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Cadmium Toxicity on Swiss Mice and Spermatogenesis

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Abstract: ***Cadmium is a widespread environmental pollutant used in industries, has been associated with reproductive abnormalities in male Swiss mice. Metal induced reactive oxygen species (ROS) impair the spermatogenesis by increasing the sperm abnormality in testis and changes the sperm morphology. The Present study, discussed that induction of oxidative stress in testis of Swiss mice overtime (i.e. 5th to 8th Weeks) after a single intra-peritonial higher dose (2mg/kg body weight) of cadmium. Animals exposed to cadmium showed decline in the sperm count and increase in sperm abnormality. Oxidative stress was measured by malonic dialdehyde content, peroxidase, catalase and amelioration by non-enzymatic antioxidants like Vitamin C and E. The changes occurs in exposed mice compared to control suggested that high dose of cadmium exposure increases the level of lipid peroxidation, decrease the catalase and peroxidase activity by causing testicular damage affecting spermatogenesis.***

Keywords: Cadmium, Swiss Mice, testis, lipid peroxidation, sperm count, sperm abnormality, Oxidative stress, ROS, Vitamin C and Vitamin E.

1. Introduction

Cadmium is a soft malleable, ductile and bluish white bivalent metal belonging to group II B of the periodic table. It is a potential member of 'notorious trio' that pollute the environment to the maximum extent (Vallee and Ulmer, 1972; Henahan, 1973). Biologically speaking, it is a non-essential element that accumulates in the tissues with well known mutagenic, carcinogenic and teratogenic effects (Waalkes, 2000). The principal application of cadmium is mainly in the production of active electrode material used in batteries, polyvinyl chloride (PVC), pigments used in plastics, and ceramics used in light engineering works (Cadmium Council, 1991).

Epidemiological data on the toxicity of cadmium in rodents revealed that testes are important targets in acute and chronic exposure to cadmium (IARC, 1993). The nature and degree of testicular damage is dose dependant. After acute exposure, cadmium-induced testicular damage is observed at interstitial and tubular levels and permeability changes in the capillary endothelium reflected in oedemas hemorrhages or necrosis (Biswas *et al.*, 2001). It reportedly induces drastic decline in sperm count (Acharya *et al.*, 2002). In experimental models, cadmium exposure can impaired sperm motility adversely affecting male fertility (Xu *et al.*, 2005; Santos *et al.*, 2004). Cadmium decreases the level of testosterone in rodent testis (Gunnarson *et al.*, 2003). Single dose of cadmium decreases expression of pro-apoptotic genes, particularly caspase - 3 and DNA repair genes (Zhou *et al.*, 2004). Exposure to cadmium can lead to testosterone suppression (Laskey *et al.*, 1984) and reduced sperm motility.

From the above findings it is clearly understood that cadmium induced cellular toxicity include interference with antioxidant defence enzymes (Shukla *et al.*, 1996). It is suggested that this metal may induce oxidative stress by producing hydroxyl radicals (O' Brien and Salasinski, 1998), superoxide, anion radicals nitric oxide and hydrogen peroxide (Stohs *et al.*, 2001; Waisberg *et al.*, 2003).. It attack the membrane polyunsaturated fatty acids (PUFA) forming lipid peroxide by a process called lipid peroxidation, which is an index of oxidative stress (Kappus, 1985). Malonaldialdehyde is the end product of lipid peroxidation and has been widely used as a marker of free radical damage in lipid molecules (Hagihara *et al.*, 1984).

To protect the cells from oxidative injury, aerobic organisms in general, are equipped with both enzymatic and non-enzymatic antioxidant defenses which potentially neutralize ROS and protect the cells from oxidative stress (Gille and Singler, 1995). The enzymatic antioxidant defences comprise of superoxide dismutase (SOD), catalase (CT) and peroxidase (PD). The activities of antioxidants including ascorbic acid (vitamin-C), α-tocopherols (vitamin E), are most important in scavenging the noxious free radicals or by terminating the long irreversible chain of lipid perioxidation and safeguard the cells from oxidative injury. Therefore, the present study was aimed at assessing the metal-induced oxidative stress, and amilioration effects, if any, of non-enzymatic antioxidants vitamin C and E in male reproductive tissue, of Swiss mice with a view to extrapolate the data of humans.

