fig. 3). Further, highly significant ($P>0.001$) increase in sperm abnormalities at 15 and 30 days post treatment in cadmium treated groups (2 and 3) were recorded (Table 1 and 2). However, more than 80% of abnormal sperms were assessed in 30 days chronic dose cadmium treated mice. Hence, chronic dose treated mice exhibited more alteration in the sperm parameters.

Discussion

Cadmium (Cd) is an environmental pollutant affecting various tissues and organs including testes (Gaurav et al., 2010). The result of this study revealed a significant decline in sperm count and sperm motility with concomitant increase in the sperm abnormalities in cadmium treated mice as compared to control group. The testicular weight was also found to be reduced in cadmium treated mice. These toxic effects induced by cadmium are in agreement with the effects of many other toxic metals (Oliveira et al., 2009, Sharma and Kaur, 2012 and Asadpour et al., 2013) which may be associated with membranous damage or macromolecular degradation incurred by reactive oxygen species, leading to significant decline in germ cell population and ultimately loss in testicular weight (Rengan et al., 2012 and Mohamed et al., 2014). Several studies have revealed that cadmium induced reactive oxygen species also disrupt leydig cell mitochondria by inhibiting the expression of steroidogenic acute

regulatory proteins (StAR) which leads to decline in testosterone synthesis and hence spermatogenesis (Berleu and Stadiman, 1997 and Huang and Liu, 2004)

The primary mechanism of cadmium induced toxicity in testes may attribute to (i) its ability to cross blood-testis barrier (Wong et al., 2005) (ii) Circulatory failure due to vascular damage (iii) decreased utilization of zinc by spermatogenic cells due to competitive action of cadmium (Amara et al., 2008). Zinc plays a very important role in spermatogenesis by enhancing testosterone synthesis and by scavenging reactive oxygen species produced in the testicular tissues (Yamaguchia et al., 2009).

In the present research work, a single dose of cadmium exhibited acute toxic effect on sperm parameters immediately after 24 hours of the treatment which continued gradually till the end of the experiment. These damaging effects on sperms may be due to accumulation of cadmium in testes, (Asadpour et al., 2013) where it directly affects the early stage VIII of the spermatogenesis to induce spermiation failure (Bench et al., 1999).

Moreover, cadmium and other heavy metals can target the vascular system in variety of ways. Several studies also strengthen this view (Karl et al., 2005 and Walter et al., 2007). They suggested that the unusual sensitivity of the testes to cadmium is related to the unique feature of its vasculature namely the pulseless and semi-stagnant flow of blood in the intracellular course of the testicular arteries, which permits cadmium to alter the capillary endothelium permeability, resulting in the oedema and pressure effects leading to anoxia. It is also found that cadmium brings about the molecular level apoptosis and necrosis of testicular tissue by changing the expression of caspase, p53, c-Jun and MT-I genes that reduces sperm motility and leads to male infertility (Jin et al., 2003).

Furthermore, the factors which contribute to decrease sperm motility in cadmium treated mice may be attributed to its potential to de-stabilize the sperm chromatin via removal of essential zinc (Suzuki et al., 1995), inhibition of respiratory activity (ATP depletion) (Christen and Gatti, 1987) as calcium channels are the targets for various heavy metals (Büsselberg 1995), alkalization of the luminal fluid of seminiferous tubules and epididymal duct due to inhibition of leydig cell androgen production and inhibition in sliding movement of microtubules (Kanous et al., 1993).

Regarding the sperm cell abnormalities, it was revealed that administration of cadmium to male mice caused significant increase in the percentage of abnormal sperms allover the experimental period in the form of detached, truncated, elongated and bifurcated heads etc. The sperm tail was in the form of curved, bent and coiled and in some cases, the midpiece was found to be thickened along with cytoplasmic droplets (Fig. 3B-E). It is postulated that the sperm cell morphology is genetically controlled by numerous autosomal and sex linked genes (Krzanowska, 1986). Hence, formation of abnormal sperm population in the present study may be due to the mutagenic effects of cadmium-induced reactive oxygen species on the specific gene loci of germ cell chromosomes involved in maintenance of the normal sperm structure.

In conclusion, cadmium negatively influenced the sperm cells as mirrored by alternation in sperm functional parameters in treated mice, might result from the generation of oxidative stress which evokes the genotoxic effects by altering specific gene loci responsible for the spermatogenesis and maintenance of the appropriate sperm shape and motility. However, this effect was more drastic in 30 days chronic dose treated group, where more pronounced overall deterioration of sperm parameters was recorded. Therefore, cadmium exhibits dose and time dependent devastating effects on the reproductive potential of the exposed animal and human populations.

