Chromium Agrian Contaminated Water may Cause Testicular Anomalies and Infertility

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Abstract

Objective: Agricultural quantitative products always depend upon soil and water quality. We aim to probe the toxicity of metal-contaminated water used for irrigation in the big cities vicinity of Pakistan. Testicular histopathology of hexavalent chromium (Cr+6) was investigated in adult male albino laboratory mice (Mus musculus). Design: Histopathological experimental study.Place and Duration: The experiment was designed at Sargodha University from 2012 to 2014.Materials and Methods: Six groups (n=15) were given 0.0, 12.5, 25, 50, 100 and 200ppm Cr+6 in drinking water. Five animals from each group were sacrificed at 1, 2, and 3-month stages to recover testes for histopathological studies. Results: A significant ($p<0.01$) increase in mean cross-sectional area (CSA) of the seminiferous tubules (ST) was noted in 12.5ppm in all durations. CSA of ST elevated in 25 and 50ppm in 1-2nd month but declined in the 3rd month, however, secondarily deteriorated in the 1-3rd month in 100 and 200ppm as compared to control. Daily average water intake and body weight were inverse while chromium intake was directly correlated to Cr+6 concentrations. The mean testicular-somatic index increased in 12.5, 25, and 50ppm groups but decreased in 100 and 200ppm groups at all stages (1-3 months) compared to control. The presence of multi-karyocytes, short-tail spermatozoa, and necrosis of interstitial cells in 50-200ppm groups indicate the signs of infertility. Conclusion: Results indicate that exposure of above 50ppm Cr in water causes various toxicological changes in the testis and such contaminated water should be banned to irrigate the crops and vegetables.

Keywords: Chromium; Testicular histopathology; multi-karyocytes and Seminiferous tubules

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Introduction

National Research Council recommended the essential dietary intake of chromium (Cr), 50-200µg/day (Bogden, Klevay, Book,2000) and its deficiency causes diabetes and cardiovascular diseases (Kobla, Volpe,2000). According to DiBona (Dibona, Love, Rhodes,2011), 1mg/kg Cr dietary supplement in rats for six months produces no effect on food intake and body weights of the animals, while Cr is considered to be an essential trace element is being redressed; rather it is gaining popularity as a common environmental toxicant (Yoshida, 2012).

Endocrine disruption by environmental pollutants has raised serious concern and the peak activities were observed in locations adjacent to industrial and shipping activities(Bayen, Gong, Chin, Lee, Leong,2004). Such factors lead to impairment of the antioxidant defense systems and increase the toxic effects, causing alterations in the cytoplasmic signaling process and producing damages at macromolecular levels (Al-Gubory, Fowler, Garrel, 2010), like alteration in body weight (Lukacinova, Racz, Lovasova, Nistiar, 2011), variation in blood profiles, and denaturation of liver enzymes (Serezli, Akhan, Fatma, 2011). It is becoming a serious environmental issue, particularly in leather industrial areas in developing countries like Pakistan (Shakir, Ejaz, Ashraf, 2012; Khan, Mushtaq, Khan, 2013).The toxicity was experimentally studied in monkeys by providing Cr (100-400ppm) in drinking water Aruldhas, Subramanian, Sekar, 2005) and in rats 10 mg/L potassium chromate for 44 weeks caused accumulation and elevation of Cr levels in the liver and testis (Sutherland, Zhitkovich, Kluz, Costa, 2000).

It has been found to be associated with various health hazards mainly mediated through oxidative stress, lipid peroxidation, and DNA damage leading to hematological, hepatic, and renal function impairment on exposure (Yousef, El-Demerdash, Kamil, Elaswad, 2006; Fatima, Mahmood, 2007). In the context of reproductive health, Cr^{+6} exposure has been proclaimed to cause the FSH levels to increase with a simultaneous decrease in LH and testosterone levels. (Marouani, Tebourbi, Mahjoub, 2012) Moreover, spermatocytes and spermatids are highly vulnerable to Cr^{+6} exposures in a dose-dependent manner. Geoffroy-Siraudin, Perrard, Chaspoul, 2010) Furthermore, it has been shown that Cr^{+6} accumulates in the testis leading to cytoplasmic vacuolations, mitochondrial degenerations, and leakage of tight junctions in Sertoli-cells thus causing impairments in spermatogenesis leading to abnormal sperm morphology and decreased sperm count. (Acharya, Mishra, Tripathy, Mishra,2006; Zhang, Liu, Wang,. et al. 2013). It has been further claimed that Cr^{+6} suppresses the antioxidant enzymes and enhances lipid peroxidation adversely affecting the testicular functions and causing infertility (Fnu, Cocuzza, Agarwal, 2008). The findings of this study and those described in the literature indicate that Cr alters the testicular function affecting the DNA induced in the testis of albino mice and ultimately such anomalies cause infertility (Vijaya, Sasikala, Karthik, Shivakumar, 2013).