2. Material and Methods

The experimental model used for the present study is the male albino Swiss mice (*Mus musculus*) with 15-25 gm. body weight procured from the live animal supply commercial farm M/S Ghosh Enterprisers, Kolkata, India. From the stock of mice, healthy males 10-12 weeks old and of approximately 15-20gm body weight each, were selected for the experiments.

Test chemical

Cadmium chloride is a known mutagen and carcinogen. The chemical manufactured by Thomas Baker chemicals Ltd. Mumbai, India, was used as a test chemical.

Vitamin C (Ascorbic Acid)
L-ascorbic Acid, a widely tested antioxidant in both genotoxicity and biochemical studies combat the toxicity of metals used in the study.

Vitamin E (α-tocopherol acetate)
Vitamin E is the most potential antioxidants and tested widely for its genotoxicity and biochemical studies has been selected to access its potentiality ameliorating the toxic effects of the metals used in the study.

3. Experimental protocol

In order to test the toxicity of the metals, single intra-peritoneal injection of cadmium (2 mg/kg body weight) is administered to groups of mice, each mouse of approximately of 15-20 gm body weight. The vehicle control group was treated with distilled water through the same route @ 1 ml /100gm body weight. The experimental groups of mice were divided into four sub-groups, each sub-group consisting of six healthy male mice. The first sub-group of mice was injected with cadmium chloride @ 2mg/kg body weight, the second sub group injected with cadmium chloride along with vitamin C (10mg/kg body weight) and the third sub-group was administered with the cadmium concentration along with vitamin E (100mg/kg body weight) and the fourth sub group was treated with usual concentration of cadmium along with the above two doses of vitamin C and vitamin E. From each batch a group of mice were scarified at 5^{th}, 6^{th}, 7^{th}, 8^{th} week post treatment to obtain the data for the estimation of lipid peroxidation potential (LPP), catalase and peroxidase, in testes. Sperm counting and abnormal sperm population were studied from the semen collected from vas deferens.

4. Sperm Parameters

Starting from the 5^{th} week to 8^{th} week post treatment a group of six mice were scarified by cervical dislocation for preparation of slides to analyze sperm head abnormalities and sperm counting following the procedure as follows:

The vas deference were dissected out and kept in phosphate buffer saline (PBS) solution. The sperm were squeezed out from the vas deferens in PBS at room temperature aspirated gently by pasture pipette and left for five minutes. It was centrifuged for one minute at 1000 rpm and the supernatant was discarded. A small amount of PBS was added and aspirated gently to prepare a thick homogenous suspension of sperm in PBS. A small drop of sperm suspension was taken on clean grease-free slide smeared gently with a glass rod and left overnight for natural drying. The dry slides were stained with 10% Giemsa diluted in fresh Sorenson's Buffer (pH-6.8) for one hour. The stained slides were washed in tap water and observed under microscope. About 1000 sperms for each specimen were scanned. Morphologically abnormal sperm were recorded following Wyrobeck and Bruce (1978). For sperm counting, sperm suspension was taken on the haemocytometer and the number of sperm heads was counted on R. B. C. counting chamber.

The slides prepared at different end points were coded separately. The students 't' test was utilized for comparision of data between control and experimental groups. The difference was considered significant at the $P \leq 0.05$ level. The data are reported here as mean ± SEM.

5. Observation and Results

In the present study, cadmium chloride was intraperitoneally injected into the mice at higher dose of 2 mg / kg b.w. and the effect on lipid peroxidation, catalase and peroxidase enzyme activities were noted in testicular tissue. Control data were compared with cadmium-treated data. Again, data on cadmium-treated individuals were compared with vitamin supplemented groups (Cd + vit C and Cd + vit E). In order to find out the efficacy of the individual vitamins, comparison was made in between the vitamin C and vitamin E groups. The vitamin supplemented groups were also compared with vitamin (E+C) supplemented groups.

