Original Article

Impact of Unplanned Disposal of Tanneries Effluents on Testes – A Dilemma of Our Society

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Abstract

The modern industrialized society faces serious problems of exposure of its population to the environmental pollutants. Rapid industrialization is beneficial to mankind but at the same time it is creating problems due to unplanned and unwise disposal of industrial effluents particularly from the tanneries in the environment. The wastes of industries utilizing the chromium as tanneries have substantial amount of chromium in it. This study was designed to analyze the effects of hexavalent chromium on diameter of seminiferous tubules of testes of mice at Animal House of Zoology Department, University of the Punjab, Lahore. This experiment was performed on forty – eight mature adult male albino mice, which were divided into eight groups. Control groups were designated as A_1, B_1, C_1 and D_1, whereas experimental groups A, B, C and D, received chromium (2 mg / kg), i.p., as potassium dichromate on alternate days for two, four, six and eight weeks respectively. Significant histological changes were recorded in the testes of treated mice, after prolonged exposure of chromium tubular diameter of the seminiferous tubules was reduced. 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, Seminiferous tubules, hexavalent chromium.

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Introduction

Hexavalent chromium (Cr (VI) is a transition element utilized in many fields of modern industries, is an important heavy metal pollutant.[1,2] The modern industrialized society faces serious problems of exposure of its population to the environmental pollutants.[3,4] 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.[5-8]

Chromium in traces being essential for living system while exposure to high concentration of hexavalent chromium is dangerous.[9,10] Hexavelent chromium compounds have mutagenic and carcinogenic effects, because chromate is rapidly taken up by the cell through the anion transport system.[11-15] 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.[16-18] 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 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 A_1, B_1, C_1 and D_1, whereas experimental groups were labeled as A, B, C and D. The animals in groups A_1, B_1, C_1 and D_1 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 (2 mg / 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 anaesthesia. All the dosages were calculated and administered with this help of disposable insulin U-100 syringes. The testes were removed by making an incision at the base of the scrotum. 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 were examined under light microscope. Diameter of the semniferous tubule was measured with the help of an ocular micrometer. Only the tubules cut at right angle to the axis of seminiferous tubule were measured. Observations were recorded at random from three different sections in each slide. For statistical analysis, the control and Cr – treated experimental mice were compared using Student's 't' test.[19]

Results

All the animals of control group at the time of sacrifice were active and healthy. Their feeding behavior was normal and showed no sign of 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 scrotum. The size of the testes was normal and looked pinkish white. The size of tubules was normal. The lumen could be marked in almost all the tubules (Fig. 1).

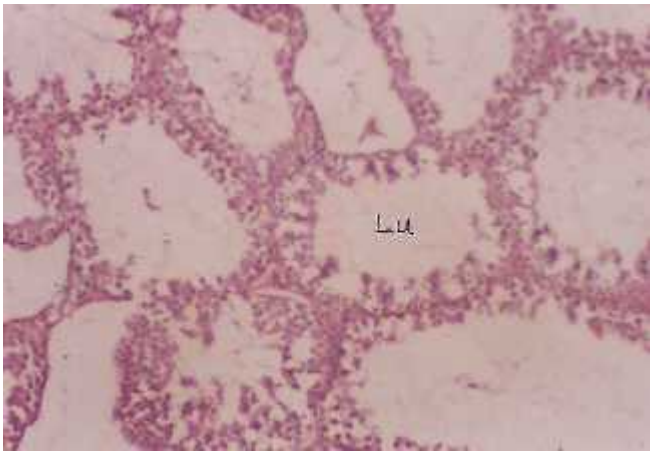

Fig. 1: H & E stain. Magnification: 200 x (control).

All the animals of group A and group B at the time of sacrifice were active and healthy looking. Their feeding behaviour was normal and showed no sign of 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 seminiferous tubules in group A and B showed no appreciable microscopic changes. The mean tubular diameter in control and ex-

perimental group A was 201.6 micrometer and 201.4 micrometer, respectively. Mean tubular diameter in control and group B was 201.6 micrometer and 200.6 micrometer, respectively (Table l).

