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RESEARCH ARTICLE

EFFECTS OF PRE AND NEONATAL CHROMIUM EXPOSURE TO THE SPERMATOGENESIS OF MALE MICE

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ABSTRACT

Chromium (Cr) is a hexavalent compound and a transitional metal used in different industries has been associated with reproductive abnormalities in male Swiss mice. Testicular dysfunctions, production of morphologically abnormal sperms, decreased sperm count, derangement of spermatogonial cells in the seminiferous tubules are the parameters of study in chromium induced Swiss mice. In the present study, an attempt has been undertaken to demonstrate whether chromium ions can traverse from the mother to the foetus and neonatal pups during gestation and lactation phases. Hence, female Swiss mice were administered with chromic acid (0.001%) through drinking water for 6 weeks from onset of pregnancy up to the end of lactation. Male pups were separately reared and 8 weeks of age the male mice were sacrificed and the testes were subjected to histological and biochemical analyses. Morphometric indices of the Cr-treated male mice indicated significant decrease in body and testes weight as compared to untreated controls. A sharp increase in lipid peroxidation potential indicated the generation of ROS due to Cr-catalysis and causes significant decline in antioxidant enzymes of the testes like peroxidation and catalase and non-enzymatic antioxidant vitamin C. As a result, these enzymes could not protect the testes from oxidative assault. The study demonstrates that maternal exposure to chromic acid during pre and neonatal stages impires spermatogenesis of male pups. Moreover, natural antioxidant system of the testes fails to protect the testes from oxidative assault resulting in impairment of spermatogenesis.

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INTRODUCTION

Chromic acid is a hexavalent compound and a strong oxidizing agent usually linked with oxygen. Chromium (VI) compounds has extensive application in welding, painting and industrial chrome plating (Katz and Salim 1994) but have carcinogenic and mutagenic effects on laboratory animals and human (Norseth 1981, Stochs and Bagchi 1995, Kawanishi *et al*. 2002). Although, the mechanism of its adverse effects on spermatogenesis is not fully understood, as a potent carcinogen/mutagen, it is allegedly involved in generating noxious ROS which damage membranes by modifying biochemical components. Based on epidemiological studies, chromium compounds are considered as more toxic (Paul *et al*., 2014, Ragab *et al*., 2014) and have great genotoxic and cytotoxic potential studied in vitamins in *in vitro* and *in vivo* system (WHO 1988, Bagchi, 2002).

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Moreover, it is also suggested that unabated pollution of the environment by chromium is considered to be a major reason for the decline human semen quality and over all reproductive health (Skakkebact *et al*., 1991). Nevertheless a number of investigations using lab animals have pointed out testicular toxicity of Cr (VI) (Saxena *et al*., 1990, Sutherland *et al*., 2000, Acharya *et al*., 2006). In the present study, an attempt has been undertaken to demonstrate whether chromium ions can traverse from the mother to the foetus and neonatal pups during gestation and lactation phases. Hence, female Swiss mice were administered with chromic acid (0.001%) through drinking water for 6 weeks from onset of pregnancy up to the end of lactation. Male pups were separately reared till sexual maturity and at 8 weeks of age they were sacrificed and the testes were subjected to histological and biochemical analyses. Morphometric indices of the Cr-treated male mice indicated significant decrease in body and testes weight as compared to untreated controls. Histological architecture of the testes revealed the formation of multinucleate cells and heavy loss of spermatogonial cells in the seminiferous tubules. Structural derangement of the tubules and a sharp increase in lipid

peroxidation potential indicated the generation of ROS (Dobroestov *et al*. 1977, Hall 1995) due to Cr-catalysis concomitant with the decrease in the antioxidant enzymes. The concentration of ascorbic acid, the non-enzymatic antioxidant of the testes also reduced markedly indicating its potential scavenging property of ROS (Aitkins 1987, Iwasaki and Gagnon, 1992). Sperm count profile was significantly reduced indicating sperm cell narcosis due to membrane damage. On the contrary, significant increase in abnormal sperm population was observed showing the mutagenic potential of chromium. This indicates the transmission of chromium from the mother to the foetus through the placenta and to the neonates through mother's milk. Chromium-induced ROS impairs the testicular development and spermatogenic process. However, reports on the transmission of Cr (VI) through placenta or mother's milk during prenatal and neonatal phases leading to testicular toxicity are scantily available. Therefore, an attempt has been made in the present study, to assess the disruption of spermatogenesis in male mice born from Cr VI exposed mothers.

