*Proceeding S.Z.P.G.M.I. Vol: 25(2): pp. 73-78, 2011.*

# Effect of Hexavalent Chromium on Leydig Cells

**Wajida Tasneem, Hassan Zaheer, Abdul Rauf Shakoori and Sabiha Riaz**
*Department of Anatomy, Allama Iqbal Medical College, Lahore*

## ABSTRACT

**Objective**: This study was designed to analyze the effects of hexavalent chromium on histology of leydig cells of mice. **Place of Study**: Animal House of Zoology Department, University of the Punjab, Lahore. **Materials and Methods**: This experiment was performed on mature adult male albino mice, which were divided into eight groups. Control groups were designated as A1, B1, C1 and D1, whereas experimental groups A, B, C and D, received chromium (2mg/kg), i.p., as potassium dichromate on alternate days for two, four, six and eight weeks, respectively. **Conclusions**: Significant histological changes were recorded in the testes of treated mice, after prolonged exposure of chromium. Leydig cells were reduced in number and size, nuclei of these cells were also reduced in size and were pyknotic. The resultant decrease in size is a matter of concern in young males. In case of chronic exposure more drastic deleterious effects will appear. Future research work will add further information in this regard.

**Key words:** Potassium dichromate, Testes, Leydig cells.

## INTRODUCTION

Occupational, industrial, environmental, therapeutic and dietary exposure to a wide range of chemicals and heavy metals have harmful effects on male fertility [1].The modern industrialized society faces serious problem of exposure of its population to the environmental pollutants.[2,3,4] Hexavalent chromium (Cr VI), used in industries is an important heavy metal pollutant. Several systemic toxicities of Cr VI have been demonstrated in experimental animals. Chromium is used in the manufacture of stainless steel, alloys, pigments, dyes, leather tanning , welding etc[5,6,7,8].

Chromium in traces being essential for living system while exposure to high concentration of Cr(VI) is dangerous[9,10,11]. Hexavelent chromium compounds have mutagenic and carcinogenic effects, because chromate is rapidly taken up by the cell through the anion transport system.[12-16] In Kasur, Kalashah kaku and Sheikhupura the industrial waste ,mainly of tanneries, are thrown in open fields resulting in exposure of its population to the environmental pollution. Occupational exposure as workers in tanneries are subjected to direct exposure to chromium. For non-occupationally exposed people the major environmental exposure to chromium occurs as a consequence of its presence in food and fresh water due to percolation if untreated or incompletely treated effluents into the soil.[17,18] A number of people who live around such industries suffer indirectly through contamination of drinking water. The greatly increased circulation of toxic metals through the soil, water and air and their inevitable transfer to the human food chains remains an important environmental issue which entails some unknown health risks for future generation.

## MATERIALS AND METHODS

Forty eight sexually mature adult male albino mice, Swiss strain of *Mus musculus*, 30-50 days of age, each weighing 20-30 grams were obtained from Veterinary Research Institute, Ghazi Road , Lahore. The animals were maintained on commercial diet (Chick Feed No. 1) and tap water *ad libtium.* All the animals were housed at the Animal House of Zoology Department, University of the Punjab, Lahore. Special care was taken regarding optimal temperature and light. Animals were given one week for acclimatization and then divided into eight

groups, each of six mice. All the animals were given respective identification marks and animals of each group were housed in separate wire screened cages.

Chromium was used in the form of potassium dichromate ($K_2 Cr_2 O_7$) salt. Potassium dichromate was dissolved in distilled water and was given as 2 mg chromium/kg body weight intraperitoneally (i.p.), on alternate days to the mice.

The Control groups were designated as A1, B1, C1 and D1 , whereas experimental groups were labeled as A, B, C and D.

The animals in groups A1, B1, C1 and D1 were given distilled water i.p., on alternate days for 2, 4, 6 and 8 weeks, respectively. Animals were sacrificed and their both testes removed.

