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CADMIUM INDUCED OXIDATIVE STRESS IN TESTIS OF MALE ALBINO RATS

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ABSTRACT

Cadmium is ubiquitous environmental and occupational pollutant. It is mainly used in the manufacture of nickel- cadmium batteries, pigments and plastic stabilizers. This study was designed to investigate the ability of Cd to induce oxidative stress in the testes of male albino rats with consequent male reproductive hormones production. Ninety adult male albino rats were used in this study. They were divided into 3 equal groups, group 1: control; group 2: received Cd at 30 mg/L in drinking water for 8 weeks and group 3: received Cd at 60 mg/L in drinking water for 8 weeks. Rats were sacrificed at the end of the 4th, 6th and 8th weeks for collection of blood and testicular samples. The results revealed that Cd induced oxidative stress in the testes of male albino rats as evidenced by marked reduction in antioxidant enzymatic activities such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and reduced glutathione (GSH) level. Exposure to Cd also resulted in lipid peroxidation as indicated by significant increase in testicular MDA level. Testosterone, LH and FSH levels were also significantly decreased in blood as a result of exposure to Cd. Marked histopathological changes ranging from detachment and sloughing to vacuolization of the seminiferous tubules appeared in the testes of rats exposed to Cd.

Key words:

Cadmium, oxidative stress, antioxidant enzymes, testosterone, rat, testes.

INTRODUCTION

Environmental pollutants, including a variety of industrial and domestic chemicals, pesticides, fertilizers, heavy metals and ionizing radiation are major factors responsible for oxidative stress. Intoxication by heavy metals, particularly lead, cadmium, arsenic and mercury constitute serious threats to human health **(WHO, 1992 and Hu, 2000)**. Cadmium (Cd) is an industrial and environmental pollutant, arising primarily from battery, electroplating, pigment,

plastic, and fertilizer industries, and cigarette smoke **(Stohs and Bagchi, 1995).** With increasing production and utilization of Cd, not only industrial workers but also the general populations are exposed to the toxic effects of Cd. This has been found to produce various pathological conditions like hepatic and renal dysfunction, testicular damage, respiratory and nervous system disorders **(Waisberg *et al.*, 2003 and Thompson and Bannigan, 2008).** A variety of experiments have suggested that Cd causes oxidative damage to cells. Cd has been demonstrated to stimulate free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals **(Waisberg *et al.*, 2003).** Testicular changes due to Cd toxicity have been seen in a variety of animal models at different stages of growth and maturity. Cd-induced testicular pathogenicity includes severe hemorrhage, edema, necrosis and atrophy, as well as reduction in counts and motility of sperm with decreased testosterone concentrations in plasma and testes **(Koizumi and Li, 1992; Santos *et al.*, 2006 and Thompson and Bannigan, 2008).**To protect the integrity of biological membranes from detrimental oxidative processes caused by free radicals, both enzymatic and non-enzymatic defense mechanisms are present in the cell **(Droge, 2002 and Halliwell and Gutteridge, 2007).** The components of these defense systems can be divided into two main groups: antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) **(Mates, 2000 and Halliwell and Gutteridge, 2007)** and small endogenous antioxidant molecules such as glutathione (GSH) **(Griffith, 1999).** Other exogenous antioxidants, such as tocopherols (Vitamin E) and ascorbate (Vitamin C), essential for the function of antioxidant enzymes, are of dietary origin **(Chen and Tappel, 1995; Tandon *et al.*, 2003 and Halliwell and Gutteridge, 2007).** The objective of this study was to investigate the toxic effect of cadmium upon testicular tissue and male sex hormones through oxidative stress and lipid peroxidation mechanism.

MATERIAL AND METHODS

Ninety adult male albino rats were used in this investigation. They were divided into 3 equal groups as follow.

The first group: used as control.

The second group:

Received Cd in dose of 30 mg/L in drinking water for 8 weeks.

The third group:

Received Cd in dose of 60 mg/L in drinking water for 8 weeks.

10 animals from each group were anaesthetized and sacrificed at the end of the 4th, 6th and 8th weeks (the end of exposure period) for collection of blood samples and testicular tissues. Serum biochemical analysis included estimation in serum of testicular total protein **(Bradford, 1976),** MDA level **(Mitsuru and Midori, 1978),** reduced glutathione level **(Ellman, 1959),** glutathione peroxidase activity (GSH-Px) **(Lawrence and burk, 1976),** superoxide dismutase activity (SOD) **(Marklund and Marklund, 1974),** serum testosterone level **(Tateiki, 1977),** serum LH level **(Sheldon and Coppenger, 1977)** and serum FSH level **(Joshi *et al.*, 1979).** Histopathological examination of testicular tissues was also performed **(Dury and Wallington, 1980).** Statistical analysis of the results was applied using ANOVA.

