REPRODUCTIVE BIOLOGY

ORIGINAL RESEARCH

Effects of hexavalent chromium on reproductive functions of male adult rats

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SUMMARY

Hexavalent chromium is an environmental contaminant which may be associated with reproductive abnormalities in male rats. In the present study, we examined the effect of hexavalent chromium on male reproductive function of rats. Male Wistar rats received a daily intraperitoneal injection of potassium dichromate (1 or 2 mg/kg body weight) for fifteen consecutive days. A decrease in testis weight and an increase in seminal vesicles and prostate weights were demonstrated after chromium treatment. Moreover, a dose-dependent increase in blood and testis chromium levels as well as an increase in FSH and a decrease in LH and testosterone serum levels were detected in treated rats. Histological analysis revealed pronounced

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morphological alterations with enlarged intracellular spaces, tissue loosening and dramatic loss of gametes in the lumen of the seminiferous tubules of treated rats. In addition, a decreased sperm motility and number of epididymal spermatozoa together with an increased sperm abnormality rate was found in chromium-treated rats in comparison to controls. In rats receiving the higher chromium dose, histological images presented considerably increased areas filled with seminal vesicle and prostate secretions. The mucosal crypts of seminal vesicles and the typical invaginations of prostate were altered. The results suggest that subacute treatment of potassium dichromate promotes reproductive system toxicity and affects testicular function of adult male rats. *Reproductive Biology 2012* ***12*** *2: 119–133.*
Key words: testis, sperm quality, seminal vesicle, prostate, hexavalent chromium, rats

INTRODUCTION

Chromium is a naturally occurring element found in rocks, volcanic dust and gases, soils as well as plants and animals. It is commonly used in three basic industries: metallurgical, chemical and refractory which leads to environmental pollution [14]. Concentrations of chromium in surface water have been increasing since 1999, with 275 μg/L being recorded near salmon spawning areas in the USA [9]. Although residual chromium in the aquatic environment has been an increasingly important issue and extensive preventive measures have been taken recently, some regional concentrations of chromium have exceeded the provisional guideline value (50 μg/L) proposed by WHO [35]. In addition, electronic devices with flat panel displays and cathode-ray TV tubes contain significant amounts of chromium, as well as many other heavy metals, which, when disposed of by land-fill or incineration, can lead to potential human health toxicity and ecotoxicity [18].

Chromium can exist in several oxidation states ranging from −2 to +6, of which the trivalent (III) and hexavalent (VI) forms are of biological

importance [31]. The two main hexavalent chromium forms dominant in the environment (CrO_7^- and $Cr_2O_4^-$) can readily cross cellular membranes with the help of nonspecific anion carriers, whereas trivalent form is poorly transported across membranes. Once inside the cell, the hexavalent form is ultimately reduced to the trivalent form through the formation of reactive intermediates like pentavalent and tetravalent forms [6]. The trivalent form complexes with intracellular macromolecules including genetic material and is ultimately responsible for the toxic and mutagenic capacities of chromium [9]. These compounds are not biodegradable and accumulate in the environment resulting in indirect and direct toxic effects to living beings [17].

Hexavalent chromium is 100–1000 times more toxic than the most common trivalent compounds [31]. It is usually linked with oxygen and is a strong oxidizing agent. It is widely known to cause allergic dermatitis as well as toxic and carcinogenic effects in humans and animals [33]. Chromium VI induced acute and chronic toxicity, neurotoxicity, dermatotoxicity, genotoxicity, carcinogenicity, immunotoxicity and general environmental toxicity [17, 33]. Soluble and insoluble chromium VI salts have been demonstrated to induce morphological and neoplastic transformation and mutagenicity in human and murine cells [23]. Exposure to chromium VI has been reported to cause reproductive toxicity in human and laboratory animals [4, 15, 32]. A decreased sperm cell count and motility as well as increased follicle stimulating hormone (FSH) serum concentration were found in men employed in electroplating [20]. Moreover, a decreased concentration of sperm cells and increase in abnormal spermatozoa were observed in mice [1], rats [15], rabbits [37] and bonnet monkeys [32] treated/exposed to chromium.