Studies on male reproductive health hazards associated with hexavalent chromium exposure indicate degeneration

of spermatids, decline in epididymal sperm count, and decrease in weight of accessory sex organs. (Chandra, Chatterjee,Ghosh, Sarkar, 2010; Akunna,Ogunmodede, Saalu, 2012)

In present research work changes in water consumption and body weight fluctuations along with the histopathological and micrometric changes in testis ST and alterations in sperm structure are being reported under a wide range of sub-chronic chromium exposure through drinking water in mice.

Materials and Methods

Animals: Male albino laboratory mice (*Mus musculus*) of 2-3 months of age, weighing 30-35g were used in this study. These were divided into six groups of 15 animals each. Potassium dichromate solutions containing 0, 12.5, 25, 50, 100, and 200 Cr^{6+} were produced in drinking water and provided to the animals of the respective six groups (*ad-libitum*). Animals were given free access to food throughout the study period. The mice in each group were maintained separately in steel gauzed iron framed (15×12×12 inches) cages. The ambient temperature, humidity, and dark-light cycles were maintained at 23±2 ^{0}C, 30-35% and 12-12hrs respectively.

Experimental design: Each animal was weighed daily to record the fluctuations in mean body weight/ group. Average water consumption for the first 30 days of the study in terms of ml/g/day for each group was obtained to find out the effect of Cr concentration on daily water consumption. Finally mean Cr^{6+} uptake for the said duration was calculated. Five animals from each group were sacrificed after 1, 2, and 3-month durations to recover testes. Separately for each animal, the testicular weight index was calculated employing the following equation.

Index = (weight of testes/weight of animal) × 100

The mean testicular indices±SEM for the above-said durations are shown in Table 1. One testis from each animal was used for smear preparation while the other was processed for histopathological studies.

Testicular smears and histology: One randomly selected testis from each animal was cut into two halves with the help of a sharp scalpel along the long axis. The two halves were placed side by side on a new clean glass slide and gently crushed with a blunt glass rod. The curdy material obtained was diluted by mixing it with 3-5 drops of normal saline. Thin smears were prepared from this material. Each smear was air-dried for a while and stained with hematoxylin and eosin. These smear slides were studied, at 100× and 400× for various structural derangements in spermatogonia, spermatocytes, spermatids, and spermatozoa. The other testis from each animal were processed for wax-embedded microtomy to obtain 6µ thick sections. The sections were also stained with hematoxylin and eosin for histopathological and micrometry studies.

For computerized micrometry of the cross-sectional area of seminiferous tubules, digital photographs of selected sections (one from each animal) were obtained at 100× by using a "Labomid" stereoscopic research trinocular microscope affixed with a Sony 7.1MP digital camera. All photographs were obtained on the same digital and optical specifications. Cross-sectional area of 10 randomly selected seminiferous tubules from each animal was obtained through

their projected images in Corel-DRAW11 under a digital grid application. A photograph of the stage micrometer obtained at 100× was used for size calibration of the grid chambers.

Statistical analysis: The testicular weight index and micrometric data obtained were analyzed statistically for a level of significance of variations among the data points on ANOVA (one way) and Duncan's Multiple Range Test (using MS Excel).

Results

Water/ Chromium daily intake: Water intake was found to be inversely proportional to the concentration of chromium present (Fig: 1). The means values of chromium intake remained 0, 1.333, 2.315, 4.433, 7.626, and 14.545 mg/kg body weight; for 0, 12.5, 25, 50, 100 and 200ppm groups respectively.