The animals in group A, B, C and D were given chromium (2mg/kg), i.p., as potassium dichromate on alternate days for two, four, six and eight weeks, respectively. The animals were sacrificed 24 hours after giving the last dose and both testes removed. The animals were sacrificed under the ether anesthesia. All the dosages were calculated and administered with the help of disposable insulin U-100 syringes.

The testes were removed by making an incision at the base of the scrotum and cleared from adherent tissue. Each testis was cut into two pieces. The tissue were fixed in 10% formalin for24 hours. The tissues were processed in auto-processor and paraffin blocks were prepared. The sections were cut at 6-8 microns by microtome and stained by haematoxylin and eosin by standard procedure. Stained sections of testes from each animal were examined under light microscope and observation were made for Leydig cells.

## RESULTS

All the animals of control groups Al, Bl, Cl and Dl at the time of sacrifice were active and healthy. Their feeding behavior was normal and showed no sign of any ailment. The testes were found to be freely hanging in the scrotum and were covered by capsule in the scrotum. The testes were soft in consistency, well vascularized and easily taken out of the scrotum. The size of the testes was normal and looked pinkish whitish. The Leydig cells were arranged in groups. The number of nucleoli in most of the leydig cells was 1-3 (Fig. 1).

All the animals of group A and group B at the time of sacrifice were active and healthy looking. Their feeding behavior was normal and showed no sign of any ailment. The testes were found to be freely hanging in the scrotum. Testes were covered by capsule in scrotum. These were soft in consistency and easily taken out of scrotum. The size of the testes seemed to be unchanged. The Leydig cells were unchanged.They were present in both clusters and scattered (Fig.2)

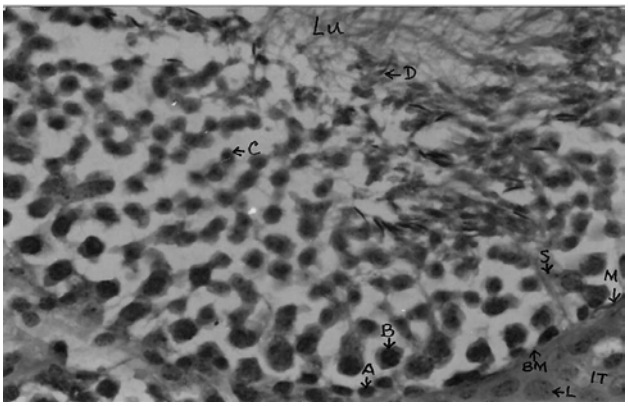


**Fig. 1: (Control) L, Leydig cell.**

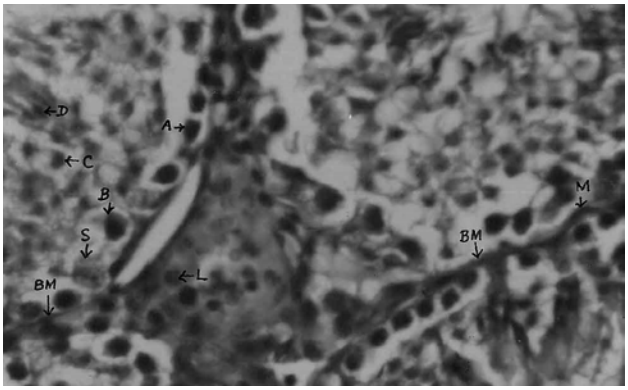


**Fig. 2: Experimental group B.**

All the animals of group C were active and healthy looking, at the time of sacrifice. Some animals of this group showed eczematous changes. There was decrease in the size of leydig cells. Nuclei were pyknotic in many cells (Fig.3).