In the current study, we have examined the effects of subacute treatment of chromium VI on different reproductive parameters of adult male rats. The examined parameters included weight and histological analysis of testis, seminal vesicles and prostate, sperm motility and morphology as well as determination of serum concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone (T). In addition, chromium content in blood and testis was studied.

MATERIALS AND METHODS

Animals and reagents

Two-month-old Wistar male rats (150–200 g) purchased from Siphat-Tunis were used in the present study. Rats were housed under controlled conditions of temperature (22±1°C) with a day/night cycle (light from 8:00 to 20:00). Food and water were provided *ad libitum*. Chromium VI as potassium dichromate ($K_2Cr_2O_7$) was purchased from Merck (Darmstadt, Germany). Earles' medium was obtained from Gibco-BRL (Paisley, Scotland, UK). Rats were randomly divided into three groups: 1/ LD rats - receiving daily 1 mg of potassium dichromate/kg of body weight (b.wt; ip) dissolved in sterile distilled water (n=13); 2/ HD rats – receiving daily 2 mg of potassium dichromate/kg b.wt, ip (n=12) and 3/ control rats (C group) receiving distilled water (vehicle; n=14). The doses were selected from results of a preliminary experiment. Rats were weighed daily throughout the experiment. After fifteen days of treatment, animals were sacrificed by decapitation; the left testis, the seminal vesicles and the prostate were dissected and weighed. The fifteen-day treatment was selected due to the duration of one seminiferous cycle (13.2 days) in albino rats [31]. Animals were cared for in compliance with the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes. The experimental protocols were approved by the Faculty Ethics Committee (Faculty of Sciences, Bizerte, Tunisia).

Chromium estimation

The testis was grinded in a mixture of $HNO_3/HClO_4$ (1:1). The residue was dissolved with 25 ml of nitric acid solution (0.2 ml HNO_3 in 100 ml ultra-pure water). The solution aliquots were used to estimate chromium using an atomic absorption spectrometer (Perkin-Elmer 306) at 357.9 nm wavelength. The values are expressed as μg/g fresh weight. Blood chromium was directly quantified after dilution with distilled deionized water [5]. The values are expressed as μg/ml of blood. The laboratory glass wares used for testis

and blood collection and processing were soaked overnight in analytical grade nitric acid and washed three times with deionized water.

Sperm preparation

Cauda of epididymis and the initial part of the spermiduct were minced and homogenized in 1ml of NaCl 0.9% with 0.5% of Triton X-100 [8] to produce epididymal sperm suspensions. The sperm suspension was incubated for 10 min at 37°C to promote the release of sperm into the medium. Sperm motility was determined in haemocytometer (Buerker, Germany; [19]). The sperm motility was determined by counting progressive, non progressive and immotile spermatozoa in the same field. The count was repeated five times for each sample to minimize the error. To assess the number of abnormal sperm, sperm suspension was pipetted onto the glass plate, dried, fixed in methanol for 5 min and stained with 1% eosin. The morphology of spermatozoa was evaluated under a light microscope and spermatozoa were classified according to Wyrobek and Bruce [36]. To assess the incidence of abnormalities in head, neck/midpiece and tail, at least 500 spermatozoa were observed per rat under 400×magnification.

Enzyme- linked immunosorbent assay and radioimmunoassay

The serum FSH, LH and testosterone were determined in the same male rats used for the examination of relative weight of testes, seminal vesicles and prostate. After decapitation, trunk blood was collected, centrifuged (500×g, 10 min, 4°C) and serum stored at -80°C until analysis. FSH and LH concentrations were determined in duplicate using an enzyme immunoassay system (enzyme-linked immunosorbent assay, ELISA) with commercial kits (Biotrak™ from Amersham Biosciences, UK). Peptide hormone levels are expressed as ng/ml. The cross-reactivity of rLH to rFSH was <0.016%. The cross reactivity of rFSH to rLH was <0.17%. The sensitivity of rLH and rFSH assay was respectively 0.1 ng/ml and 8.66 ng/ml. Testosterone level, expressed as ng/ml, was determined in duplicate using a radioimmunoassay (RIA) kit (Immunotech, France). The sensitivity of the testosterone assay was 0.025 ng/ml.