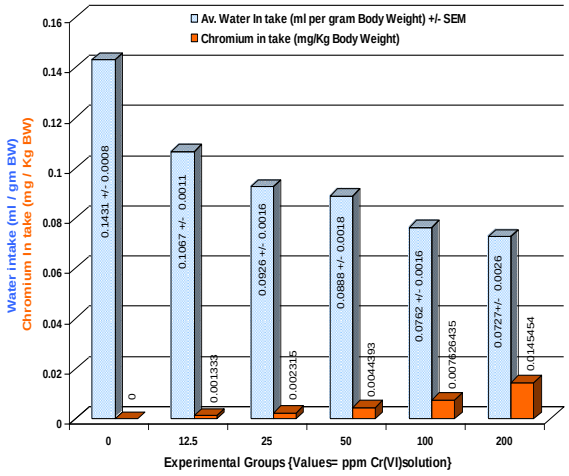

Fig 1: Average water and chromium intake in control and experimental groups.

Daily body weight fluctuations: Fluctuations in mean body weight for each group are shown in Fig.2. Mean initial and final weights for the control group animals remained between 34.4- 39g; whereas values for the same remained between 33.52- 30.5, 33.125-26, 33- 24.5, 32.86-22 and 32.64-20.5g respectively for 12.5, 25, 50, 100 and

200ppm groups; clearly indicating a dose-dependent inverse effect of Cr^{+6} intake on the body weight.

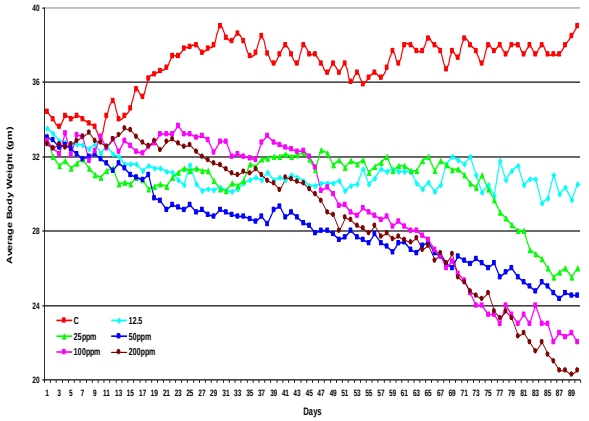

Fig 2: Daily average body weight fluctuation for three months.

Testicular/Body Mass Index: Testicular weight expressed in terms of mg /g body weight in Control and Cr^{+6} treated groups are given in Table 1. Statistical analysis (ANOVA-one way) has shown a significant ($p < 0.01$) difference in the testicular weight among the groups. However, the post hoc analysis (Duncan's multiple range tests) indicated no significant variation between any two of the experimental groups.

Table 1: Average testicular weight (expressed in mg/g body weight ± Standard Deviation) in control and experimental groups

Organ	Duration	Experimental Groups					
		Control	Chromium Treated Groups				
		0ppm	12.5ppm	25ppm	50ppm	100ppm	200ppm
Testes weight	One month*	0.6601± 0.02257	0.6669± 0.04424	0.6668 ± 0.04983	0.6798 ± 0.0311	0.6549 ± 0.0414	0.6432 ± 0.0985
	Two months*	0.6528 ± 0.0401	0.70465 ± 0.0409	0.6838 ± 0.0317	0.6879 ± 0.0570	0.6485 ± 0.0995	0.6394 ± 0.1138
	Three months*	0.6646 ± 0.0403	0.7101± 0.5119	0.7025 ± 0.0490	0.6905 ± 0.0516	0.6119 ± 0.0555	0.6045 ± 0.0593

*: ANOVA Single factor ($p < 0.01$)

Cross-Sectional Area of the Seminiferous Tubules: The mean cross-sectional area (CSA) of the seminiferous tubules in the control group remained 9905.8, 10067.4, and 9997.8μm² for 1, 2 and 3 months of the study respectively. A significant increase in the mean CSA was noted in Cr^{+6} treated groups to that of the control at the month stage. This trend continued for 12.5, 25, and 50ppm groups at 2-month stage however in 100 and 200ppm groups a secondary decline (indicative of secondary atrophy) was observed. At the 3-month stage all Cr^{+6} treated groups except 12.5ppm showed a decline in mean CSA values to their respective mean values at the 2-month stage (Fig: 3).

