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Cadmium Induced Spermatogenesis On Swiss Mice And Modifying Effect Of Vitamins

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Abstract: ***Cadmium is a non-essential element which is profusely used in industries. It is soft malleable, ductile and bluish white bivalent metal belonging to group II B of the periodic table. Although it is 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 the 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 dose (0.5 mg/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 possible treatment by non-enzymatic antioxidants like Vitamin C and E. The changes occurs in exposed mice compared to control suggested that 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.***

I. INTRODUCTION

Cadmium is a non essential element with great concern as it accumulates in the environment due to industrial practices. It is often considered as the metal of the 20th centaury. Cadmium is a potential member of 'notorious trio' that pollute the environment to the maximum extent (Vallee and Ulmer, 1972; Henahan, 1973). Occupational exposure of cadmium and effects on the male reproductive system Queiroz EK, Waissmann W: (2006) It accumulates in the tissues with well known mutagenic, carcinogenic and teratogenic effects (Waalkes, 2000). Testes are important targets in acute and chronic exposure to cadmium (IARC, 1993). The nature and degree of testicular damage is dose dependant. Lower dose of the metal affects reproductivesystem (Thompson and Bennigan (2008), induces apoptosis in spermatogenic cells (Xu *et al.,* 1996; Zhou *et al.,* 1999), inhibits spermiation (Hew *et al.,* 1993) and increases the risks of prostrate cancer (Waalkes *et al.,* 1989).

In experimental models, cadmium exposure can also affect testis weight and induce pathogenesis leading to reduced sperm count and impaired sperm motility adversely affecting male fertility (Shiraishi and Waalkes, 1996; Xu *et al.,* 2001; Santos *et al.,* 2004). Single dose of cadmium decreases expression of pro-apoptotic genes, particularly caspase - 3 and DNA repair genes (Zhou *et al.,* 2004). From the above findings it is clearly understood 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). Since these are highly reactive and unspecific, they 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). In humans, oxidative damage to lipids has been assessed traditionally by measuring of thio-barbituric acid reactive substances (TBA-Rs) which are thought to reflect the production of malondialdehyde (Halliwell and Chirico, 1993). Excessive generation of ROS in response to metal catalysis also depresses the activity of catalase and peroxidase (Acharya *et al.,* 2004).

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To protect the cells from oxidative injury, aerobic organisms in general, are equipped with both enzymatic and non-enzymatic antioxidant defences which potentially neutralize ROS and protect the cells from oxidative stress (Yu, 1994; Gille and Singler, 1995). The enzymatic antioxidant defences comprise of 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. Several studies demonstrated that extraneous supplementation of ascorbic acid to metal-treated rodents could increase sperm count by declining oxidative stress and significantly decreasing the production of morphologically abnormal sperm population (Acharya *et al.*, 2003, 2004a, 2004b, 2004c, 2006, 2008). Vitamin E supplementation to cadmium induced mice could increased sperm count and reduced sperm abnormality, thus, maintaining normal spermatogenesis by reducing metal-induced oxidative stress (Acharya *et al.,* 2002, 2008; Mishra and Acharya, 2004).

Evidences indicated that the sperm shape morphology is under the control of specific genes present in the autosomes and sex chromosomes (Hunt and Johannssen, 1971, Benett, 1975; Krazanowaska 1976). Sperm abnormality assay is utilized for genotoxicity test and risk assessment studies (Wyrobek and Bruce, 1975). The present study was aimed at assessing the metal-induced oxidative stress, and modifying effects of non-enzymatic antioxidants vitamin C and E in the somatic and male reproductive tissue of Swiss mice with a view to extrapolate the data of humans.

II. 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. They were procured from the live animal supply commercial farm M/S Ghosh Enterprisers, Kolkata, India. Mice were acclimatized in the animal house in perfect hygienic condition at a temperature of 23 ± 2^0C. They were provided with balanced diet of prepared food cakes and tap water *ad libitum*. 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.

III. EXPERIMENTAL PROTOCOL

In order to test the toxicity of the metals, single intra-peritoneal injection of cadmium (0.5mg/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 @ 0.5 mg/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.

The syringes were sterilized properly before injection and the volume of each injection adjusted to 1ml /100gm b. w. of mouse. All the batches of mice were caged separately. 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.