Table 1. Comparison of sperm parameters of control and cadmium treated mice (acute dose).

Parameters (n=6)	Control	Autopsy days			
		1	7	15	30
Sperm motility (%)	82.50±0.92	71.66±0.77*	66.83±1.80*	62.83±0.86**[a]	63.00±0.86**
Sperm count ($x10^6$/ml)	24.83±0.79	21.50±2.15#	17.33±1.07**	16.33±1.77**	15.66±2.72**[a]
Abnormal sperms (%)	46.83±2.26	63.33±3.67*	74.33±2.26**[a]	75.00±2.18**[a]	77.16±1.66**

** Significant variations at P<0.001, * P<0.01 and # P<0.05 (control vs cadmium-treated group).
[a] P<0.05 (between group)

Table 2. Comparison of sperm parameters of control and cadmium treated mice (chronic dose).

Parameters (n=6)	Control	Autopsy days	
		15	30
Sperm motility (%)	82.50±0.92	56.16±0.64**	54.83±0.59**
Sperm count ($x10^6$/ml)	24.83±0.79	18.00±6.55**	13.66±1.28**[a]
Abnormal sperms (%)	46.83±2.26	69.33±3.88**	80.66±1.26**[a]

** Significant variations at P<0.001 (Control vs cadmium-treated).
[a] P<0.05 (between group)

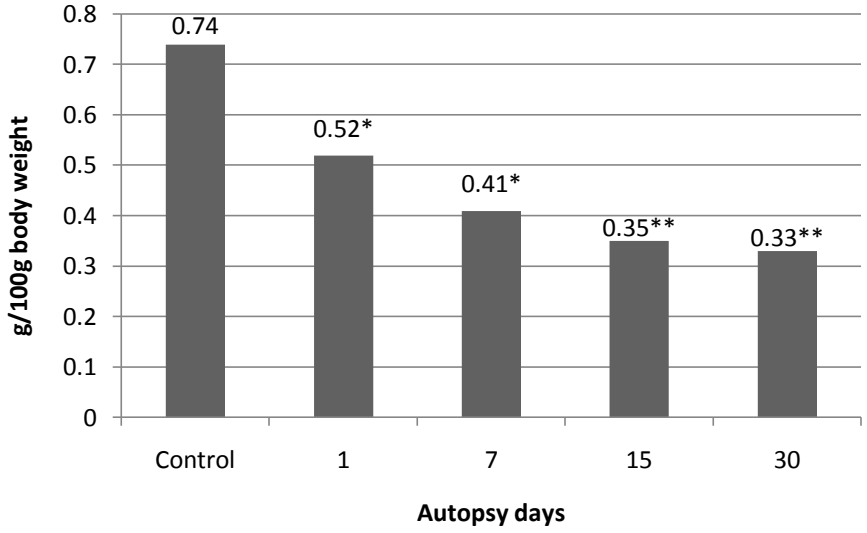

Fig. 1 Comparison of weight of testes in control and cadmium treated mice (acute dose) . *Significant at P<0.05 , **P<0.001.

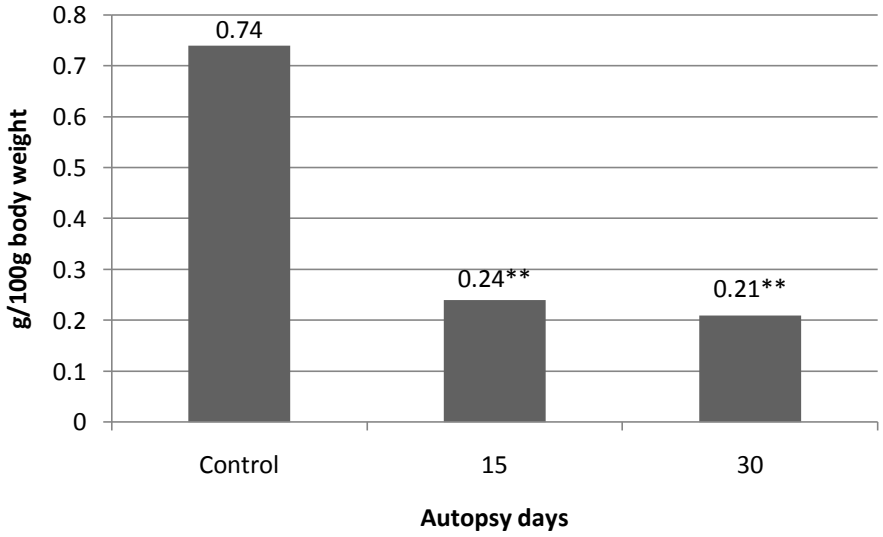

Fig. 2 Comparison of weight of testes in control and cadmium treated mice (chronic dose). ** Significant at P<0.001

