

Effects of high and low dose of cadmium chloride on male Reproductive system in mice

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Summary:

Background: Cadmium is highly toxic and carcinogenic environmental contaminant exposure to cadmium has been reported to reduce male fertility and there are several hypotheses that suggest how reduced male fertility may result from incorporation of cadmium. The purpose of this study was to determined the role of testicular ascorbic acid level in cadmium mediated oxidative damage on mice testis.

Objectives: To know the extent of toxicity of cadmium chloride and its adverse effects on male reproductive system in mice .

Methods: Fifteen adult albino male mice were divided into three groups, group one control and two experimental groups (low dose and high dose).the experimental groups were injected with single dose of (0.14mg/BW) cadmium chloride in low dose and with (0.28mg/BW) cadmium chloride in high dose group ,intraperitoneally (ip) . The control group has given normal saline (0.9%) only .Animals were sacrificed after 15 days.The epididymal sperm count, sperm abnormalities, tissue level of lipid peroxidation and testicular ascorbic acid level were estimated.

Results: Exposure of mice to different doses of cadmium showed a decrease in the testicular weight (0.219±0.03), sperm count (124.6±0.2), ascorbic acid level (0.023±0.04), and increase in the testicular level of lipid peroxidation (0.49±0.1) and in the incidence of abnormal sperms. Exposure to high dose of cadmium showed a significant decrease in testicular weight (0.140±0.03), sperm count, (83.8±0.5) and increase in lipid peroxidation (0.79±2.1) compared to low dose group. Ascorbic acid level decreased significantly in high dose group compared to low dose group.

Conclusion: Therefore the present study suggests that cadmium has deleterious effect on spermatogenesis and one of the possible mechanism in cadmium induced oxidative damage on mice testis cell might be mediated through its effect on reducing ascorbic acid level.

Key words: Cadmium chloride, ascorbic acid, lipid peroxidation.

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Introduction:

Infertility affects approximately 15% of all couples trying to conceive and male factor infertility is implicated in almost half of these cases[1].It has long been suggested that at least half of the cases of human male infertility of unknown etiology may be attributable to various environmental and occupational exposure to toxic metals [2] .In recent years there has been an increasing interest in the contribution of occupational and environmental exposures to toxic metals in declining sperm concentration and human male fertility [3].Cadmium has been reported to damage the testis of many mammals[4,5].because there is no biological function attributed to cadmium, this metal is toxic for the cell, even in low concentrations[6].Free radicals and lipid peroxidation are potentially important mediators in testicular physiology and pathology .Exposure to cadmium metal is known to induce the formation of reactive oxygen species (ROS) like superoxide radical hydroxyl ion and hydrogen peroxide[7].Reaction of these reactive oxygen species with cellular biomolecules has been shown to result in lipid peroxidation, membrane protein and DNA damage [8, 9]. Various studies link cadmium

with oxidative stress, since this metal can alter the antioxidant defense system in several tissues of several animals, causing a depletion in the levels of reduced glutathione , as well as an alteration in the activity of antioxidant enzymes , and a change in the structure of the cellular membrane through a process of lipid peroxidation [10,11].Cell membranes are rich sources of polyunsaturated fatty acids(PUFAs) , which are readily attacked by oxygen free radicals .The oxidative destruction of PUFA , is known as lipid peroxidation[12]. The ascorbic acid is a known antioxidant present in the testis with precise role of protecting the latter from the oxidative damage [13].Several other agents are known to decrease the ascorbic acid level in the testis thus provoking free radical induced damage [14, 15].Moreover, in men who had decreased sperm counts , motility and increased abnormal sperms, the ascorbic acid level was also found decreased [16].These effects have been reversed after ascorbic acid supplementation in animals, indicating a direct role for this vitamin in the normal spermatogenesis [13,17]. In view of above findings. we have hypothesized that effect of cadmium intoxication on sperms might also have an impact on ascorbic acid level in the testis .Till date there is no

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evidence elucidating the role of ascorbic acid correlating the effects of cadmium toxicity on male reproductive function. Thus, the present study was undertaken to evaluate the effects cadmium induced oxidative damage on rat testis and their possible relation with ascorbic acid level.