Animals of group D showed eczematous change. The leydig cells were reduced in number and were quite scattered, and were occasionally seen

**Table 1.- Outcome in experimental and control group.**

| | Experimental Group (n=24) | Outcome | | | | Control group (n = 24) | Outcome | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Yes | % | No | % | | Yes | % | No | % |
| **1.** | **A (n=6) (2 weeks)** | | | | | **A1 (n=6) (2 weeks)** | | | | |
| | Leydig cells change | | | 6 | 100 | Leydig cells change | | | 6 | 100 |
| | Eczematous changes | | | 6 | 100 | Eczematous changes | | | 6 | 100 |
| **2.** | **B (n=6) (4 weeks)** | | | | | **B1 (n=6) (4 weeks)** | | | | |
| | Leydig cells change | | | 6 | 100 | Leydig cells change | | | 6 | 100 |
| | Eczematous changes | | | 6 | 100 | Eczematous changes | | | 6 | 100 |
| **3.** | **C (n=6) (6 weeks)** | | | | | **C1 (n=6) (6 weeks)** | | | | |
| | Leydig cells change | 6 | 100 | | | Leydig cells change | | | 6 | 100 |
| | Eczematous changes | 3 | 50 | | | Eczematous changes | | | 6 | 100 |
| **4.** | **D (n=6) (8 weeks)** | | | | | **D1 (n=6) (8 weeks)** | | | | |
| | Leydig cells change | 6 | 100 | | | Leydig cells change | | | 6 | 100 |
| | Eczematous changes | 6 | 100 | | | Eczematous changes | | | 6 | 100 |

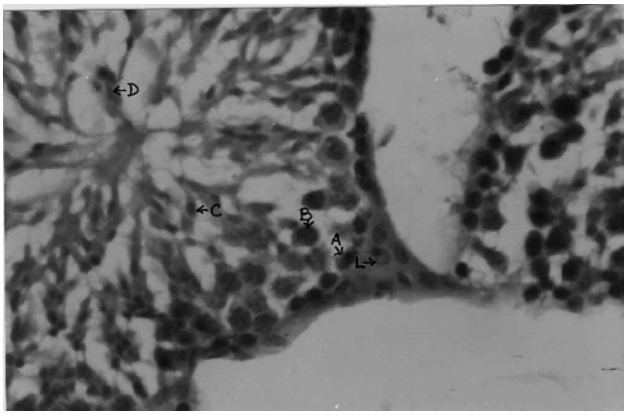


**Fig. 3: (Experimental group C) L, Leydig cell size is reduced with pyknotic nucleus.**

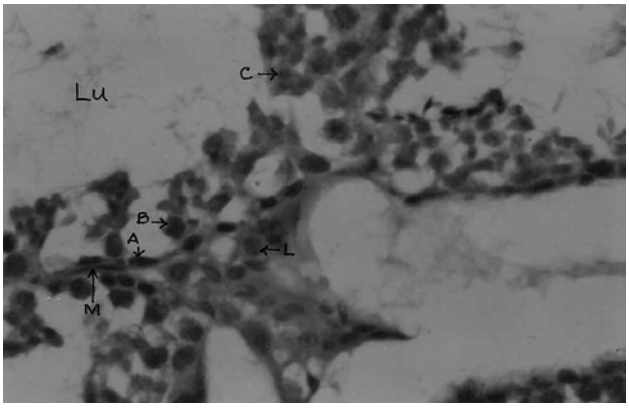


**Fig. 4: (Experimental group D) L, Leydig cell with pyknotic nucleus. H & S stain.Magnification:400x**

in the form of clumps or groups. These were relatively small and nuclei of these cells were also reduced in size and were pyknotic, suggestive of atrophy of leydig cells (Fig.4).

## DISCUSSION

All kinds of life *i.e.* humans, animals and plants are continuously being exposed to heavy metals. Rapid industrialization is beneficial to mankind but at the same time it is creating problems due to unplanned and unwise disposal of industrial wastes in the environment[19]. These wastes gain entry into the food chain directly or indirectly, thus causing serious health problems[1,20]. The present study is aimed at highlighting the damage caused by hexavalent chromium on testes of albino mice. Hexavalent chromium is readily taken up by the cells and passes through the membranes by the sulfate anion transport system[21]. These compounds are actively transported through the cell membranes. After absorption hexavalent chromium is readily reduced by a number of metabolic pathways and transported by blood to target organs. Derivatives of Cr(lll) are water insoluble at neutral pH and can be removed from media in the form of chromium hydroxide, where as hexavalent forms are highly soluble. Valences of chromium are very important for determining their physiological effects [22].Toxic

effects are due to greater permeability of biological membranes and strong oxidative effect of hexavalent chromium on membrane phospholipids, proteins, nucleic acid and other macromolecular complexes [23].