MATERIALS AND METHODS

The experimental model used for the present study is the male and female 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.

Test Chemicals

Chromic acid a known carcinogen (IARC, 1982) manufactured by Qualigens fine chemicals, Glaxo India Ltd., Mumbai was used as the 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.

Experimental Protocol

Experiments were performed using female Swiss mice 24 nos. which were exposed to chromic acid (0.001%) in drinking water daily from the 1st day of pregnancy up to six weeks (includes gestation and lactation phase). Mating was performed by keeping together non-exposed males and females at least for 12-14 days. Virgin males and females not mated previously were used in the study. Females were checked daily during five days of mating, for the presence of vaginal plugs. Females having vaginal blocks (24 nos.) were divided into two groups (12 nos.) each. One group of female was given 0.01% chromic acid daily in drinking water, which constitutes the experimental group. The other groups were given normal water. All pups were weighed on the first day after birth from both group of mothers. About five pups born from Cr-exposed mothers showed prenatal death. No visible structural deformities were received in the pups. After lactation phase the male pups were separated from the mother and given normal diet and water till sexual maturity is attained. Additionally litter size and viability were accessed. All pups were weighed after birth. The sexes of the pups were assessed at weaning. Male mice were sacrificed by cervical dislocation of 9th week. Testes were removed, freed from accessory tissues and weighed. A part of the testes was trimmed in ice cold saline and homogenized in ice cold PO4 buffer (7.4) at 0-40^{0}C using a glass-potter type homogenizer and centrifuged for 30 min. at 40^{0}C to obtain tissue homogenate which was used for the assay of enzymes.

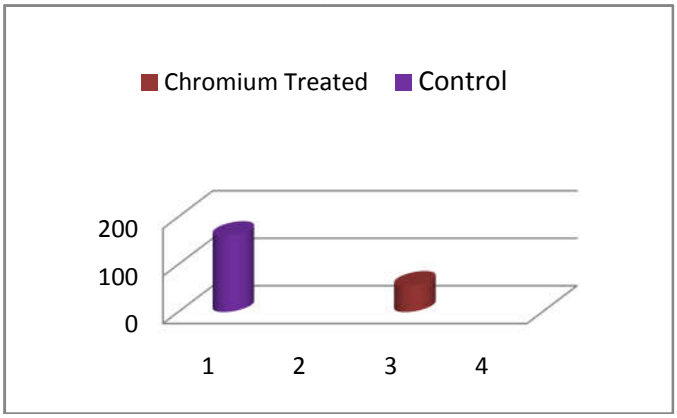


Fig. 1. Effect of Chromium (0.01 mg) on testes weight in grams of Swiss mice

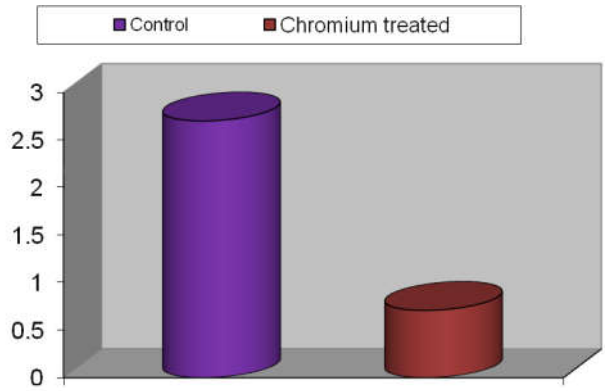


Fig. 2. Effect of Chromium (0.01 mg) on body weight of pups in grams of Swiss mice

Assay of Peroxidase (PD) activity

Peroxidase activity of the testes is determined by Michly and Chance (1976) with little modification. The concentration of purpurogalin formed was determined spectophotomatrically at 430nm. Enzyme activity was expressed in U/mg of tissue protein.