Cadmium as a heavy metal can induce the formation of reactive oxygen species (ROS) in concerned tissues which can effectively peroxidise polyunsaturated lipids of the membrane systems of both cellular and sub-cellular components. This process is known as lipid peroxidation which can be measured in the tissues as thiobarbituric acid reactive substances (TBA-Rs) as MDA equivalents and expressed (as n moles/ gm wet tissue) in both control and cadmium injected Swiss mice groups. In testes tissue concentration-dependant increases in TBA-Rs was observed following all the four weeks of treatment. At the highest dose (2 mg/kg. b.w.) non-significant decline in testicular LPP was observed in vitamin E supplemented groups when compared with Cd-treated groups (Fig I).

Mice treated with 2mg/Kg b.w. of cadmium chloride indicated declined catalase enzyme activity ($P \leq 0.001$) in testis compared to respective control groups (Fig II). A non-significant increase in enzyme activity was observed in between Cd-treated and Cd+E groups, Cd+ C vs Cd + vit (E+C) groups.

Mice treated with 2mg/kg b.w. of cadmium chloride demonstrated significant decline ($P \leq 0.001$) in peroxidase activity compared to controls both in testes (Fig III). In the testes, however, significant increase in peroxidase activity was observed in between Cd-treated Vs Cd+C and Cd+E groups. But non-significant increase was observed when Cd+C and Cd+E groups when compared. Also non-significant rise in peroxidase activity was observed in between Cd + C and Cd + (E +C) groups Cadmium chloride treatment (2mg/kg b.w.) decreased sperm count significantly ($P \leq 0.001$). Vitamin C supplementation could increase sperm count significantly in all the weeks except 5^{th} week. Similarly, vitamin E supplementation could increase sperm count significantly in all the weeks except 6^{th} week in 2mg/kg b.w. Cadmium injected to different groups recorded decreased sperm count compared to controls ($P \leq 0.001$). However, a significant increase ($P \leq 0.05$) in sperm count was recorded following vitamin supplementation. Vitamin C was found to be more potent than vitamin E. Combined vitamin therapy was most effective in increasing sperm count in cadmium-treated mice (Fig IV).

Estimation of the percentage of abnormal sperm population in cadmium-treated mice groups (2mg/kg b.w.) were compared with controls and the data are presented in Fig. V). At every concentration of cadmium treatment, there was significant increase (P≤0.001) in the percentage of abnormal sperm compared to controls. The frequency of sperm abnormality was dose-dependent. Supplementation with vitamins significantly minimized the frequency of abnormal sperm production

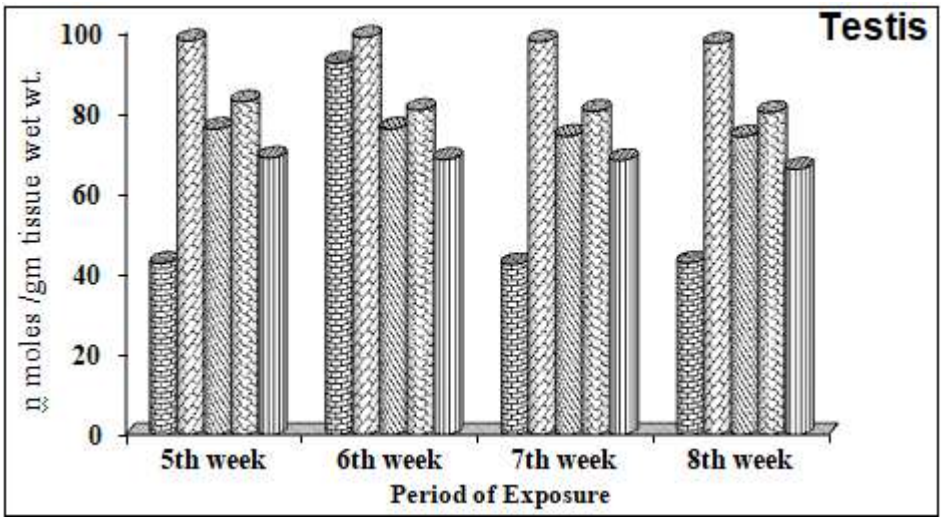

Figure I: Effect of single intraperitoneal injection of cadmium chloride (2mg/kg b.w.) on malonicdialdehyde (n moles/gm tissue wet wt.) in testis of Swiss mice.