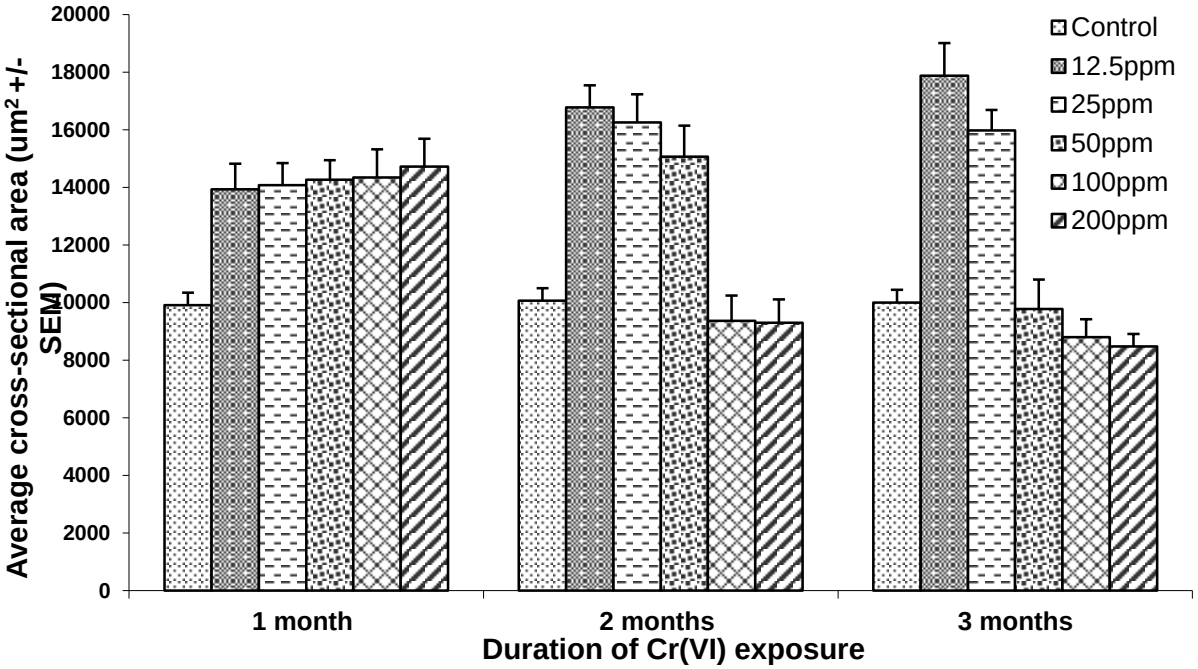

Fig 3: Variations in mean cross-sectional area of the seminiferous tubules among the experimental groups after 1, 2, and 3 months of Cr^{+6} exposures (+bars indicate SEM; * indicate significant variation)

Histology and Histopathology of Testis
Control Group:
Seminiferous tubules appeared almost rounded and filled with spermatogenic cells. Interstitial tissue seemed to be quite well placed (Fig: 4A).

Chromium Treated Groups:

Depending upon concentration and duration of exposure shrunken and centrally hollowed seminiferous tubules with concurrent necrosis of the interstitial tissue were seen in all Cr^{+6} exposure groups (Fig: 4B and C). Necrosis of Sertoli cells with a resultant dislodged spermatogonia and spermatocytes were seen in 100 and 200ppm groups at 2 and 3 month's exposure (Fig: 4D). Certain megakaryocytic spermatogonia /spermatocytes were visible at 2 and 3 months of exposure in 100 and 200ppm groups indicating a probable endoploidy.