Material and methods:

A total 15 adult albino male mice, weight (25-35) gm, were used in this study, and were isolated in a relatively controlled environment at temperature of about 25°C, in the "Animal breeding center" / college of medicine / university of Baghdad. They were given food and free access to water. Mice were divided into three groups, one control group and two experimental groups (low dose and high dose). The experimental groups were injected with a single dose of (0.14mg/BW) cadmium chloride in low dose and with (0.28mg/BW) cadmium chloride in high dose group, intraperitoneally (ip). The control group has given normal saline (0.9%) only. Animals were sacrificed after 15 days cadmium chloride and saline injection. Animals were sacrificed through cervical dislocation. Testes were removed, cleaned of accessory tissues and weighed. The epididymis was carefully separated from the testis. The epididymis was minced with a scissor and suspended in 0.8ml of 10% neutral buffered formalin [18]. Both chambers of the hemocytometer were charged with 5µl of sperm. The sperm in five small square were then counted and the total number of sperms per mm³ were calculated. Also large samples of these sperms were examined carefully for morphological abnormalities according to the Wyrobeck and Bruce [19].

The left testis was removed and placed in normal saline and the tunica albuginea was removed. The testis was homogenized in the same solution and the homogenate was used for the estimation of ascorbic acid level by 2,4-dinitrophenyl hydrazine method calorimetrically [20]. Briefly, the ascorbic acid in the homogenate is oxidized by Cu²⁺ to form dihydro-ascorbic acid, which reacts with acidic 4-dinitrophenyl hydrazine to form a red hydrazone, which is measured at 520 nm. The testicular tissue (1g) was transferred to a homogenizer containing 10 ml of 10 mM cold potassium phosphate buffer pH (7.4). The tissue was homogenized using a manual homogenizer. The unbroken cells were removed by centrifugation at 3,000 rpm for 10 min and the obtained supernatant was used for the estimation of lipid peroxidation level. This assay is based upon the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA), one of the aldehyde products of lipid peroxidation. Values were expressed as nanogram of MDA/gm tissue. [21] The data was expressed as Mean ± SD. The differences between groups were compared for statistical significance by t-test with the level of significance set at P < 0.01.

Results:

Cadmium intoxication decreased the testicular weight sperm count, ascorbic acid level and increased the incidence of abnormal sperms and testicular lipid peroxidation in both the experimental group. The significant decrease observed in parameters was dose dependent. Testicular weight (0.219±0.03*) significant decrease (P<0.01) compared to control group (0.620 ± 0.04) and Sperm count (124.6 ± 0.2*) was significantly (P<0.01) low in experimental groups compared to control group (210 .05 ± 0.9) (Table 1). Testicular weight and sperm count was significantly low (P<0.01) in high dose experimental group (0.140±0.03*), (83.8± 0.5*) respectively versus low dose experimental group (Table 1). A significant increase in total sperm shape abnormality was recorded in animals exposed cadmium chloride over control groups (P<0.01) (Table 2) as was shown in fig 1 & fig 2. In all the experiments total abnormal sperms were several times greater in numbers than those in normal control. Head and tail abnormalities have been observed with both doses of cadmium chloride (Table 2). Ascorbic acid level decreased significantly (0.023 ± 0.04*), (0.015 ± 0.01*) in experimental groups compared to normal control (1.86 ± 0.4) (Table 1). High dose group showed a significant decrease in ascorbic acid level compared to low dose group (Table 1). Significant increase in the level of lipid peroxidation was observed in both the cadmium treated group (0.49 ± 0.1*), (0.79 ± 2.1*) and again was significantly high with high dose of cadmium chloride compared to low dose (Table 1).

Table 1 : Effect of cadmium chloride on testicular weight (g), testicular lipid peroxidation testicular ascorbic acid level (mg/g tissue) and sperm count (×10⁶) albino male mice Mean ± SD, n = 5 in each group

Parameters	Control group	cadmium chloride Low dose group 0.14mg /BW	cadmium chloride High dose group 0.28mg /BW
Testicular weight	0.620 ± 0.04	0.219±0.03*	0.140±0.03**
Testicular lipid peroxidation	0.22 ± 0.6	0.49 ± 0.1*	0.79 ± 2.1**
Testicular ascorbic acid level	1.86 ± 0.4	0.023 ± 0.04*	0.015± 0.01**
Sperm count	210 .05 ± 0.9	124.6 ± 0.2*	83.8± 0.5**

*Significant at P<0.01 Low dose group and high dose group versus control group

** Significant at P<0.01 high dose group versus Low dose group

Table 2: Effect of cadmium chloride on sperm morphology in mice; Mean±SD , n=5 in each group

Group	Normal sperm	Abnormality sperm	
		HA	TA
Control	170.8 ± 2.28	12.6± 0.1	11.5±0.5
cadmium chloride Low dose group 0.14mg /BW	120.3±1.25*	33.6±2.6*	32.17±1.2**
cadmium chloride High dose group 0.28mg /BW	34.6±1.3*	55.20±0.8*	58±0.2**

* Significant at $P<0.01$ Low dose group and high dose group versus control group