The process of intracellular chromium reduction occurs either in cytoplasm or in the nucleus and yields several reactive intermediates, which interact with DNA.DNA lesions are capable of obstructing DNA replication[24,21]. Hexavalent chromium has been reported to cause severe side effects, besides impotence. Lindbohm *et al.*[25] discovered that semen analysis of more than 50% of welders had a sperm count of less than 4 million sperm/ml. Mortensen[26] investigated a greater risk of sperm quality among welders. Al-Hammod *et al.* [27] and Elbetieha *et al*.[28] also reported results resembling those of Mortensen. Not much is known regarding the mechanisms responsible for the sperm count reduction. Several potential mechanism exists beside a direct toxic effect on spermatogonia. These include a toxic effect on some later stages in speramtogenesis within the testes, on the epididymal maturation of spermatozoa or on transport along the excretory –duct system [7].

In the present experiment by giving 2 mg chromium/kg body weight *i.p.* on alternate days to the mice, histological changes were observed for two to eight weeks. The histological appearance of two to four weeks chromium (Vl) administration showed picture very near to the control level. After two months of chromium administration histological examination revealed significant reduction in the size of leydig cells.

During reduction of hexavalent chromium to trivalent chromium highly reactive radical species are released, including the hydroxyl radical, thiyl and pentavalent chromium, which are capable of causing DNA damage.

Spermatogenesis is activated by testosterone, which is synthesized in the leydig cells. Testosteron deficiency causes spermatogenic impairment. Biosynthesis of testosterone from leydig cells is an essential physiological process to promote post miotic spermatogenic activity[29]. The atrophied leydig cells after chromium administration and reduced diameter of their nuclei indicate the possibility of disturbed endrogen biosynthesis and thus affecting the spermatogenic cycle. Several other metals, such as lead and cadmium have also been reported to effect the testes by interfering with hormones synthesies[30].

Results of this study resemble with those of Chowdhury and Mitra.[29,31-33,4] Chromate has been shown to concentrate in the testes following intraperitoneal injections.[34] The increase in chromium in the testes has been proposed to be responsible for the testicular damage. Chromium might be toxic to spermatogenic epithelium or exert its effect via interruption of hormonal control of spermatogenesis.[35] In spite of chromium in traces being essential for living system exposure to high concentration of Cr(VI) is hazardous [36].

## CONCLUSIONS

The present study indicates that Cr(VI) has caused toxic effects even at low concentration , when they were given for a long time. Significant histological changes were recorded in the testes of treated mice, after prolonged exposure of chromium. Leydig cells were reduced in number and size, nuclei of these cells were also reduced in size and were pyknotic. The resultant decrease in size is a matter of concern in young males. It can be speculated that with higher amount of metal and/or in case of chronic exposure more drastic deleterious effects will appear. Future research work in these directions will add further information in this regard.

## REFERENCES

1. Aruldhas MM, Subramanian S, Sekhar P, Vengatesh G, Chandrahassan G, Govindarajulu P and Akbarsha MA. Chronic chromium exposure-induced changes in testicular histoarchitecture are associated with oxidative stress: study in a non-human primate. *Human Reprod.* 2005; 20(10): 2801-13.
2. Linos A, Petralias A, Christophi CA, Christoforidon E, Kouroutou P, Stoltidis M, Veloudaki A, Tzala E, Makris KC and Karagas MR. Oral ingestion of hexavalent chromium through drinking water Greece.

An ecological study. *Environ Hlth.,* 2011; 10: 50.