RESULTS

Effect of cadmium on testicular MDA level and antioxidants activity:

Exposure to Cd (30 mg/L and 60 mg/L in drinking water) for 4, 6 and 8 weeks resulted in significant increase in testicular MDA level and significant decrease in testicular GSH level and SOD activity compared to control group (Table 1). On the other hand, exposure to Cd (30 mg/L and 60 mg/L in drinking water) for 4 weeks resulted in nonsignificant increase in testicular GSH-Px activity, while exposure for 6 and 8 weeks resulted in significant decrease in testicular GSH-Px activity compared to control group (Table 1).

Table (1): Effect of cadmium on testicular MDA level (nM/g wet tissue), GSH level (nM/mg protein),SOD activity (U/mg protein) and GSH-Px activity (U/g protein) in male albino rats.

Group		MDA	GSH	SOD	GSH-Px
Control	4th week	5.08 ± 0.19	6.23 ± 0.14	20.54 ± 1.28	309.8 ± 9.98
	6th week	6.59 ± 0.21	5.95 ± 0.17	17.62 ± 1.9	306.2 ± 8.64
	8th week	8.50 ± 0.37	6.23 ± 0.33	17.96 ± 1.76	310.8 ± 12.96
Cadmium (30 mg/L)	4th week	26.78 ± 0.55 *	4.08 ±0.14 *	9.12 ± 0.54 *	342.4 ± 11.94
	6th week	31.58 ± 1.11 *	3.86 ± 0.26 *	8.28 ± 0.38 *	201.2 ± 10 *
	8th week	37.40 ± 2.02 *	3.49 ± 0.08 *	7.44 ± 0.43 *	194.2 ± 17.04 *
Cadmium (60 mg/L)	4th week	32.64 ± 2.40 *	3.21 ± 0.1 *	8.56 ± 0.37 *	316.6 ± 18.32
	6th week	51.17 ± 2.32 *	2.91 ± 0.05 *	7.52 ± 0.50 *	180.2 ± 19.10 *
	8th week	48.71 ± 4.40 *	3.09 ± 0.09 *	8.38 ± 0.69 *	166.4 ± 15.58 *

All values represented as mean ± SE (n = 10).

*** Means significantly different from corresponding control group.**

Effect of cadmium on serum FSH, LH and testosterone hormone levels:

The results also revealed that exposure to Cd (30 mg/L and 60 mg/L in drinking water) for 4, 6 and 8 weeks resulted in significant decrease in serum FSH and LH levels as compared to control group (Table 2). Exposure to Cd (30 mg/L in drinking water) for 4 and 6 weeks resulted in significant decrease in serum testosterone. While exposure for 8 weeks resulted in nonsignificant decrease in serum testosterone compared to control group. On the other hand, exposure to Cd (60 mg/L in drinking water) for 4, 6 and 8 weeks resulted in significant decrease in serum testosterone compared to control group (Table 2).

Table (2): Effect of cadmium on serum FSH (IU/L), LH (IU/L) and testosterone hormone (ng/ml) levels in male albino rats.

Group		FSH	LH	Testosterone
Control	4th week	4.92 ± 0.40	3.08 ± 0.30	1.62 ± 0.11
	6th week	4.55 ± 0.72	2.78 ± 0.15	1.63 ± 0.18
	8th week	4.46 ± 0.75	2.98 ± 0.29	1.02 ± 0.07
Cadmium (30 mg/L)	4th week	2.83 ± 0.41 *	0.89 ± 0.15 *	0.89 ± 0.10 *
	6th week	2.41 ± 0.63 *	0.89 ± 0.14 *	0.50 ± 0.07 *
	8th week	2.25 ± 0.73 *	0.81 ± 0.12 *	0.95 ± 0.09
Cadmium (60 mg/L)	4th week	1.88 ± 0.57 *	0.93 ± 0.28 *	0.41 ± 0.12 *
	6th week	2.23 ± 0.56 *	0.71 ± 0.10 *	0.62 ± 0.15 *
	8th week	1.59 ± 0.38 *	0.89 ± 0.10 *	0.60 ± 0.15 *

All values represented as mean ± SE (n = 10).

*** Means significantly different from corresponding control group.**

Histopathological examination:

Testicular sections from control group showed normal histology with normal spermatogenesis. While, testicular sections from rats exposed to Cd (30 mg/L in drinking water) showed a low incidence of detached, sloughed or vacuolated seminiferous tubules. On the other hand, testicular sections from rats exposed to Cd (60 mg/L in drinking water) showed varying degrees of germ cell degenerative changes, ranging from detachment and sloughing to vacuolization of the seminiferous tubules Fig. (1, 2 & 3).

