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Original Article

Cadmium Chloride Toxicity Revisited: Effect on Certain Andrological, Endocrinological and Biochemical Parameters of Adult Male Rabbits

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Short Title: Cadmium Chloride Toxicity and Physiological Parameters

Summary

The present study was devised to assess the effects of cadmium chloride ($CdCl_2$) administration on certain andrological, endocrinological and biochemical alterations in adult male rabbits (n=24). The animals were assigned to control (n=8) and experimental (16) group. Experimental group was orally administered with 1.5 mg/kg body weight of $CdCl_2$. The trials were carried out for a total of 5 weeks and blood sampling was carried out on weekly basis. A gradual decrease was noticed for body weight in the experimental group from week 1 to 5, being significantly lower in week 4 and 5 ($P<0.05$). A similar decremented trend was noticed for serum testosterone level being significantly lower in experimental group in week 4 and 5 ($P<0.001$). Significantly lower values were noticed for Prolactin in experimental group in week 4 and 5 ($P<0.05$), than in the control. On the contrary, serum cortisol level showed a gradual increase in experimental group, from week 1 to 5, being significantly higher in week 4 and 5 ($P<0.05$). Regarding the biochemical attributes, all the parameters under study revealed a gradually ascending trend. Statistical significance was, however, achieved in varying weeks and at varying levels. The total protein and albumin were significantly higher in week 4 and 5 ($P<0.01$); Alanine aminotransferase in week 2 ($P<0.01$), 3 ($P<0.001$), 4 ($P<0.01$) and 5 ($P<0.001$); Aspartate aminotransferase in week 1, 2, 3, 4 and 5 ($P<0.01$); and Alkaline phosphatase in week 1, 2 ($P<0.01$), 3, 4 and 5 ($P<0.0001$), respectively. Overall mortality rate in experimental group was 68.75 (11/16). In a nutshell, Cd exposure results in adverse effects on all physiological parameters of body and may lead to lethal consequences.

Key words

Cadmium • Endocrine disruptors • Prolactin • Cortisol

Introduction

An Endocrine Disruptor/Endocrine Disrupting Chemical (EDC) is an exogenous substance that causes adverse effects on health in intact organisms and their progeny, secondary to changes in endocrine function (European Environment Agency 1997). The possible threat posed by the EDCs to health in human beings and animals at an equal pace, has now globally been accepted through extensive *in vitro* and *in vivo* experimentation (Safe *et al.* 2000). In humans, their effects are on male and female reproduction, breast development and cancer, prostate cancer, neuroendocrinology, thyroid, metabolism and obesity, and cardiovascular endocrinology (Maffinni *et al.* 2006). However, in animals, feminization of males, and various degrees of male genital abnormalities from mild to severe hypospadias, unilateral or bilateral cryptorchidism, and poorly developed testes are common manifestations in dogs, horses, goats and other domestic animals (Lange *et al.* 2002a, Meyer *et al.* 2006).

Cadmium (Cd) is one such naturally occurring toxic metal, a potential pollutant emanating from industrial and agricultural sources and an EDC (Jarup and Akesson 2009). It is a contaminant in most human foodstuffs because of its high rates of soil-to-plant transfer, rendering diet as a primary source of exposure (Satarug *et al.* 2005). The toxicity of Cd at various tissue levels such as kidneys, liver and testes has previously been elucidated (Meyer *et al.* 2006). However, a detailed account of its basis of toxicity is yet to be unearthed. In Pakistan during recent years, air pollution has emerged an alarming issue because of the presence of toxic traces in the air owing to rapid industrialization and transportation (GOP 2006). The Cd level in various species of vegetables from Peshawar, Punjab, Pakistan have been reported to be <0.008ppm (Hussain *et al.* 2009). Similarly, alarming levels of 0.62mg/L and 27.7ng/m^3 of Cd have been reported for waste water and Airborne Particulate Matter, respectively in Pakistan (Khan *et al.* 2009; GOP 2006). However, an elaborative detail regarding Cd as environmental toxicant from Pakistan is still lacking. The present study was, therefore, designed with an

objective to investigate the toxicity of cadmium chloride ($CdCl_2$) in terms of certain androlological, endocrinological and serum biochemical markers in adult male rabbits.