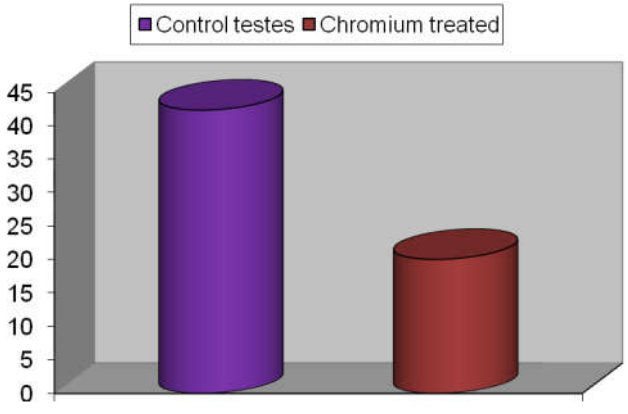


Fig. 3. Effect of Chromium (0.01 mg) on Peroxidase in Units/mg of protein in testes of Swiss mice

Assay of Catalase activity

Testicular catalase activity was determined by the method of Mittal and Dubey (1995) spectophotomatrically at 570 nm. Enzyme activity expressed in U/mg of tissue protein.

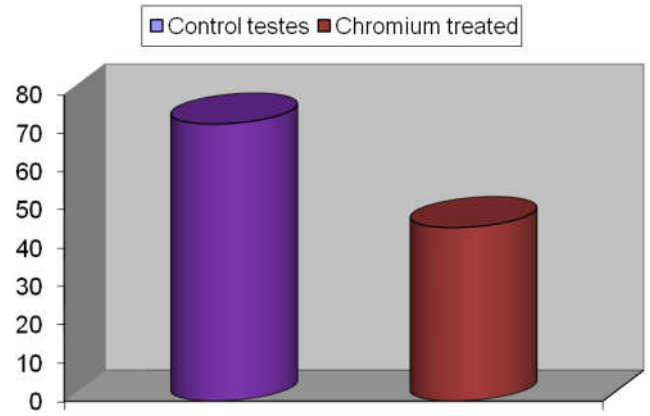


Fig. 4. Effect of Chromium (0.01 mg) on Catalase in Units/mg of protein in testesof Swiss mice

Assay of Lipid Peroxidation

A part of the testes was processed for the spectophotomatric determination of LPP following the method of Stroev and Makavova (1989). Amount of TBA reactive species (TBA-Rs) produces in mole/gm of wet tissue was calculated.

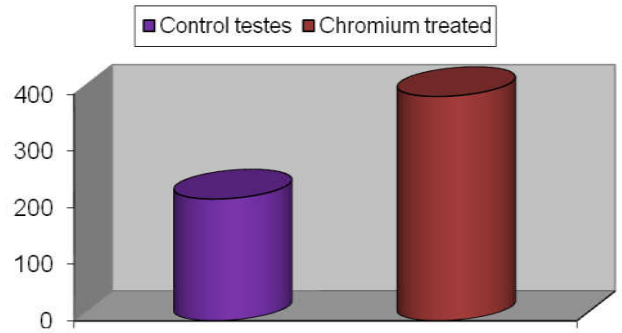


Fig. 5. Effect of Chromium (0.01mg) on Malonicdialdehyde content (n moles/gm wet Weight tissue) in testes of Swiss mice

Sperm count and sperm abnormality test

Sperm sample was collected from vas deferens and centrifuged in PBS and centrifuged following the method (Acharya, 2003). The extract was used for sperm counting and sperm abnormality studies. Sperm counting was performed using a haemocytometer. For the study of abnormal sperm population sperm smear was made and stained with giemsa and categorization of sperm was made following Wyrobeak and Bruce (1975). Histological examination of the testes of treated males (born from Cr-exposed mothers) demonstrated evacuated seminiferous tubules and dearrangements in the membranes of the tubules. Apoptotic and phagocytic cells are more frequent indicating the death of spermatids. Multinucleate giant cells are also observed.