SPERM PARAMETERS

Starting from the 5^{th} week to 8^{th} week post treatment a group of six mice were sacrified 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 (1975). 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 student's 't' test was utilized for comparision

of data between control and experimental groups. The difference was considered significant at the P≤0.05 level. The data are reported here as mean ± SEM.

OBSERVATION

In the present study, cadmium chloride was intra-peritoneally injected into three different groups of mice @ 0.5mg / kg body weight. and the effect on lipid peroxidation, catalase and peroxidase enzyme activities were noted in testes. 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.

Lipid peroxidation potential (LPP) of testes in cadmium treated animals also increased significantly in a given concentration (0.5mg/kg body weight) of cadmium chloride (Table-1). Highly significant increase in LPP was observed in cadmium-treated mice compared to controls in all the weeks of post-treatment.

Activity of catalase enzyme was significantly increased (P≤0.001) in all the weeks in Cd treated (0.5mg/kg b.w) mice compared to untreated controls (Fig 2). But it was decreased significantly when compared with either of the vitamins (C or E) supplemented groups or when compared with Cd + Vit (E+C) groups. When the data from Cd + Vit C and Cd + Vit E mice were compared, the enzyme activity in the testes presented significantly (P≤0.001). However, in both the tissues, catalase activity increased non-significantly in Cd + vit (E+C) groups over Cd + vit C mice. But in both the tissues combined vitamin therapy could increase the catalase activity in the Cd + Vit (E+C) mice over Cd + E groups.

The peroxidase activity of the Cd-treated (0.5mg/kg b.w) testis was significantly increased (P≤0.001) with respect to controls (Fig 3). In all other comparison, either with Cd-treated groups with vitamin supplemented groups or among the vitamin supplemented groups, peroxidase activity was found to be significantly decreased throughout the post-treatment phase (Fig-3).

Cadmium chloride treatment (0.5mg/kg b.w.) decreased sperm count significantly (P≤0.001) (Fig-4). Vitamin C supplementation could increase sperm count significantly in all the weeks except 5th week. Similarly, vitamin E supplementation could increase sperm count significantly in all the weeks except 6th week. Cadmium injected to different groups recorded decreased sperm count compared to controls (P≤0.001). However, a significant increase (P≤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.

Estimation of the percentage of abnormal sperm population in cadmium-treated mice groups (0.5mg/kg body weight.) were compared with controls and the data are presented in Fig-5). 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 (Fig 5). Abnormal sperm population is presented in plate-I which is compared with the standard sperm abnormality as indicated by Wyrobeck and Bruce (1975).

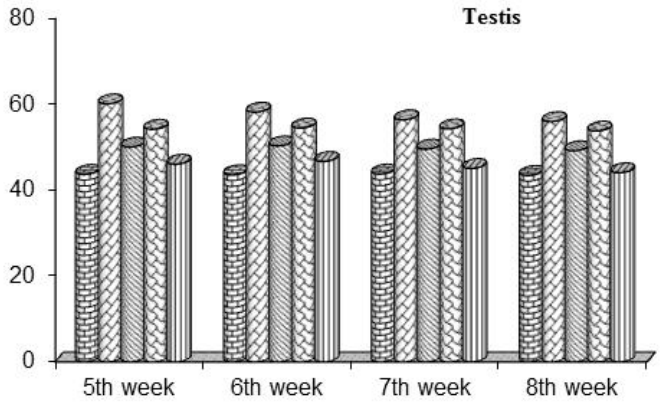

Figure 1: Effect of single intraperitoneal injection of cadmium chloride (0.5mg/kg b.w.) on malonicdialdehyde (n moles/gm tissue wet wt.) in testis of Swiss mice

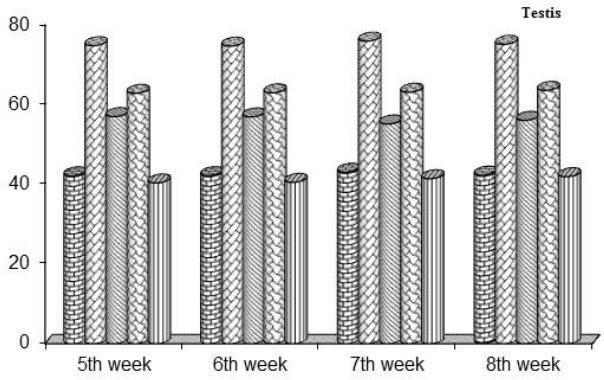

Figure 2: Effect of single intraperitoneal injection of cadmium chloride (0.5mg/kg b.w.) on catalase in Units/mg of protein in testis of Swiss mice