3. Vitale RJ, Mussoline GR and Rinehimer KA. Environmental monitoring of chromium in air, soil, and water.*Regul. Toxicol. Pharmacol.,* 1997; 26(1 Pt 2): S80-5.
4. Tebourbi O, Mahjoub S, Yacoubi MT, Sakly M, Benkhalifa M, and Rhouma KB. Effects of hexavalent chromium on reproductive function of male adult rats. *Reprod Biol.,* 2012; 12(2):119-33.
5. Burg RV. and Liu D, Toxicology update. *J. Appl. Toxicol.,* 1993; 13(3): 225-30.
6. Dreisbach RH. *Handbook of poisoning: Diagnosis* treatment. 9th ed. Lange Medical Publication. Los Altos, California, 1977. pp. 218.
7. OEHHA. Proposition 65 Oral Maximum Allowable Dose Level (MADL) for Developmental and Reproductive Toxicity for Chromium (Hexavalent Compound). Office of Environmental Health Hazards Assessment Reproductive and Cancer Hazard Assessment Branch. 2010.
8. Oliveira H, Spano M, Guevara MA, Santos TM, Santos C. and Pereiraes ML. Evaluation of in vivo reproductive toxicity of potassium chromate in male mice. *Experi Toxicol Pathol.* 2009;10: 1016-
9. Kirmani MZ, Sheikh M, Farah N, Iftikhar IN. and Erum Z. Determination of some toxic and essential trace metals in some medicinal and edible plants of Karachi city. *J Basic App Sci.* 2011; 7(2):89-95.
10. Silva M, Barrosi JVP, Silva S, Silva J, Pinto de SL, Alves de J. Thamires and Silvano Jose, Testicular morphometry hexavalent chromium. *Vistant*e 2012; 12:47.
11. Rankov J. and Trif, A. The consequences of potassium dichromate intake on some reproductive toxicity morphological biomarkers in male rats. *Lucrari Stiintifice Medicina Veterinava,* 2009;
12. Blankenship LJ, Carlisle DL, Wise JP, Sr Induction of apoptotic cell death by particulate *lead* chromate: differential effects of vitamins C and E on genotoxicity and survival. *Toxicol Appl Pharmacol.* 1997; 146: 270-80.
13. Saikia SK, Mishra, AK, Tiwari S. and Pandey R; Hexavalent chromium induced histological alterations in Bacopamonnieri and assessment of genetic varience. *J Cytol Histol.* 2012; 5:
14. Stern AH. A quantitativei assessment of the carcinogenicity of hexavalent chromium by the oral route its relevance to human exposure. *Environ Res,* 2010; 110: 798-807.
15. Smith AH. and Steinmaus CM. Health effects of arsenic and chromium in drinking water:recent human findings. *Annu Rev Pub. Hlth.* 2009; 30:107-207.
16. Kerger BD, Butler WJ, Paustenbach DJ, Zhang J. and Li S. Cancer mortality in Chinese populations surrounding an alloy plant with chromium smelting operation. *J. Toxicol Environ Hlth A.,* 2009; 72:329-44.
17. Aruldhas MM, Subramanian S, Sekhar P, Hasan GC, Govindarajulu P, and Akbarsha MA. Microcanalization in the epididymis to overcome ductal obstruction caused by chronic exposure to chromium- a study in the mature bonnet monkey .*Reproduction.* 2004; 128: 127-37.
18. Nriagu JO and Pacyna JM. Quantitative assessment of world contamination of air,water and soils by trace metals. *Nature,* 1998; 333(6169): 134-39.
19. Brindha K, Elango L. Impact of Tanning industries on Groundwater Quality near a Metropolitan City in India. *Water Resources Management* 2112*;* 26(6):1747-61.
20. Samuel JB, Stanley JA, Roopha DP, Vengatesh G, Anbalagan J, Banu SK and Aruldhas MM. Lactational hexavlent chromium exposure-induced oxidative stress in rat uterus is associated with delayed puberty and impaired gonadotropin levels. *Hum Exp Toxicol.,* 2011; 30: 91-101.
21. Xu J, Manning FCR and Patierno SR, Preferential formation and repair of chromium-induced DNA adducts and DNA-protein crosslinks in nuclear matrix DNA. *Carcinogenesis,* 1994; 15(7): 1443-50.
22. Vishniakov SI, Levantovskii SA and Ryzhkova GF. The biological action of chromium in relation to its valency. *Biol.*