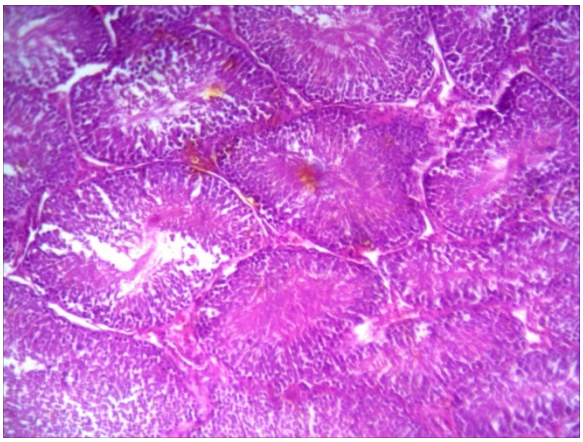

Fig. (1): Testis of a control rat showing normal seminiferous tubules **(H&E, X100).**

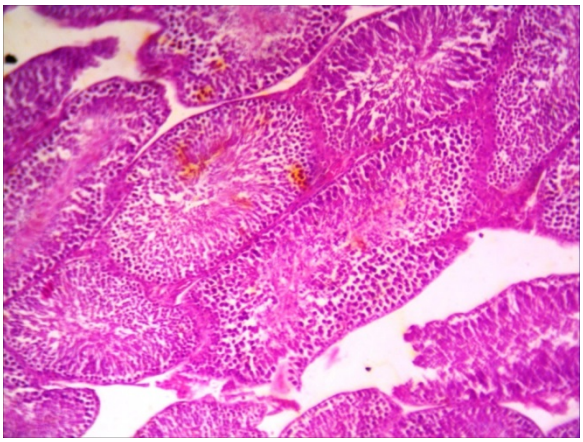

Fig. (2): Testis of a rat exposed to Cd (30mg/L) showing a low incidence of detached (A), sloughed or vacuolized (B) seminiferous tubules **(H&E, X100).**

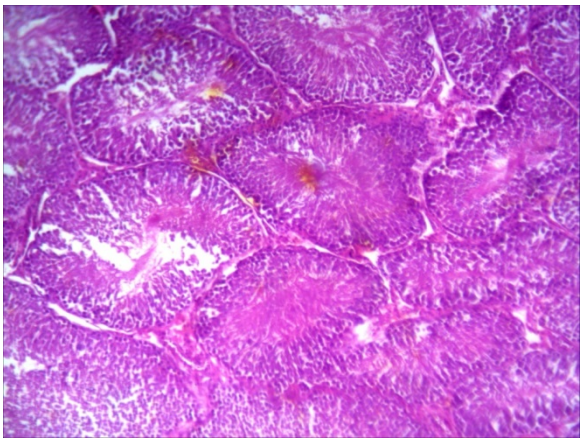

Fig. (3): Testis of a rat exposed to Cd (60mg/L) showing varying degrees of germ cell degenerative changes indicated by arrows **(H&E, X100).**

DISCUSSION

It has been generally accepted that, the pathogenesis of testicular damage upon Cd exposure is a generation of reactive oxygen species (ROS). Decreased activity of antioxidant enzymes including SOD, GPx and CAT and subsequent increased lipid peroxidation strongly suggested that Cd induces oxidative stress in the testis **(Agarwal *et al.*, 1997 and Siu *et al.*,**