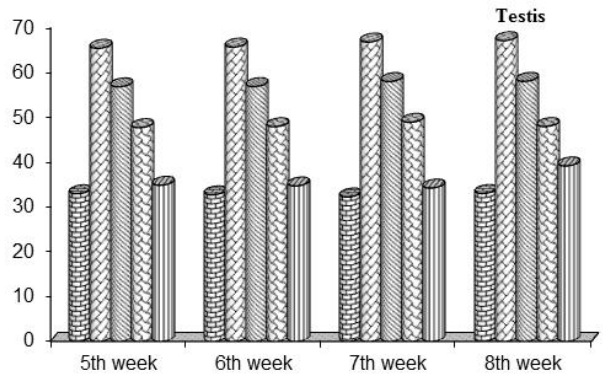

Figure 3: Effect of single intraperitoneal injection of cadmium chloride (0.5mg/kg b.w.) on peroxidase in Units/mg of protein in testis of Swiss mice

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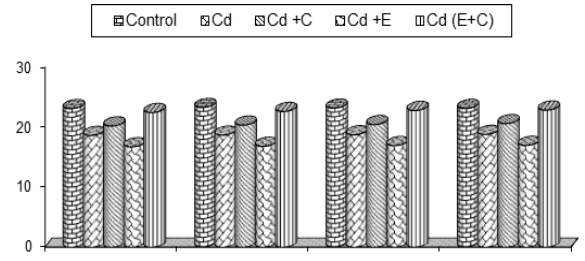


Figure 4: Effect of single intraperitoneal injection of cadmium chloride (0.5mg/kg b.w.) on x 10^6 spermatozoa/ml of Swiss mice

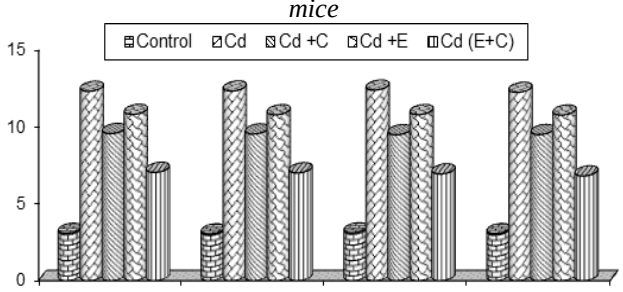
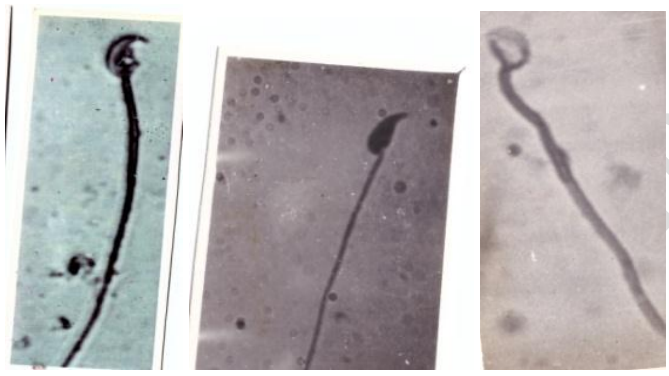
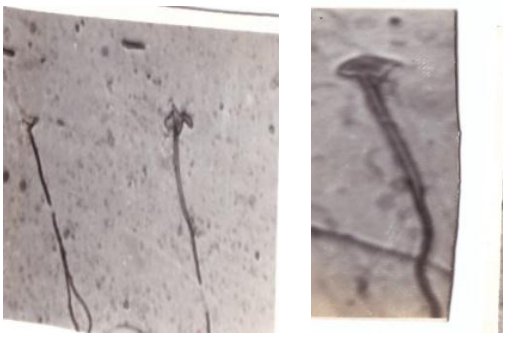


Figure 5: Effect of single intraperitoneal injection of cadmium chloride (0.5mg b.w.) on % of sperm abnormality of Swiss mice.