Material and Methods

Study Animals and Management

A total of 42 adult male rabbits (*Oryctolagus cuniculus*) ranging in age from 10 to 12 months of age, used for the toxicity tests, were purchased from the local market of Lahore, Pakistan. They were acclimatized to their housing and feeding regimen. Proper diet consisting of green fodder given thrice a day; vegetables fruits and dry feed (grains *i.e.* wheat, corn and oat) twice a week and free access to water *ad libitum* was maintained.

Determination of Lethal Doze (LD_{50})

For the determination of LD_{50} for $CdCl_2$, 18 adult male rabbits were kept under the appropriate conditions of ventilation and feeding for 15 days. They were divided into 3 groups with 6 animals in each. Three different dozes of $CdCl_2$ *i.e.*1, 1.5 and 2mg/Kg were given to these groups, respectively through the oral administration. The dose-dependent effects in terms of body weight, physical evaluation and mortality were observed daily (Ghosh and Bhattacharya 1992). Ultimately, 1.5mg/Kg of $CdCl_2$ was found to be the LD_{50}.

Toxicity Trials

A total of 24 rabbits were divided into 2 groups *i.e.* control and experimental with 8 and 16 animals per group, respectively. The dosage administration was carried out on the daily basis. All animals in the experimental group were orally administered with 1.5 mg/ kg body weight of $CdCl_2$ dissolved in equivalent amount of saline (0.9% NaCl, Otsuka Pakistan Ltd., F/4-9, H.I.T.E, Hub, Balochistan, Pakistan), whereas the control group was given 0.9% normal saline only. The trials were carried out for a total of 5 weeks.

Blood/Serum Collection and Analyses

Blood samples (4mL) were collected aseptically from the marginal ear vein of each animal, on a weekly basis, under proper restraining protocol. It was transferred into vaccutainers containing thixotropic gel separator for serum separation. Serum was harvested and analyzed for testosterone, prolactin (PRL) and cortisol through MiniVIDAS 12 Compact Automated Immunoanalyzer. The biochemical attributes *viz.* total protein (TP), Albumin, Globulin, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) were determined through semiautomatic chemistry analyzer (Chem 7, Germany). Detail of commercial kits used in the study is given in Table 1.

Statistical Analysis

Statistical analysis was conducted with the Statistical Package for Social Science (SPSS for Windows version 12, SPSS Inc., Chicago, IL, USA). Results were expressed as mean ±SE. Group differences were tested for statistical significance by using repeated measures analysis of variance, followed by Bonferroni post-hoc test.

Results

Andrological Parameters

The comparative mean (±SE) values for andrological parameters *viz.* body weight and serum testosterone level in control and experimental group of adult male rabbits are presented in Table 2. A gradual decrease was noticed for body weight in the experimental group from week 1 to 5, being significantly lower in week 4 and 5 ($P<0.05$). A similar decremented trend was noticed for testosterone level across the study weeks being significantly lower in experimental group in week 4 and 5 ($P<0.001$).

Endocrinological Parameters

The comparative mean (±SE) values for endocrinological parameters *viz.* prolactin (PRL) and serum cortisol level in control and experimental group of adult male rabbits are presented in Table 3. Prolactin was found be in a close range for both groups under study during the study weeks. However, a significantly lower value was noticed in experimental group in week 4 and 5 ($P<0.05$), than in the control. On the contrary, serum cortisol level showed a gradual increase in experimental group, from week 1 to 5, being significantly higher in week 4 and 5 ($P<0.05$).

Biochemical Parameters

The comparative mean (±SE) values for biochemical parameters in control and experimental groups of adult male rabbits are presented in Table 4. It was noted that all the parameters were altered in Cd-administered group during the study weeks revealing a gradually ascending trend. Statistical significance was, however, achieved in varying weeks and at varying level. The TP and albumin were significantly higher in week 4 and 5 ($P<0.01$); ALT in week 2 ($P<0.01$), 3 ($P<0.001$), 4 ($P<0.01$) and 5 ($P<0.001$); AST in week 1, 2, 3, 4 and 5 ($P<0.01$); and ALP in week 1, 2 ($P<0.01$), 3, 4 and 5 ($P<0.0001$), respectively.

Mortality

There was no mortality in case of control animals (n=8) as they survived throughout the study. However, the experimental animals started showing mortality from week 3 onwards with 4, 5 and 2 rabbits in week 3, 4 and 5, respectively. An overall mortality rate of 68.75 (11/16) was noticed in the study.