Statistical analysis

Data generated from the control and experimental groups were calculated and compared following students't' test. Data generated at the level of ($p \leq 0.05$) was considered significant.

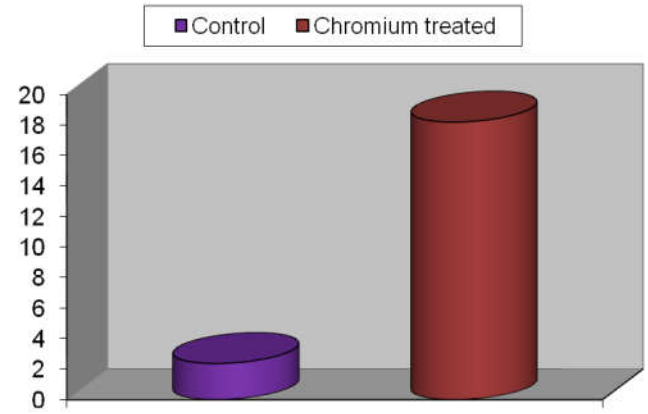


Fig 6. Effect of Chromium (0.01 mg) on % of sperm abnormality in testes of Swiss mice

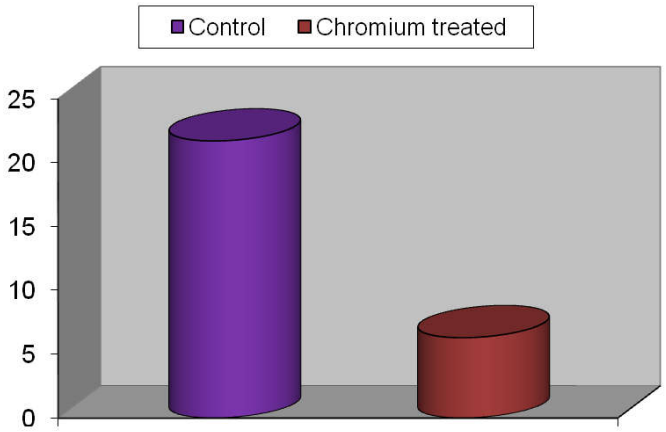
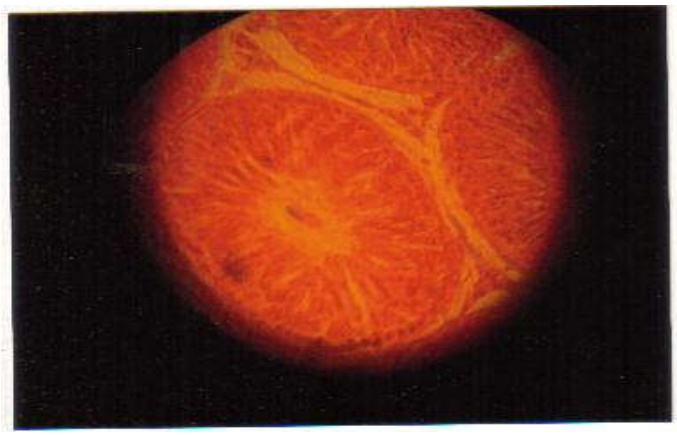
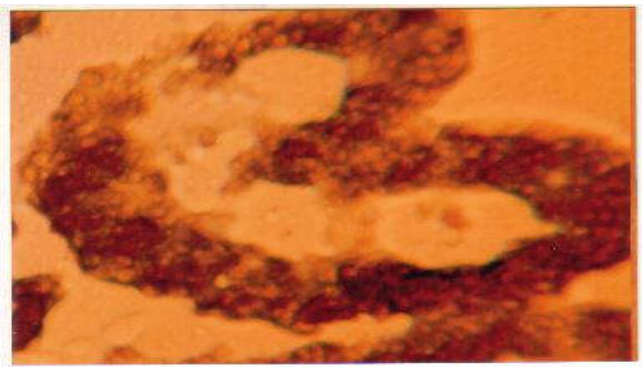


