Chromium induced testicular impairment in relation to adrenocortical activities in adult albino rats

Amar K. Chandra a,*, Aparajita Chatterjee a, Rituparna Ghosh a, Mahitosh Sarkar b, Shail K. Chaube c

a Endocrinology & Reproductive Physiology Laboratory, Department of Physiology, University of Calcutta,
University College of Science & Technology, 92, Acharya Prafulla Chandra Road, Kolkata 700009, West Bengal, India
b Department of Physiology, Gurunanak Institute of Dental Science & Research, Kolkata, India
c Department of Reproductive Biomedicine, National Institute of Health and Family Welfare,
New Mehrauli Road, Munirka, New Delhi 110067, India

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Abstract

The present study investigates the testicular and adrenocortical activities under different doses and durations of chromium (Cr) exposure and their interactions. Mature male Sprague Dawley rats were injected daily with three different doses (0.2, 0.4, and 0.6 mg/kg bw) of Cr salt (K2Cr2O7) intraperitoneally for 13 and 26 days, respectively. The medium (0.4 mg/kg bw/day) and higher dose (0.6 mg/kg bw/day) of Cr significantly (p < 0.05) decrease accessory sex organs weight, testicular \(\Delta^5\)-3\(\beta\)-hydroxysteroid dehydrogenase (HSD) and 17\(\beta\)-HSD activities, epididymal sperm count, effective spermatid degeneration, serum testosterone, LH level, testicular catalase and superoxide dismutase (SOD) activities while testicular lipid peroxidation, serum FSH, corticosterone level, adrenal weight and adrenal \(\Delta^5\)-3\(\beta\)-HSD activity increased significantly than that of control and lower dose (0.2 mg/kg bw/day) Cr exposed animals. Testicular histoarchitecture shows deterioration after critical dose (0.4 mg/kg bw/day) and duration (26 days) of Cr exposure. Cr induced alterations on testicular and adrenocortical activities are dose and duration dependent. Adrecortical hyperactivity accompanied by testicular oxidative stress might have a crucial role for Cr induced male reproductive impairment.

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Keywords: Chromium; Testis; Adrenal cortex; Corticosterone; Testosterone; Oxidative stress; Steroidogenic enzymes

1. Introduction

Chromium is the sixth most abundant element in the earth’s crust, where it is combined with iron and oxygen in the form of chromites ore [1]. It occurs in the environment mainly in two states, tri (Cr(III)) and hexavalent (Cr(VI)). Cr(III) is recognized as an essential trace element whereas Cr(VI) is considered as highly toxic to living beings [2,3]. Cr is used in three basic industries: metallurgical, chemical and refractory. These industrial wastes produce the second largest source of ambient Cr [4]. Industrial workers are directly exposed to Cr. Residents near these industries might be exposed to higher than background levels of Cr. Slag from smelter or chromate-producing facilities is used as landfill. Residents on these landfills are exposed to Cr through inhalation, dermal contacts and through ground water [1]. Other environmental sources of Cr include road dust contaminated by emission of Cr based catalytic converters, cement dust, tobacco smoke and food stuff [1]. The US National Academy of Science has established safe and adequate daily intake of Cr in adults of 50–200 \(\mu\)g/day [5].

Cr(VI) can easily enter the cell through \(SO_4^{2-}\) and \(HPO_4^{2-}\) channels [5]. After entering the cell, it undergoes a chain reaction with the production of reactive Cr intermediates such as Cr(V) and Cr(IV) by cellular reductants (e.g. ascorbic acid, riboflavin, etc.). During this reduction process, Cr produces reactive oxygen species (ROS) [6]; generate oxidative stress that in turn is responsible for defective sperm function and male infertility [7]. It is well established that Cr(VI) is a strong oxidant, which causes metabolic and cellular dysfunction and cell death [8]. High dose
Cr(VI) is also responsible for spermatogenic and steroidogenic impairments in rats [9].