Table 1: Effect of Potassium Dichromate Administration at a Dose of 2 mg/kg Body Weight i.p., for 2, 4, 6 and 8 Weeks on the Diameter of Seminiferous Tubules (μm) of Albino Mice, *Mus Musculus.*

Weeks	Control (n = 6)	Experimental (n = 6)
2	201.6 ± 0.558	201.4 ± 0.507
4	201.6 ± 0.620	200.6 ± 0.551
6	201.7 ± 0.815	199.4 ± 0.480*
8	201.5 ± 0.580	197.1 ± 0.709***

Values given are Mean ± SEM.
Values with asterick(s) are significantly different from their respective controls.
*P < 0.05; **P < 0.01; ***P < 0.001

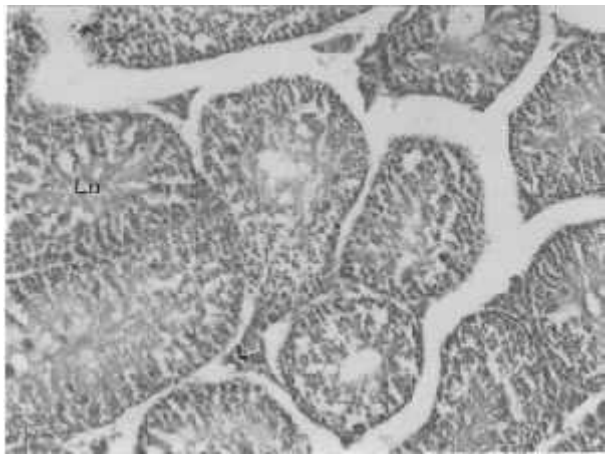

Fig. 2: H & E stain. Magnification: 200 x (experimental).

All the animals of group C were active and healthy looking, at the time of sacrifice. Some animals of this group showed eczematous changes. Size of the seminiferous tubules was slightly smaller than control size. Mean tubular diameter in control and treated group was 201.7 micrometer and 199.4 μm, respectively (Table l).

All the animals of group D at the time of sacrifice were active and healthy looking. Animals of this group showed eczematous change. Size of the seminiferous tubules was smaller than the control size (Fig. 2). The mean tubular diameter in control and treated group was 201.5 μm and 197.1 μm, respectively (table 1).

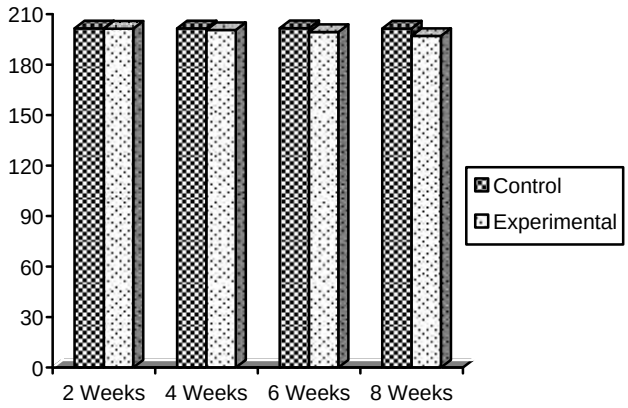

Fig. 3: Comparison of diameter of seminiferous tubules of control and experimental (treatment with potassium dichromate at a dose of 2 mg / kg body wt.) albino mice.

Discussion

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. These wastes gain entry into the food chain directly or indirectly, thus causing serious health problems.[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.[23,24]

The process of intracellular chromium reduction occur either in cytoplasm or in the nucleus and yields several reactive intermediates, which interact with

DNA. DNA lesions are capable of obstructing DNA replication.[25,21] Hexavalent chromium has been reported to cause severe side effects, besides impotence. Lindbohm ML *et al*[26] discovered that semen analysis of more than 50% of welders had a sperm count of less than 4 million sperm / ml. Mortensen JT et al[27] investigated a greater risk of sperm quality among welders. Al-Hammod *et al*[28] and Elbetieha *et al*[29] also reported results resembling those of Mortensen. Seminiferous tubules diameter was reduced after chromium exposure.[30,31] These results suggest that occupational exposure to Cr (VI) leads to alteration of semen status and may affect the reproductive success of exposed workers.

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 (VI) administration showed picture very near to the control level. After two months of chromium administration histological examination revealed significant decrease in the diameter of seminiferou tubules ($P < 0.05$ to 0.001).

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 to DNA damage.

Several other metals, such as lead and cadmium have also been reported to effect the testes by interfering with hormones synthesies.[32,33] 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 spermatogenisis.[35] The effects observed are perhaps due to direct toxic affect of chromium on the seminiferous tubules.

The present studies indicate that Cr (VI) has toxic effects even at low concentration, when they were given for a long time. These findings are quiet in conformity with some previous studies of Hong Li *et al*[30] and Chandra *et al*[31,36] done to evaluate intoxication of hexavalent chromium in biological systems. Inspite of chromium in traces being essential for living system exposure to high concentration of Cr (VI) is hazardous.[37]

Conclusion

The present study indicates that Cr (VI) has 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 tubular diameter of the seminiferous tubules was reduced. 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.

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