Table 1. Effect of chromium treatment on the number, motility and morphology of epididymal spermatozoa (mean±SEM) in adult rats

Group	Sperm number $\times 10^6$/ml	Sperm motility (%)	Sperm abnormality (%)
Control group	19.3 ± 1.6^a	39.2 ± 4.0^a	4.5 ± 0.8^a
LD group	11.0 ± 1.3^b	28.2 ± 2.6^b	8.4 ± 1.2^b
HD group	6.7 ± 1.0^c	24.9 ± 2.8^b	13.9 ± 1.0^c

n=14; different superscripts depict significant differences ($p < 0.05$)

Histological analysis

The testis, seminal vesicles and prostate were fixed overnight at room temperature by direct immersion in 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4. The samples were dehydrated with ethanol and toluene series and embedded in paraffin. Serial sections (4 μm) were mounted on gelatin-coated glass slides cut and stained with haematoxylin and eosin.

Statistical analysis

Data were analyzed using Stat View 512^+ Software (Abacus Concept, Inc. Calabasas, CA, USA). Differences among groups were measured using one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls as post hoc test. The results were expressed as means±SEM and differences were considered statistically significant at $p<0.05$.

RESULTS

No cases of mortality and abnormal behavior as well as no macroscopic injuries were observed in the examined rats. The fifteen-day chromium treatment caused a significant decrease in relative weight of the testis in both LD (18%) and HD (24%) groups in comparison to the control group.

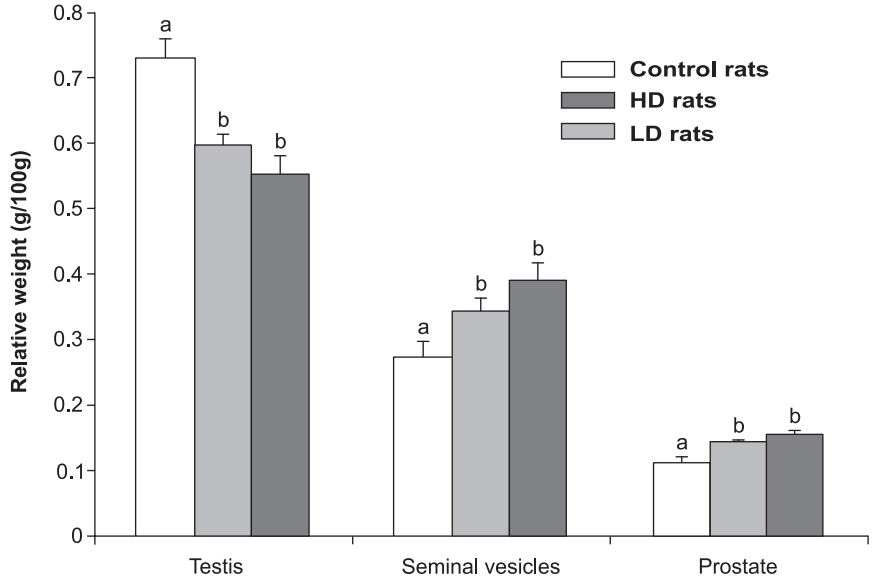

Figure 1. Effect of chromium treatment on the testis, seminal vesicles and prostate relative weight (mean±SEM) in male adult rats (n=12–14). Different superscripts depict significant differences among groups ($p< 0.05$)

In contrast, a significant increase in relative weight of seminal vesicles (LD: 26%; HD: 43%) and prostate (LD: 26%; HD: 35%) was observed in these animals (fig. 1). A higher concentration of chromium was found in blood (LD: 0.057±0.004 µg/ml; HD: 0.087±0.013 µg/ml) and testis (LD: 0.286±0.030 µg/g; HD: 0.423±0.017 µg/g) of treated than control rats (blood: 0.027±0.004 µg/ml; testis: 0.130±0.046 µg/g; fig. 2).