Discussion

Cd is ubiquitous environmental occupational pollutant which is adding up to our environment at a fast pace owing to both the natural and man-made activities. This might be more alarming for the flora and fauna of developing countries. Its true basis of toxicity yet needs to be unearthed. However, its direct action in free ionic form, and indirect action through hampering the oxidative-antioxidant balance of the body have been proposed through earlier studies (Stoths *et al.* 2001, Kowalczyk *et al.* 2003). The present study was devised to assess the effect of $CdCl_2$ toxicity on certain andrological, endocrinological and biochemical parameters of adult male rabbits.

Andrological Parameters

Our study revealed that $CdCl_2$ administration decreased the body weight in the rabbits in week 4 and 5 of the trial. Previous studies (Zeng *et al.* 2003, Amara *et al.* 2008) have also revealed a reduction in body weight after a loss in appetite, as a primary manifestation of CdCl2 administration in rats. This decrease in body weight in post-pubertal Cd-treated animals might be correlated to the greater accumulation of Cd both in hypothalamus and pituitary glands of these animals. It is worth mentioning here that earlier studies on assessment of growth hormone (GH) levels in Cd-administered rabbits have revealed that this decrease in body weight is not caused by alterations in the GH levels (Lafuente and Esquifino 2000). Contrary to our work, Jabeen and Chaudhry (2011) reported a non-significant effect of Cd administration on body weight of Sprague-Dawley rats. Difference in species under study and variation in dose rate could be plausible justifications for this variation.

It has been identified in various species of animals that Cd can act as an EDC exerting androgenic as well as oestrogenic effects (Ronchetti *et al.* 2013). Testes in males have been considered as one of the major targets of Cd in many species of animals such as rats, rabbits and dogs (Biswas *et al.* 2001, Amara *et al.* 2008). Serum testosterone levels in the present study

were decreased in $CdCl_2$ administered group. Similar results have been reported by various workers on different species of animals; however, its mechanism of this effect has not yet been elucidated. Lafuente *et al.* (2000) reported increased Cd accumulation in the hypothalamus, pituitary, and testis and decreased plasma levels of follicle stimulating hormone in rats, suggesting a possible effect of Cd on the hypothalamic-pituitary-testicular axis. Others have quoted the Cd-induced oxidative stress in the testicular tissue as a prime cause for hampered steroidogenesis (Valko *et al.* 2005). Cd-induced toxicity to the testis is probably the result of inter-digitating complex interactions which involve the disruption of the blood–testis barrier via specific signal transduction pathways and signaling molecules (Siu *et al.* 2009).

Endocrinological Parameters

The endocrinological parameters studied for the affect of Cd administration in the present study were serum PRL and cortisol levels. A gradual decrease was noticed in serum PRL level in Cd-induced experimental group, whereas; a gradual increase was seen for serum cortisol. Our results are consistent with previous study in rats which demonstrated that cadmium accumulation differentially altered PRL and Adrenocorticotropic hormone (ACTH) episodic pattern of secretion in males (Lafuente *et al.* 2003). The serum PRL levels are under the dopaminergic system of the body. Cd has been shown to interfere with the genesis of biogenic amines from hypothalamus in rats, and hence inhibiting the PRL secretion (Lafuente *et al.* 2003). In a novel study on renal and neurogenic effects of various toxic metals in children, Burbure *et al.* (2006) reported a negative correlation of Cd with serum PRL levels.

Regarding serum cortisol level as affected by Cd exposure, various studies (Chowhdury *et al.* 2004, Wu *et al.* 2007) have indicated that the primary response to Cd-induced stress is an indication of the elevation of serum cortisol level. The general adaptation response of the animal to stressors is the increase of cortisol secretion. It has been reported that animals living in contaminated environments for longer duration experiences periods of high metabolic activity

that could eventually lead to impaired cortisol secretion and cellular alterations. The direct toxic effect of Cd on adrenal tissue causes an elevated ACTH and hence, an increased serum cortisol level (Yin *et al.* 2000). However, the results of our study are not in line with studies conducted on fish which have reported the absence of Cd-induced cortisol response in fish (Dang *et al.* 2001, Lange *et al.* 2002b). The cortisol response might be more of a general phenomenon during exposure to various combinations to environmental stressors rather than a Cd-related specific effect (Lange *et al.* 2002b).