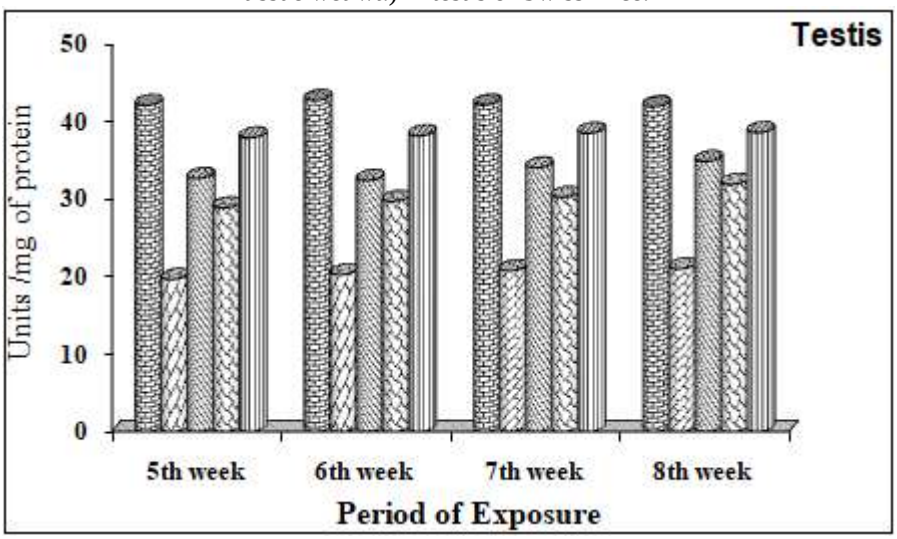

Figure II: Effect of single intraperitoneal injection of cadmium chloride (2mg/kg b.w.) on catalase in Units/mg of protein in testis of Swiss mice.

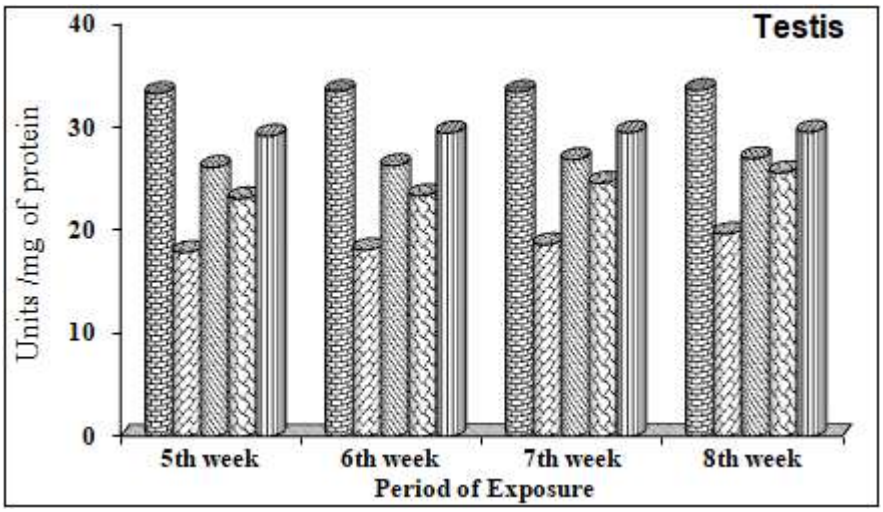

Figure III: Effect of single intraperitoneal injection of cadmium chloride (2mg/kg b.w.) on peroxidase in Units/mg of protein in testis of Swiss mice.