The quality of sperm from caudal epididymis was severely affected (tab. 1). The number of epididymal spermatozoa and epididymal sperm motility were significantly decreased in chromium-treated rats compared to those of the control group. In contrast, the incidence of abnormal spermatozoa was significantly higher in LD and HD rats compared to control rats. Light microscopy revealed the signs of active spermatogenesis in the seminiferous tubules of untreated rats (fig. 3A). In contrast, a dramatic reduction of spermatozoa number in the lumen of the seminiferous tubules was found in HD rats. This was accompanied by the disappearance

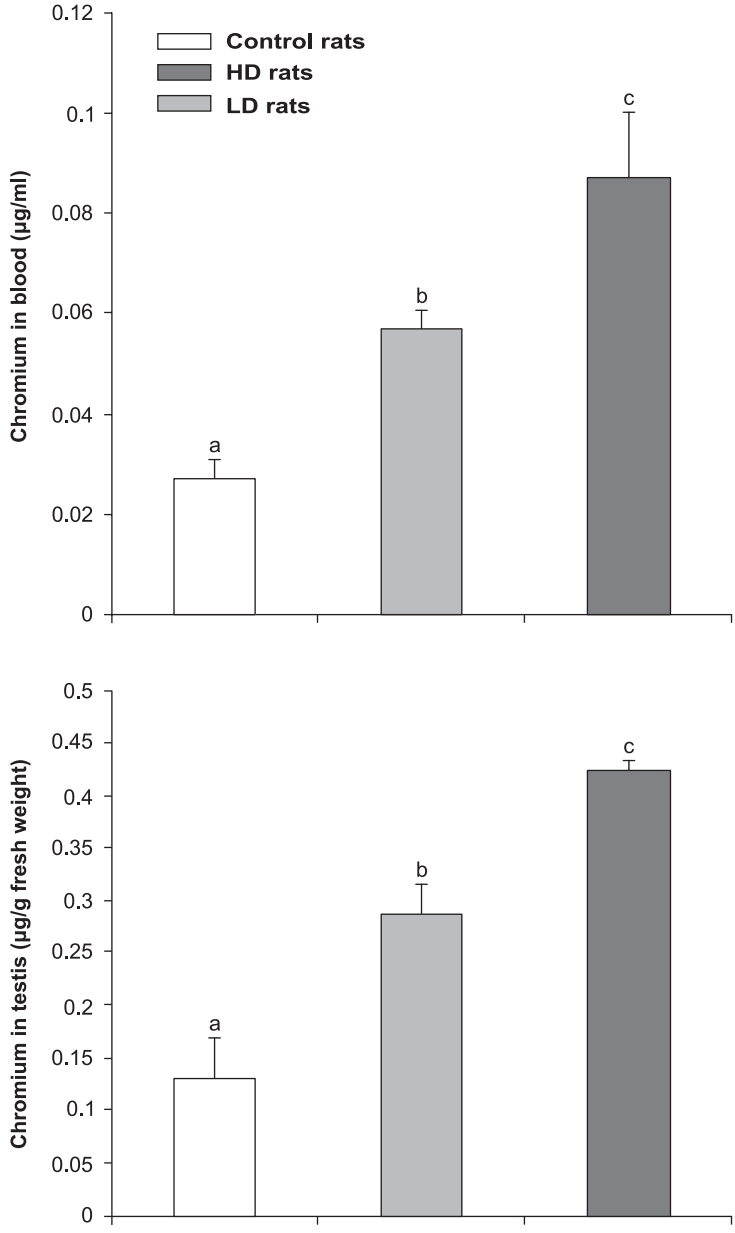

Figure 2. Chromium content (mean±SEM) in the blood and testis after intraperitoneal administration of chromium VI in male adult rats (n=10). Different superscripts depict significant differences among groups ($p< 0.05$)

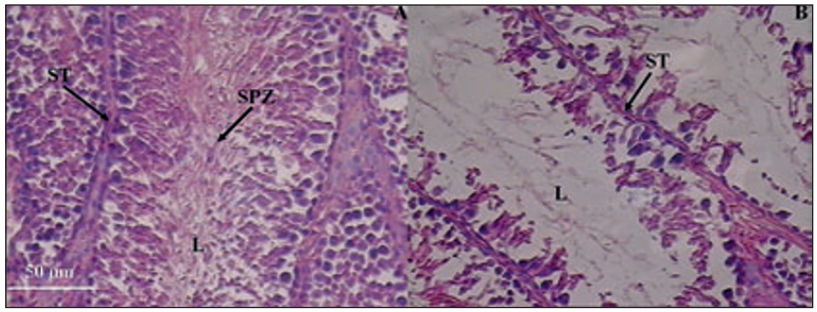

Figure 3. Photomicrographs of testicular sections of control (A) and chromium-treated (B) rats. The treated rats received 2 mg/kg body weight of potassium dichromate (ip). L: lumen; ST: seminiferous tubule; SPZ: spermatozoa.