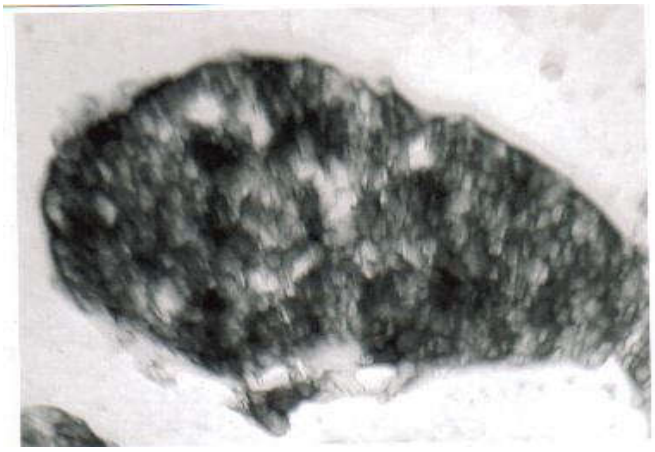
Fig. 7. Effect of Chromium (0.01 mg) on X 106 spermatozoa/ml in testes of Swiss mice



Control



Cr treated Testes showing histological de-arrangement



Testicular damage and apoptotic cells

RESULTS

Results of the present study indicate a sharp and significant decline ($p \leq 0.01$) in body weight of the pups born from chromic acid treated mothers than those of the vehicle-treated controls. Six dead pups were born from the Cr treated mothers. Similarly testes weight was significantly declined ($p \leq 0.05$) in the males born from Cr (VI) treated from mothers than controls. Most of the testes of the treated males were shrunken and filled with watery substances only. Biochemical analysis of the testes for LPP in both the groups indicated a significant increase ($p \leq 0.01$) TBA-Rs substance in treated groups. Similarly significant decrease in testicular peroxidase activity ($p \leq 0.05$) and catalase activity ($p \leq 0.01$) in chromate induced groups were observed when compared with controls. A testicular ascorbic acid content also declined significantly ($p \leq 0.01$) in treated mice than the vehicle injected groups. Sperm count studies indicated sharp decline in treated groups while sperm abnormality was increased significantly.

DISCUSSION

Results of the present study indicate a significant increase in testicular LPP in Cr-induced males than the controls. Increased LPP indicates increased oxidative stress (Kappus 1985, Janero DR 1990) with the generation singlet oxygen radicals. Cr-induced oxidative stress releasing ROS happens to be the causative factor for sperm cell death and membrane fluidity loss and formation of apoptotic cells. This is fairly related with the stable and significant decline in sperm count. Also the ROS induced by chromium must have been instrumental in bringing chromosomal and gene alterations leading to the formation of abnormal sperm population (Wyrobeck, and Bruce, 1978). The role of chromium causing gene mutations are well documented (Sugiyama 1992). Moreover morphologically altered sperm formations in mammals are genetically controlled. In the present study, significant percentage of abnormal sperm justifies the Cr-induced oxidative stress. Like all other heavy metal ions chromium must have been crossed the placenta competing with other ions for transport proteins (Semczuk and Semczuk-Sikora, 2001) and reached the foetal blood system and can solve oxidative stress affecting the spermatological stem cells. Aditionally during infancy, breast feeding also could be a source of mobilization of chromium from mother to neonates (Corpas *et al*., 2002). Increased oxidative stress in the chromium exposed males after 3-4 weeks chromium withdrawal is possibly due to the presence of increased abnormal sperm in the testes. Reports indicate that abnormal spermatozoa could be a source ROS generation leading to infertility in mammals (De Lanirande and Gagnon, 1995). The study also demonstrates significant decline in antioxidant enzymes of the testes like peroxidation and catalase and non-enzymatic antioxidant vitamin C. It is suggested that chromium induced ROS Possibly have demonstrated the protein structure of these enzymes. As a result, these enzymes could not protect the testes from oxidative assault. On the contrast, significant decline in ascorbic acid content lead to the impaired spermatogenesis in the neonates (Mukkadam 1980, Dawson *et al*., 1990). The study demonstrates that maternal exposure to chromic acid during pre and neonatal stages impires spermatogenesis of male pups. Moreover, natural antioxidant system of the testes fails to protect the testes from oxidative assault resulting in impairment of spermatogenesis.

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