Biochemical Parameters

For Cd, the first organ reached after entry into the blood is liver where it causes direct effects of consecutive necrosis and apoptosis of hepatocytes and indirect effect of carcinogenesis (Zalups and Ahmad 2003). Biochemical parameters assessed in the present study revealed pronounced elevation at a gradual level in the weeks of study in Cd-administered group.

Assessment of TP is one of the vital diagnostic tools for hepatic injury. A gradual increase in its level in Cd-treated group in the present study is in line with previous work reported for various animal species (Zalups and Ahmad 2003, Sant'Ana *et al.* 2005). The increasing trend in protein concentration with exposure duration might be the result of Cd interrupting with cellular mechanisms as it accumulates in liver and conjugates with metallothionine (Godt *et al.* 2006). Contrary to our results, Abou El-Naga *et al.* (2005) reported no effect of Cd administration on TP concentration of marine fish. Further comparative research is needed on different animal models to assess these variations.

The albumin level also showed a gradual increase in Cd-treated groups in the present study. The results are not in line with those published earlier which have reported a significant hypoalbuminema in Cd-administered rabbits (Hristev *et al.* 2007, Hassan *et al.* 2012). This decrease was attributed to the focal haemorrhages in liver parenchyma which ultimately resulted

in decreased albumin genesis from hepatocytes. These variations could be because of varying experimental procedures in terms of dosage and time of trials conducted.

The concentration of liver enzymes in blood provides a reliable diagnostic approach to the degree of hepatic injury and status of hepatic physiology. The three enzymes studied here *i.e.* ALT, AST and ALP revealed an ascending trend in Cd-administered rabbits during the study period. Interestingly, significant difference for these enzymes was evident from the very first week of study. Similar results have been reported earlier (Hristev *et al.* 2007, Hassan *et al.* 2012). Accumulative affinity of Cd for liver causing focal haemorrhages and lipid peroxidation of its parenchyma has been given as plausible justification for this increase in hepatic enzymes (Godt *et al.* 2006). An early onset of difference in the liver enzyme levels between control and experimental group in the present study is in line with previous work (Hassan *et al.* 2012). This is a clear indication of liver being the primary site of accumulation for Cd and a major target organ.

Mortality

In the present study, no mortality was seen in rabbits of control group. However, it started in Cd-administered group from week 3 onwards with an overall rate of 68.75. A lower mortality rate of 39.4 has been reported for $CdCl_2$ treated rabbits (Hassan *et al.* 2012). Variation in these results could be because of different in dosage rate of $CdCl_2$ or breed. The mortality is not a direct effect of cadmium as such. It is rather attributed to its nephropathic/nephrotoxic ability which causes kidney failure and hence death (Godt *et al.* 2006).

In a nutshell, Cd exposure results in adverse effects on all physiological parameters of body and may lead to lethal consequences. Developing countries, in specific, need to devise directional protocols for monitoring the factors which could be responsible for Cd addition in

environment. Furthermore, surveillance needs to be carried out, in order to assess the Cd exposure both at human and animal level.

Conflict of Interest

There is no conflict of interest.

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TABLES

Table 1. Detail of commercial kits used for assessment of various biochemical analytes of the study

Attribute	Make and Batch No.	Minimum Detection Limit	Precision %		Sensitivity
			Within Assay	Between Assay	
Testosterone	AccuBind Testosterone ELISA Kit (3725-300A), Monobind Inc. Lake Forest, CA 92630, USA	0.1ng/mL	4.5-7.3	6.0-6.3	0.38pg/mL-0.038ng/mL
Prolactin	AccuBind Active PRL ELISA Kit (725-300A), Monobind Inc. Lake Forest, CA 92630, USA	5ng/mL	2.5-3.5	3.5-4.7	0.05ng/mL-1ng/mL
Cortisol	AccuBind Active Cortisol ELISA Kit (3625-300A), Monobind Inc. Lake Forest, CA 92630, USA	0.1µg/dL	6.1-8.2	7.3-9.7	6.25pg-0.25µ/dL
Total Protein	Total Protein FS Kit (123119910920), DiaSys Diagnostic Systems GmbH, Alte Strasse 9, 65558 Holzheim, Germany	0.05g/dL	1.14-1.25	0.86-2.39	0.05g/dL-15g/dL
Albumin	Albumin Kit (IUS-7-5UK), Chema Diagnostica, Via Campania 2/4, 60030 Monsano (AN), Italy-EU	0.2g/dL	0.3-0.42	0.7-1.14	0.2g/dL-6g/dL
Alanine Aminotransferase	ALAT Kit (127019910962), DiaSys Diagnostic Systems GmbH, Alte Strasse 9, 65558 Holzheim, Germany	4U/L	1-6.22	0.92-3.08	-
Aspartate Aminotransferase	ASAT Kit (126019910021), DiaSys Diagnostic Systems GmbH, Alte Strasse 9, 65558 Holzheim, Germany	3U/L	1,40-2,02	1,34-2,29	
Alkaline Phosphatase	ALP Kit (104419910021), DiaSys Diagnostic Systems GmbH, Alte Strasse 9, 65558 Holzheim, Germany	3U/L	1.06-1.50	0.85-1.60	