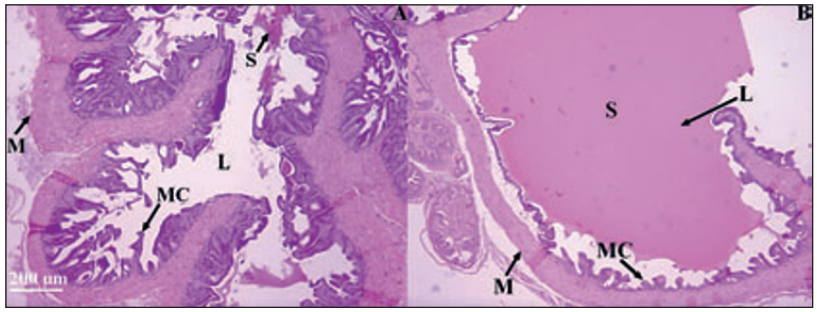

Figure 4. Photomicrographs of seminal vesicle sections of control (A) and chromium-treated rats (B). L: lumen; M: muscular layer; MC: mucosal crypt; S: secretion.

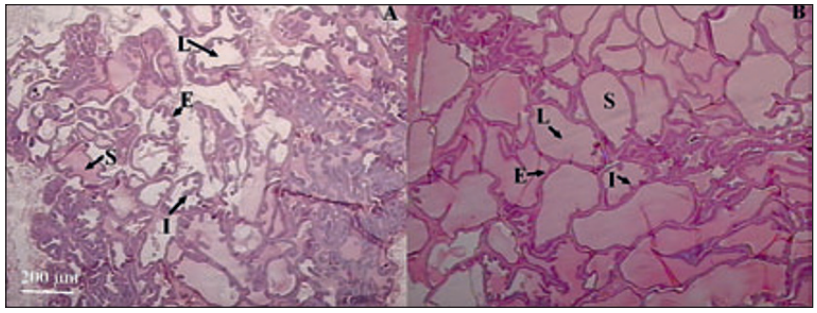

Figure 5. Photomicrographs of prostate sections of control (A) and chromium-treated rats (B). L: lumen; E: epithelium; I: invagination; S: secretion.

Table 2. Effect of chromium treatment on testosterone, FSH and LH serum level (mean±SEM) in male adult rats

Group	Testosterone (ng/ml)	FSH (ng/ml)	LH (ng/ml)
Control group	1.6±0.1[a]	25.6±2.8 [a]	3.3±0.2[a]
LD group	0.8±0.1[b]	35.6±1.8[b]	2.5±0.1[b]
HD group	0.5±0.1[c]	71.9±17.5[c]	1.6±0.2[c]

n=6 rats per group; different superscripts depict significant differences ($p< 0.05$)

of the Sertoli cells (fig. 3B). The effect of chromium in the LD group was less pronounced than in the HD group (data not shown).

A remarkable increase in seminal vesicle secretion was observed in HD rats. In addition, epithelial cells became short and the muscle layer surrounding the epithelium became thinner (fig. 4B). Some morphological alterations enhancing the luminal space were observed in the mucosal crypts of HD rats (fig. 4B) when compared to those of control rats (fig. 4A). In LD rats, the seminal vesicle modifications were less pronounced than in HD rats (data not shown). In prostate parenchyma of HD rats the cell height of epithelium was smaller than in control rats and lacked the typical invaginations (fig. 5A). Moreover, the alveolar lumen of HD prostate was filled with secretion (fig. 5B). Chromium treatment significantly ($p<0.05$) diminished serum testosterone and LH levels in a dose-dependent manner. In contrast, serum FSH was significantly higher in the LD group compared to the control group and in the HD group compared to the LD group (tab. 2).