A *B* *C*

Normal Sperm - A



D *E*

Abnormal Sperms - B, C, D, E

Plate 1

IV. DISCUSSION

In the present study increased lipid peroxidation in cadmium-treated mice hints at the possible generation of lipid peroxides and singlet oxygen species in testis. The malonicdialdehyde (MDA) level in testis is higher in the cadmium-treated animal compared to the controls (Fig 1). MDA is the end product of lipid peroxidation and has been used widely as a marker of free radical damage in lipid molecules and, therefore, an index of oxidative stress (Hagihara *et al.*, 1984; Kappus, 1985). It directly impact on the lipid membranes of the sperm acrosome, 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). The lowest dose of 0.5 mg / kg body weight can elicit lipid peroxides as demonstrated by increased MDA. In order to combat the deleterious effects of Cadmium-induced ROS in the tissues, intracellular defense systems like Enzymatic antioxidant defense include an array of antioxidant enzymes like catalase (CAT) and peroxidase (PD).

In the testes, low dose of cadmium (0.5mg/kg b.w.) increased the catalase activity and higher doses inhibited the enzyme activity. It is also assumed that increase or decrease in the amount of such enzymes are marked through their modification in gene expression, decreased uptake or when cells are over-loaded with oxidants (Vuillaume, 1987; Barber and Harris, 1994). The reactive oxygen molecules, on the other hand, possess the ability to sufficiently modify a protein leading to altered enzyme activity (White *et al.*, 1976; Bellomo *et. al.*, 1983). The enzyme inhibition is therefore, may be due to conformational alteration/modification of protein structure of the enzymes leading to their inactivation.

Another antioxidant enzyme which is adversely affected by cadmium toxicity, is the peroxidase. Glutathione peroxidase (GPX) is a seleno protein with a tetrameric structure. This enzyme reduces lipidic and non-lipidic hydroperoxides as well as H_2O_2 by oxidizing two molecules of glutathione (GSH) (Larewance and Burk, 1976).

Cadmium-induced ROS allegedly disrupt the microtubule assembly of Sertoli cells, thereby; causing destabilization in the membrane bound hormone receptor proteins, resulting finally in the significant decline in the sperm count. The abnormal sperm population in cadmium-treated mice may be due to the involvement of damaging oxygen radicals generated through metal catalysis causing chromosomal aberrations (Roy and Rossnan, 1992, Chaudhury *et al.*, 1995, 1996; Hsu *et al.*, 1998; Acharya *et al.*, 2002, 2003, 2004, 2006). In the present findings, increased oxidative stress leading to the generation of lipid peroxides and oxygen radicals possibly have caused damage to the DNA complements resulting in increased sperm abnormality.

Vitamin C is a potential scavenger of free radicals that can restore the activity of the cell by scavenging the noxious radicals and/or by increasing the antioxidant enzymes. As a result, the sperm count has been increased and the percentage of abnormal sperm population declined significantly (Yousef, 2005; Yousef *et al.*, 2005; El-Demardash, 2005; Sonmez *et al.*, 2005; Chang *et al.*, 2006; Acharya *et al.*, 2008). Vitamin E (Tocopherol) acts within lipid membranes to prevent oxidation and formation of free radicals that may damage cellular membranes (Burton *et al.*, 1982). Vitamin E is demonstrated as an androgenic stimulant (Hew *et al.*, 1993).

Activity of anti- oxidative enzymes like catalase and peroxidase, which neutralize the hydroxyl and H_2O_2 radicals to molecular oxygen and water were also sensitive to oxidative stress of the tissue. At lower dose i.e. in 0.5 mg of $CdCl_2$ activity of both the enzymes increased significantly indicating adoptive response. However, on supplementation with vitamins, either individually or combination, the activity of both the enzymes, almost came back to control levels. Reduced sperm count and sperm abnormality were observed following treatment with different doses of the compounds. After vitamin supplementation sperm count was close to control value when the mice were treated with the lowest doses of the compounds. Individual vitamins or their combination reduced the frequency of abnormal sperms.

The present study is based on the analysis of oxidative stress due to the metal catalysis and its damaging effects on testicular tissue. The study focuses on the activity and status of intracellular defense system (both enzymatic and non-enzymatic) due to oxidative stress. supplementation of potential vitamin antioxidants like vitamin C and vitamin E to the metal induced exposed Swiss mice to investigate the modifying 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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