2009). Lipid peroxidation is one of the main manifestations of oxidative damage, which plays an important role in the toxicity of many xenobiotics **(Stohs and Bagchi, 1995 and Anane and Creppy, 2001).** Cell membranes, which are structurally made up of large amounts of poly unsaturated fatty acids are highly susceptible to oxidative attack and consequently changes in membrane fluidity, permeability and cellular metabolic functions **(Bandyopaddhyay *et al.*, 1999).** Moreover, the end products of lipid peroxidation like malondialdehyde (MDA) can also cause tissue injury by interacting with bio macromolecules **(Freeman and Crapo, 1982 and Valentine *et al.*, 1998). Onwuka *et al.* (2010)** showed that Cd treatment induced as much as 60% of lipid peroxidation in the testes of rats compared to control. There are numerous reports concerning Cd influence on the various antioxidant enzyme activities and the beneficial effects of different antioxidant agents in amelioration of Cd toxicity. For instance, it have been shown that Cd decreases testicular SOD or GPx **(Sen Gupta *et al.*, 2004; Kara *et al.*, 2007 and Messaoudi *et al.*, 2010)** and CAT activities **(Amara *et al.*, 2008 and Ognjanovic *et al.*, 2010)** as well as glutathione level **(Koyuturk *et al.*, 2006).** All of the above investigations also confirmed the increased lipid peroxidation level after Cd intoxication. The interaction between Cd and essential trace elements could be one of the reasons for decreased enzymatic activity in the rat testis. Cd can displace iron (Fe) from its binding sites, as CAT contains Fe in its active center; therefore, decline in the activity of CAT might be a result of Fe deficiency. On the other hand, Cd is able to occupy the site of zinc (Zn) in zinc/copper SOD molecule, which creates an inactive form of this enzyme. Plasma Zn depletion associated with Cd injection also supports the causative role of Cd in SOD decreased activity **(Bauer *et al.*, 1980).** SOD rapidly converts superoxide anion ($^{\bullet}O_2$) to less dangerous hydrogen peroxide (H_2O_2). GPx and CAT can decompose H_2O_2 to water. Decreased SOD activity may imply reduce H_2O_2 production followed by subsequent decline in the CAT activity **(Kono and Fridovic, 1982).** It is well documented that the activity of CAT is directly proportional to the substrate level assumed to be produced by SOD **(Patra *et al.*, 1999 and Aitken and Roman, 2008).** GPx is highly dependent on glutathione concentration. Cd binds to cysteine in reduced glutathione (GSH), resulting in decrease in GPx activity which therefore fails to metabolize H_2O_2 to water. Moreover, there is increasing evidence that Cd interacts with Se leading to GPx inactivation **(Acharya *et al.*, 2008).** GSH is

the most abundant cellular thiol, which serves to protect against various forms of metal toxicity **(Dalton *et al.*, 2004)**. GSH is also known as free radical scavenger and potent inhibitor of lipid peroxidation **(Arthur, 2000).** It has been proved that Cd induces GSH depletion with concomitant ROS generation. It was also indicated that GSH concentration might be direct or indirect targets for Cd toxicity in the testis **(Bagchi *et al.*, 1996).** In male rodents, it is well established that Cd significantly alters the circulating levels of several hormones, e.g. FSH, LH and testosterone **(Lafuente *et al.*, 2004). AlAzemi *et al.* (2010)** stated that cadmium significantly reduced the circulating levels of FSH and LH by 60% and testosterone by 70%. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are required for quantitatively normal spermatogenesis in pubertal rats **(Russell *et al.*, 1987).** LH is a prime regulator of testicular androgenic enzyme activities **(Shaw *et al.*, 1979).** In male rats, circulating LH is responsible for maintaining normal plasma testosterone concentrations **(Ellis and Desjardins, 1982).** Adult mammalian spermatogenesis is a testosterone dependent process **(Sinha-Hikim and Swerdloff, 1999).** Massive testicular germ cell apoptosis is known to result directly either from exposure to cadmium **(Zhou *et al.*, 1999)** or alterations of hormonal support from Leydig cells **(Nandi *et al.*, 1999).** Previous studies have shown that Cd impairs the testosterone production in isolated Leydig cells without affecting their viability **(Laskey and Phelps, 1991),** demonstrating that steroidogenic disruption in Leydig cells is likely to be an initial target of Cd toxicity as an endocrine modulator. Cd also decreased steroidogenic acute regulatory protein (StAR), LH receptor and cAMP levels in the testis **(Gunnarsson *et al.*, 2007).** Lower serum testosterone levels seen in experimental animals might be due to the direct effect of cadmium on Leydig cells **(Yang *et al.*, 2003)** or an indirect effect by altering gonadotrophin secretion from pituitary, because cadmium has been shown to get accumulated in hypothalamus and pituitary and thereby implicating serious consequences on spermatogenesis process.The Cd-induced hypo-cellularity observed in our study is in agreement with the other investigations **(Yang *et al.*, 2006; Kara *et al.*, 2007).** The most of tubules exposed to Cd exhibit massive germ cells loss, and only spermatogonia remained in some of tubules to cause spermatogenesis resumption after Cd removal. It is thought that ROS are responsible for testicular degeneration in Cd toxicity **(Mathur *et al.*, 2011).** Oxidative stress can conflict direct oxidative damage to genomic DNA or upregulate

apoptotic proteins, which leads to germ cell loss and impaired spermatogenesis **(Saradha and Mathur, 2006).** Oxidative stress and the release of ROS have been linked by many studies for description of germ cell apoptosis and are a direct inducer of apoptosis in the testis **(Erkkila *et al.*, 1999 and Doreswamy *et al.*, 2004).** On conclusion; cadmium administration caused oxidative stress arising from increased lipid peroxidation and decreased antioxidant mechanism. That oxidative stress deteriorates the testicular tissue Fig.(2,3) with subsequent spermatogenesis disturbance. Also Cd affects spermatogenic process indirectly through its lowering effect upon FSH, LH and testosterone levels in blood. This study confirms the previous investigation on our native rat breeds.

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