DISCUSSION

Our results showed that fifteen-day exposure of adult male rats to chromium induced reproductive toxicity. The relative weight of testis, the number of epididymal spermatozoa and sperm motility were reduced in chromium-treated rats. Similar to previously published data [21, 30], serum and testis

chromium levels increased in a dose-dependent manner in treated rats, confirming the absorption of the metal after ip administration.

Moreover, histological changes were observed in the gonads of treated animals, including enlarged intracellular spaces resulting from the disappearance of Sertoli cells as well as a dramatic loss of gametes in the lumen of the seminiferous tubule. This might be caused by the disruption of the blood–testis barrier with a consequent metal accumulation in tissue [2]. Changes in the permeability of the blood-testis barrier [24] and alterations in testicular and epididymal histoarchitecture [25] were also demonstrated in mice exposed to chromium. In rats [22] and humans [12], the number of Sertoli cells is correlated with spermatogenesis level and testicular weight. Previous studies have demonstrated that in rats chromium had caused testicular atrophy, reduced sperm count and motility [2, 25] and induced degenerative changes in seminiferous epithelium and Leydig cells [2, 15].

The present study showed that the sperm abnormality level was significantly higher in chromium-exposed rats than in control animals. Our animal model corroborated epidemiological findings on the increased prevalence of sperm abnormalities among workers occupationally exposed to chromium compounds [4]. Although it has been demonstrated that sperm morphology is genetically controlled by complex autosomal and Y specific genes [13], oxygen-dependent mechanisms are clearly important factors [29]. Spermatogonial cells with gross DNA damage may be naturally eliminated, while cells with more subtle defects may survive and are finally reflected as abnormal sperm [36]. Excessive generation of reactive oxygen species (ROS) in semen may be associated with reduced sperm fertilizing potential and impaired sperm metabolism, morphology, and motility [28]. Therefore, ROS generated during chromium VI reduction may induce lipid oxidative damage and may reduce spermatozoa motility by affecting phosphorylation of axonemal proteins essential for spermatozoa movement [7, 16].

Testicular activity is governed by gonadotrophic hormones, FSH and LH [20]. In our study, serum LH and testosterone levels were significantly decreased and FSH level was increased in rats treated with chromium for fifteen days. It is possible that Sertoli cells of treated animals failed to produce inhibin which provided a negative feedback on FSH secretion

[34]. During intracellular reduction of chromium VI, the pentavalent form of chromium is produced; chromium V is known to disrupt the blood-testis barrier and affect the functional properties of Sertoli cells [24], which leads to decreased inhibin production and reduced negative feedback on the hypothalamic-pituitary axis [34].

Semen data in humans and animals exposed to chromium clearly indicate a drastic depletion in sperm counts, which may lead to male infertility [3, 27]. The decrease in sperm count by chromium may be related to disruption in steroidogenesis and changes in serum gonadotropin and testosterone levels [15, 27]. Previous studies on chromium-exposed rats [2, 3] demonstrated that low sperm count and low serum LH level were associated with decreased activities of testicular Δ^5 3β-hydroxy steroid dehydrogenase (3β-HSD) and 17β-hydroxy steroid dehydrogenase (17β-HSD), enzymes responsible for testosterone synthesis. Moreover, studies on virile oligospermics have shown a correlation between a high serum FSH level and a low sperm count [26].

It is of interest that the relative weights of seminal vesicles and prostate were higher in rats exposed to chromium compared to untreated animals. Moreover, the histological images of seminal vesicle and prostate of chromium-treated animals displayed enlarged areas filled with secretions. This could be caused by the generation of reactive intermediates and inflammation which was related to chromium VI toxicity [11]. In addition, testosterone was reported to be essential for the maintenance of height of the mucosal epithelium cells important for seminal vesicle and prostate functions. This steroid was also found to affect the function of smooth muscle in the ductus deferens and seminal vesicles [10]. In conclusion, our results demonstrated that subacute treatment of male rats with chromium VI caused reproductive system toxicity. More studies are required to understand the mechanism underlying this toxicity.

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