** Significant at $P<0.01$ high dose group versus Low dose group

HA -Head abnormality, TA-Tail abnormality

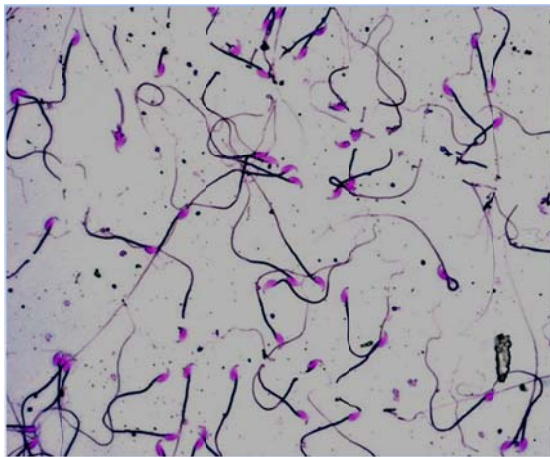


Fig 1: normal sperm (Control)
Eosin Y stain, 400x



Fig 2: abnormal sperm (HA -Head abnormality)
Eosin Y stain , 400x
➡➡➡ Showed Head abnormality

Discussion:

Cadmium is an environmental toxic pollutant affecting various tissues and organ including testes [22]. Exposure cadmium chloride in mice resulted in decreased sperm count and increased sperm abnormalities concomitantly with decreased ascorbic acid level in the dose dependent pattern. These toxic

effects induced by cadmium are in agreement with the effects of many other toxic metals [23]. The testicular weight significantly decreased in the cadmium exposed mouse compared to control group. In the cadmium treated mice most of the germ cells might have been destroyed either due the membranous damage or macromolecular degradation incurred by formation of reactive oxygen species (ROS) leading to significant decline in the sperm count and ultimately testicular weight loss[23]. In the present study the testicular lipid peroxidation level significantly increased in the dose dependent pattern which implies the generation of reactive oxygen radicals. Conceptually , reactive oxygen radicals are detrimental to the organ and therefore are regularly being scavenged by a variety of endogenous antioxidants and quenchers including vitamins, enzymes, tripeptides [24] and ascorbic acid could be one of them. Ascorbic acid is a hydrophilic and functions as a most important free radical scavenger trapping free radicals in the aqueous phase thus protecting biomembrane from oxidative damage[25]. In the present context ascorbic acid contents in the cadmium treated rat testes have been declined significantly possibly indicating it's role as a potential scavenger of ROS. The ascorbic acid has long been established as an agent to play a crucial role in the differentiation process of the spermatogonial cells to sperm .Consequent upon its use as an antioxidant to fight against cadmium toxicity eventual insufficiency of the vitamin is incurred in the cadmium treated rat testes, possibly failing to participate appropriately in the array of differentiation programme leading to transformation of sperm thereby resulting in a significant decline in the sperm count. Statistically significant increase in the percentage of sperm abnormalities in the cadmium treated mice coupled with increased lipid peroxidation level in the testicular tissue emphasizes the possibility of gene alteration in germ cells induced by ROS generated through cadmium toxicity. Cadmium compounds do not appear to damage DNA directly but through generating ROS which apparently causes DNA breaks [25]. Moreover available literature during past have revealed the causation gene mutation induced by heavy metals[29,31]. On the contrary testicular germ cells carrying minor gene mutations are not eliminated but are manifested as morphologically deformed sperm. It is also documented that certain metals including cadmium are germ cell mutagens affecting specific gene loci in spermatogonial cells thereby increasing the percentage of sperm abnormality[26, 27]. It is further stated that sperm cell morphology is genetically controlled by numerous autosomal and sex-linked genes[28]. Hence formation of abnormal sperm population in the present study is very likely due to mutagenic effects of cadmium induced ROS on specific gene loci of germ cell chromosomes involved in the maintenance of normal sperm structure. The testicular germ cells might have been

destroyed either due to membrane damage or macromolecular degradation incurred by ROS leading to a significant decline in sperm count increased the incidence of abnormal sperms and ultimately testicular weight loss. Findings of the present study demonstrate the genotoxic activity of cadmium-induced ROS in damaging the germ cells leading to significant decline in sperm count. On the other hand cadmium-induced ROS have apparently involved in gene alteration of the germ cells producing varieties of abnormal sperm. Depletion of endogenous ascorbic acid level suggests its constant use in scavenging the ROS, there by protecting the organ from potential injury. Heavy loss of germ cells in the testes is reflected from its significant weight loss. Therefore, the present study suggest that at least one of the more possible mechanism in the cadmium induced toxic effect on the male reproductive system might be mediated through its effect on reducing ascorbic acid level and generating ROS.

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