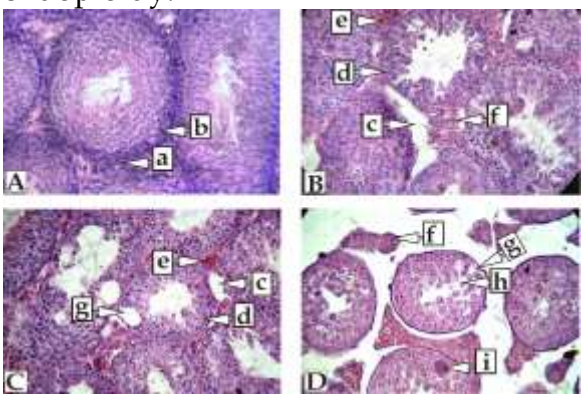

Fig 4: TS Testis (400×) (3months stage) Cr^{6+} exposure groups;

A: 0.0 ppm; B: 50ppm; C: 100ppm; D: 200ppm.

a:interstitial tissue; **b:**seminiferous tubule with whorls of spermatogonia, spermatocytes, and centrally placed spermatozoa; **c:**adjacent seminiferous tubules gap (secondary shrinkage); **d:**centrally hollowed seminiferous tubule with no spermatozoa; **e:** interstitial vein; **f:**necrosis of the interstitial tissue; **g:**basement membrane with no spermatogonia and spermatocytes (necrosis of Sertoli); **h:** dislodged spermatocyte; **i:** megakaryocytic (endoploid) spermatocyte.

Study of Testicular Smears:

Normal spermatozoa (with well-developed heads and long tails) were seen in the control group. All normal stages of meiosis (primary spermatocytes with enlarged nuclei, secondary spermatocytes with 2 haploid nuclei, and pro-spermatid cells with 4 haploid nuclei were clearly visible) (Fig: 5A).

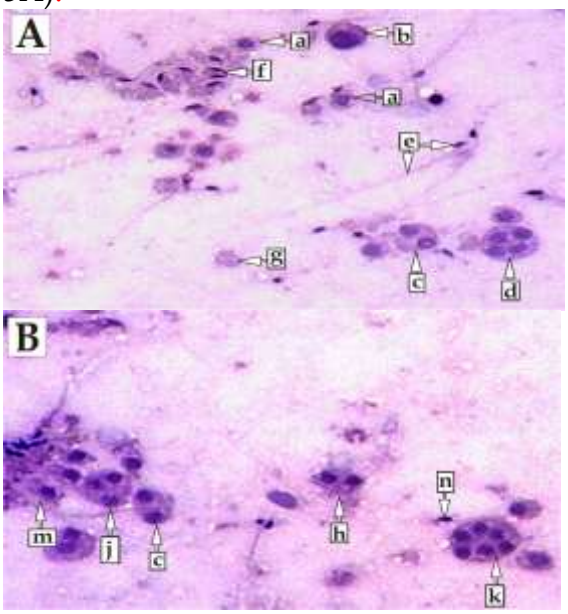

Fig 5: Testicular smears (400×) (3month stage) Cr^{+6} exposures; A: 0.0 ppm; B: 200ppm

a: spermatocyte, b: spermatogonium, c: binucleated cell, d: tetranucleated cell, e: sperm with tail, f: spermeogenesis, g: cell after producing sperms, h: necrotic cell, j: trinucleated cell; k: multinucleated cell, m: halted spermeogenesis, n: tail-less sperm

The density of mature spermatozoa decreased gradually with increased duration (1, 2, and 3 months) of exposure in all chromium-treated groups. Tail development in spermatozoa was badly affected by Cr^{+6} exposures. Spermatozoa with very short or no tail were seen at 2 and 3 months exposure stages in 50, 100, and 200ppm groups (Fig: 5B). Meiotic/Mitotic errors in terms of 3, 5, 6, 7, and multinucleated syncytial cells were visible most frequently in 50ppm group never the less these cells were also present in testicular smears of 100 and 200ppm groups. On the other hand spermatocyte necrosis was seen increasing dose-dependently (Fig 5B and Fig 6).