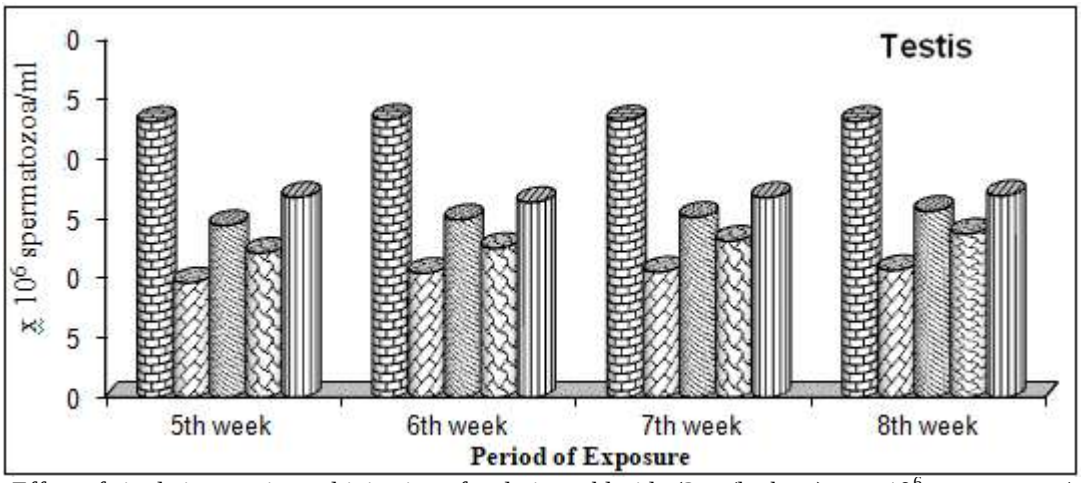

Figure IV: Effect of single intraperitoneal injection of cadmium chloride (2mg/kg b.w.) on x 10^6 spermatozoa/ml of Swiss mice.

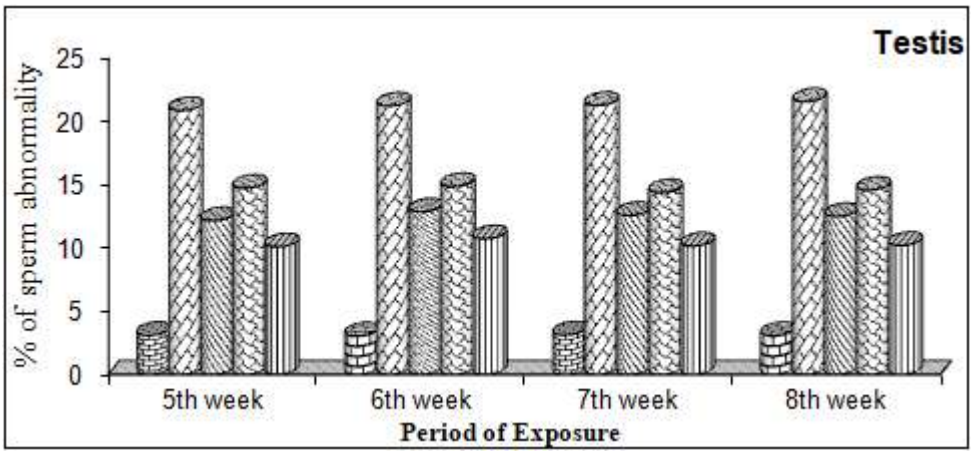

Figure V: Effect of single intraperitoneal injection of cadmium chloride (2mg/kg b.w.) on % of sperm abnormality of Swiss mice.