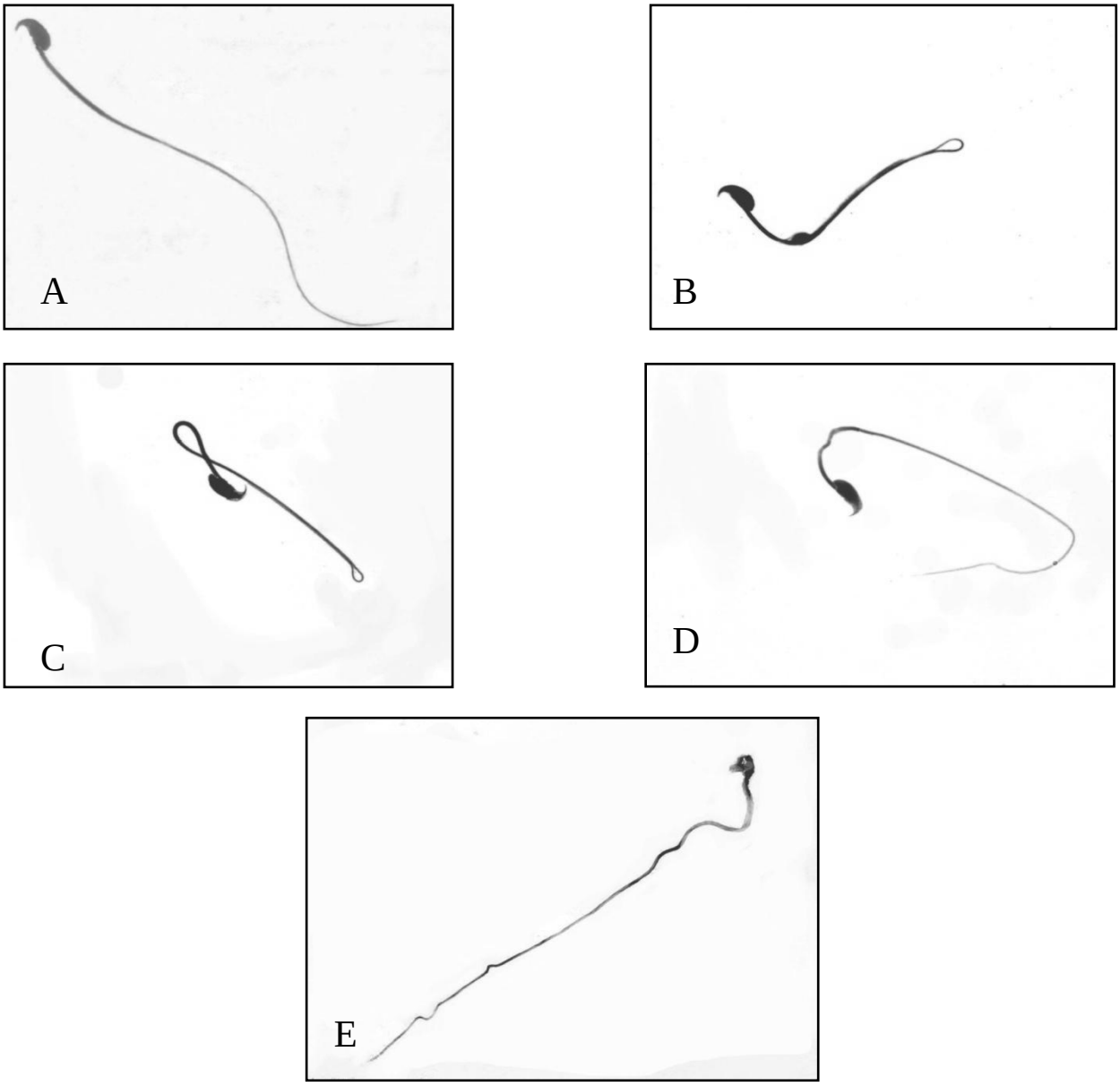

Fig. 3 Photomicrographs showing abnormal sperms in cadmium treated mice. X 400.
A. Normal sperm with scimitar head and long flagellum tail.
B. Sperm showing thickened mid piece with cytoplasmic droplet and folded tail.
C. Sperm showing coiling in mid piece and tail.
D. Sperm showing bends in mid piece and tail.
E. Sperm showing degenerated head and wavy mid piece and tail.

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