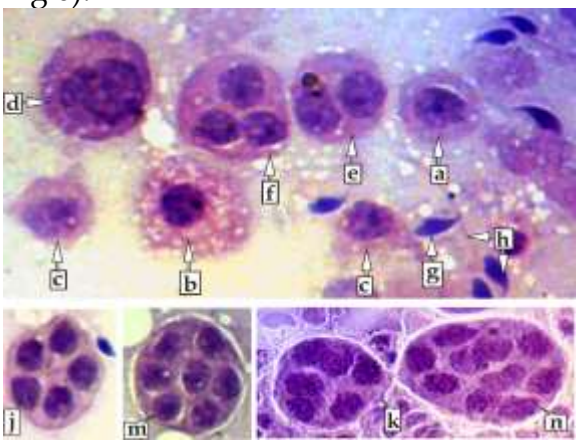

Fig 6: Meiotic/Mitotic Error in Testicular smears (400×) by Cr^{+6} exposures

a: spermatocyte; b,c: necrotic spermatocytes; d: megakaryocyte (endoploid spermatocyte/spermatogonium); e: binucleated cell; f: trinuceated cell; g: tail-less sperm; h: sperm with tail; j: pentanucleated, m: heptanucleated, k and n: multinucleated syncytial cells.

Discussion

The present study was planned to investigate the effects of sub-chronic exposure to hexavalent chromium for 1-3 months durations under a wide range of chromium concentrations (0, 12.5, 50, 100, and 200ppm) in drinking water instead of force-feeding or IP injections (Chandra, Chatterjee, Ghosh, Sarkar, 2010). Interestingly it was seen that the water intake decreased depending on the concentration of chromium in drinking water. The most likely reason for the concentration-dependent decline in body weight may lie in the chromium-related persistent oxidative stress and lipids per-oxidation (Acharya, Mishra, Tripathy, & Mishra, 2006) and concomitant hyperactivity and hostility of the animals in Cr groups than that of the control group. Likewise, the concentration and duration-dependent gross histologic and micrometric changes in the testis are most logically attributable to the oxidative stress and lipid peroxidation inflicted on the animals due to Cr^{+6} exposures. The accumulation of Cr^{+6} in the liver, kidney, spleen, and bone causes the enhancement of lipids, triglycerides, and phospholipids and inhibits membrane enzymes (Burczynski, Southard, et al,2001).

Hexavalent chromium has recently been reported to cause various genetic and cytological abnormalities in germ cells (Carette, Perrard,& Prisant, 2013) Available literature gives a clear preview that Cr^{+6} is highly important in terms of its reproductive toxicological implications; it has damaged testicular tissues and distorted the process of spermatogenesis[11,17,21]. Murthy has pointed out that late-stage spermatids are the most affected cells following Cr treatment(Murthy, Saxena,1991)Cr^{+6}

treated group with abnormal sperm heads and tail-less spermatids, and dislodged appearance indicates the possible deformities at the androgen receptor in cells during terminal differentiation of spermatids. The appearance of considerably short-tailed and tailless spermatozoa in the present study indicates some sort of inhibition of the biosynthesis of tubulin and/or polymerization of the tubulins into a microtubular array of the sperm tail on Cr^{+6} exposures. The presence of poly-nucleated (usually in odd number) spermatocytes in 50ppm or more indicates a synchronous DNA replication in daughter nuclei and/ or inability of the cytoplasm to divide which again may be attributable to the cytoskeletal (mainly microfilaments in this case) production and/ or function. Sertoli cell damage caused by Cr^{+6} exposures can be a possible cause of this arrested spermiogenesis (Pereira, Das, Oliveira, Santos, & De-Jesus,2005) like histo-pathological effects of cadmium chloride which induce seminiferous atrophy, testicular architecture disorganization, and germinal epithelium disruption (Sharma, Vyas, Tamot, & Manhor, 2013). Interstitial cell necrosis has been seen as the most persistent toxicological manifestation of chromium (Fig: 4) and is directly involved in the biosynthesis and release of male sex hormones (steroidogenesis). As androgens are directly involved in spermatogenesis, the loss of interstitial cells thus brings about the permanent loss of the natural source of androgenic steroids; hence, it may affect the process of spermatogenesis in general and spermiogenesis in particular to create the possible probabilities of infertility.

Conclusion

The findings indicate that chronic exposure to Cr^{+6} leads to several critical histopathological changes in the testis including a gradual size reduction (CSA) in the seminiferous tubules and impairments in spermatogenesis along with a concurrent necrosis in the interstitial tissue and the Sertoli cells. Thus the continued practice of the release of untreated industrial effluents particularly containing Cr^{+6} is a source of groundwater pollution that may particularly be injurious to male reproductive health.

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Compliance With Ethics Requirements

All institutional and national guidelines for the care and use of laboratory animals were followed.

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