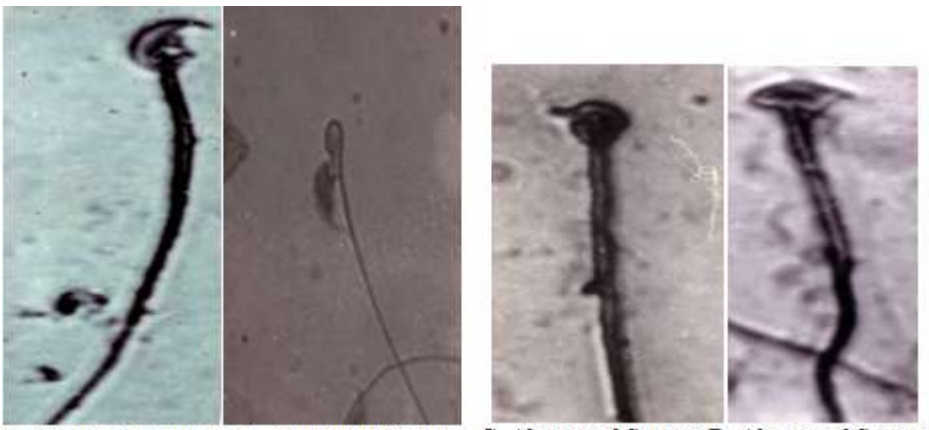

Figure A: Normal Sperm**, B, C, D:** Abnormal Sperms after Cadmium exposure

6. Discussion

Cadmium, as a metal, is highly toxic even in small concentrations. Increased oxidative stress in cadmium-induced organisms is related to the suppression of free radical scavenging functions of cellular antioxidants (Waisterg *et al.*, 2003). In the present study, malonicdialdehyde (MDA) levels in testis are higher in high dose of cadmium-treated mice than the controls. Cadmium-induced ROS enhance lipid peroxidation of sperm cell membrane, damage the mid piece and axonemal structure leading to mal-functioning, capacitation, disterted acrosomal reaction and loss of motility resulting in infertility (Atiken and Clarkson, 1987).

In order to combat the deleterious effects of Cadmium-induced ROS in the tissues, intracellular defense system normally become stimulated to protect the cell and safeguard

the intracellular environment (Yu, 1994). Enzymatic antioxidant defense like superoxide dismutase (SOD), catalase (CAT) and peroxidase (PD) that scavenge the intracellular free radicals. Results of present study also demonstrate a decline in the activity of the antioxidant enzymes in mice treated with higher doses of cadmium (2mg/kg b.w.) a comparatively higher enzyme activity than controls. In the testes, catalase activity in higher doses inhibited the enzyme activity. Inhibition of testicular catalase activity in response to metal exposure has already been demonstrated (Acharya *et al.*, 2004, 2006). In the present study higher doses of cadmium treatment to mice has lead to a decrease in the peroxidase activity in testis this is in agreement with the earlier findings (Acharya *et al.*, 2004)

Cadmium toxicity declines the sperm count and increase of morphologically abnormal sperm population. Consequently, membrane of spermatogonial cells at all stages including mature spermatozoa are reportedly degraded which is ultimately reflected in the decline of sperm count (Aitken *et al.*, 1989). Another key finding of the present study is to assess the abnormal sperm population in cadmium-treated mice (Wyrobek and Bruce, 1975). It is indicated that occurrence of sperm abnormality might be due to the involvement of damaging oxygen radicals generated through metal catalysis causing chromosomal aberrations (Acharya *et al.*,2006).

In order to combat the damaging effects culminated by the oxygen radicals, vitamin C and vitamin E were injected to cadmium-treated mice separately and in combination. Vitamin C is that it acts as a potential antioxidant which scavenges different free radicals (Nordberg and Arner, 2001). It can protect the oxidative DNA damage through its free-radical scavenging activity (Lee, 2002). Vitamin C, along with other small antioxidant molecules plays a significant role in neutralizing ROS. As a result, the sperm count has been increased and the percentage of abnormal sperm population declined significantly.

Vitamin E (Tocopherol) helps to protect the male germ cells from degeneration and maintains normal reproductive health of animals (Willis, 1985). Vitamin E is demonstrated as an androgenic stimulant (Hew *et al.*, 1993). Hence impairment of testicular functions can be reversed by supplementation of Vit E. From this point of view, testicular impairment by different metals is reportedly reduced by supplementation of vitamin E (Acharya *et al.*, 2004, 2006, 2008)

The study focuses on the activity and status of intracellular defense system like vitamin C and vitamin E to the metal induced Swiss mice to investigate the action of these vitamins over the damaging effects of metal induced oxidative stress. The findings of above study demonstrate the modifying activity of the vitamins individually or in combination to protect the tissues from oxidative injury by neutralizing the oxygen radicals generated through metal catalysis.

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