Table 2. Values for andrological parameters in control and cadmium chloride treated groups

Parameters	Weeks					Overall
	Groups	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	
Body Weight (kg)	Control	1.2±0.1	1.1±0.1	1.1±0.1	1.3±0.1	1.1±0.05	1.2±0.02
	Experimental	1.4±0.2	1.2±0.1	1.03±0.09	0.9±0.1*	0.9±0.03*	1.1±0.08
Testosterone (ng/mL)	Control	3.9±0.3	3.7±0.3	3.7±0.2	3.6±0.2	3.8±0.3	3.8±0.05
	Experimental	3.9±0.2	3.2±0.2	2.5±0.4	1.04±0.3**	1.1±0.4**	2.3±0.5

Values are presented as means (±SE). * P<0.05; **P<0.001

Table 3. Values for endocrinological parameters in control and cadmium chloride treated groups

Parameters	Weeks					Overall
	Groups	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	
Prolactin (ng/mL)	Control	0.5±0.02	0.5±0.01	0.5±0.02	0.5±0.01	0.6±0.02	0.5±0.01
	Experimental	0.5±0.01	0.5±0.01	0.5±0.01	0.4±0.01*	0.4±0.01*	0.4±0.01
Cortisol (µg/dL)	Control	3.4±0.1	3.7±0.3	4.01±0.1	4.6±0.19	4.7±0.3	4.1±0.2
	Experimental	3.6±0.05	4.1±0.1	4.4±0.07	4.8±0.09*	5.6±0.1*	4.5±0.3

Values are presented as means (±SE). *P<0.05

Table 4. Values for biochemical parameters in control and cadmium chloride treated groups

Parameters	Weeks						Overall
	Groups	Week 1	Week 2	Week 3	Week 4	Week 5	
Total protein (g/L)	Control	4.7±0.3	5.0±0.2	4.9±0.3	5.3±0.1	5.3±0.2	5.1 ±0.1
	Experimental	5.1±0.2	5.2±0.2	5.2±0.1	5.8±0.1*	5.8±0.1*	5.4±0.1
Albumin (g/L)	Control	3.1±0.1	3.3±0.1	3.2±0.7	3.1±0.1	3.4±0.12	3.2±0.05
	Experimental	3.6±0.1	3.7±0.09	3.8±0.06	3.9±0.08*	3.9±0.07*	3.8±0.06
Alanine Aminotransferase (U/L)	Control	22.6±0.5	23±0.4	23.2±0.3	24.1±0.5	24.6±0.6	23.5±0.3
	Experimental	41.0±18.1	42.4±4.4*	46.7±4.6**	55.1±6.2*	61.8±6.1**	49.4±3.9
Aspartate Aminotransferase (U/L)	Control	32.2±0.03	33.5±0.1	33.0±0.6	35.5±0.04	38.6±0.03	34.6 ±1.1
	Experimental	62.9±6.9*	65.0±7.0*	65.6±6.9*	71.5±7.7*	78.0 ± 11.0*	68.6±2.7
Alkaline Phosphatase (U/L)	Control	19.4±0.7	19.6±0.7	19.9±0.7	20.2±1.1	20.6±1.1	20.0±0.2
	Experimental	55.7±7.5*	55.5±7.7*	61.6±7.9**	71.4±5.7**	74.7±6.7**	63.8±3.99

Values are presented as means (±SE). *P<0.01; **P<0.001