*Nauki*, 1992; 9: 105-108.
23. Ginter E, Chorvatovicova D and Kosinova A. Vitamin C lowers mutagenic and toxic effects of hexavalent chromium in guinea pigs. *Int. J. Vit. Nutr. Res.*, 1989; 59:161-66.
24. Bridgewater LC, Manning FCR, and Patierno SR. Base specific arrest of *in vitro* DNA replication by carcinogenic chromium: relationship to DNA interstrand crosslinking. *Carcinogenesis*, 1994; 15(11): 2421-27.
25. Lindbohm ML, Hemminki K, Kyyronen P, Parental occupational exposure and spontaneous abortions in Finland. *Am. J. Epidemiol.*, 1984; 120: 370-78.
26. Mortensen JT. Risk for reduced sperm quality among metal workers, with special reference to welders. *Scand. J. Work Environ. Hlth.* 1988; 14(1): 27-30.
27. Al-Hamood MH, Elbetieha A and Bataineh H. Sexual maturation and fertility of male and female mice exposed prenatally and postnatally to trivalent and hexavalent chromium compounds. *Reprod. Fertil. Dev.*, 1998; 10(2):179-83.
28. Elbetieha A and Al-Hamood MH. Long term exposure of male and female mice to trivalent and hexavalent chromium compounds: effect on fertility. *Toxiclogy*, 1997; 116: 39-47.
29. Chowdhury AR and Mitra C. Spermatogenic and steroidogenic impairment after chromium treatment in rats. *Indian J. Exp. Biol.* 1995; 33: 480-84.
30. Wiebe JP. Effect of lead on testicular endocrinology in the developing male. *Am.* Zool. 1981; 20: 899.
31. Liu KJ, Mader K, Shi X. and Swartz HM. Reduction of carcinogenic chromium (VI) on the skin of living rats. *MRM* 1997; 33: 524-26.
32. Saxena DK, Murthy RC, Lal B, Srivastava RS and Chandra SV. Effect of hexavalent chromium on testicular maturation in the rat. *Reprod. Toxicol.* 1990; 4: 223-28.
33. Chandra, AK, Chatterjee A, Ghosh R, Sarkar M. and Chaube SK. Chromium induced testicular impairment in relation to adrenocortical activities in adult albino rats. *Reprod Toxicol.*, 2007; 24(3-4):388-96.
34. Vittorio PV, Wight EW and Sinnott BE. The distribution of chromium-51 in mice after intraperitoneal injection. *Canadian J. Biochem. Physiol.* 1962: 40: 1677-83.
35. Danielsson BRG, Dencker L, Lindgren A and Tjalve H. Accumulation of toxic metal in male reproductive organs. *Toxicol. Suppl.* 1984; 7: 177-80.
36. Chorvatovicova D, Kovacikova Z, Sandula J. and Navarova J. Protective effect of sulfoethylglucan against hexavalent chromium. *Mut. Res.* 1993: 302: 207-11.

**The Authors:**

Wajida Tasneem,
Assistant Professor,
Department of Anatomy,
Allama Iqbal Medical College,
Lahore.

Hassan Zaheer,
House Officer,
Services Hospital,
Lahore.

Abdul Rauf Shakoori
Director,
School of Biological Sciences,
University of the Punjab,
Lahore

Sabiha Riaz
Professor
Department of Histopathology,
Fatima Memorial Hospital,
Lahore.

**Address for Correspondence:**

Dr. Wajida Tasneem.
Assistant Professor,
Department of Anatomy.
Allama Iqbal Medical College,
Lahore.
Cell# 0300-4268930