Common people are exposed to Cr at low levels. There are hardly any data available on prolonged exposure of Cr at a low dose on male reproductive system. Present investigation also focused on the mechanism of gonadal and adrenal interactions in response to Cr exposure. The hypothalamo-pituitary-gonadal axis (HPG) and hypothalamo-pituitary-adrenal axis (HPA) closely interact with each other [10]. Stress induced elevation of glucocorticoids help to mediate many critical processes during stressful condition [11]. These elevated glucocorticoids can potentially interfere with the release of reproductive hormones from HPG axis [12]. Therefore, the present study was conducted to evaluate the effect of Cr exposure at different doses and durations on testicular and adrenocortical activities and the possible mechanism.

2. Material and methods

Most of the chemicals used in the present study were purchased from Sigma Chemical Company, St. Louis, MO, USA. Potassium dichromate (K2Cr2O7) was purchased from E-Merck, Mumbai, India. Sixty-four adult (90 ± 10 days) male albino rats (Rattus norvegicus) of Sprague Dawley strain weighing 200 ± 10 g were used in the present study. The animals were maintained as per national guidelines and protocols, approved by the Institutional Animal Ethics Committee. The animals were housed in clean polypropylene cages and maintained in an air-conditioned animal house (temperature: 22 ± 2 °C; relative humidity: 40–60%) with constant 12-h light:12-h dark schedule. The animals were fed on standardized normal diet (20% protein) which consisted of 70% wheat, 20% Bengal gram, 5% fish meal powder, 4% dry yeast powder, 0.75% refined til oil, and 0.25% shark liver oil and water ad libitum [13]. For the selection of dose dependent effect of Cr, the experiment was carried out in the laboratory using different doses of hexavalent Cr salt (K2Cr2O7). The animals were divided into two groups for 13 and 26 days treatment schedule, respectively. Treatment schedule was selected according to the duration of one seminiferous cycle, which is 13.2 days in albino rats [14]. These two groups were again divided into four subgroups consisted of eight animals in each.

Absorption of chromium depends on its valance state. Hexavalent chromium can rapidly enter the cell than its trivalent form. Cr(VI) is less readily absorbed by oral route than by the other routes. Cr(VI) is reduced to Cr(III) by gastric juices inside the stomach which significantly decreases its absorption capacity by oral route. Dermal exposure to chromium has been demonstrated to produce irritant and allergic contact dermatitis [15,16]. Therefore, intraperitoneal (i.p.) route was selected for Cr administration in the present study to enhance the absorption capacity.

One group from first treatment schedule was kept as control and received i.p. injection of 0.1 ml distilled water/kg bw/day for 13 days. The other three groups received i.p. injection of Cr dissolved in sterile distilled water at a dose of 0.2 mg K2Cr2O7/kg bw/day (low dose group), 0.4 mg K2Cr2O7/kg bw/day (medium dose group), 0.6 mg K2Cr2O7/kg bw/day (high dose group), respectively, for 13 days.

The four subgroups of second treatment schedule were treated in the same way as mentioned above except that the duration of treatment was 26 days. Body weight of experimental animals was recorded on the day before first injection and on the day of sacrifice. All the animals were sacrificed 24 h after the last treatment following protocols and ethical procedures. Blood samples for hormone assay were collected from the hepatic portal vein under light ether anesthesia. Plasma samples were separated by centrifugation, frozen and stored at −20 °C for different assay. The testis and accessory sex organs were dissected out, trimmed off the attached tissues and weighed. The left testis of each rat was immediately fixed in Bouin’s fluid for histological study and the right kept for other biochemical estimations.

The tissue was embedded in paraffin for histological observation. Sections of 5 μm thicknesses were taken from the mid portion of each testis and stained with hematoxylin–eosin and examined under a light microscope. Quantitative analysis of spermatogenesis was carried out by counting the relative number of each variety of germ cells at stage VII of the seminiferous epithelium cycle, i.e. type-A spermatagonia (ASg), preleptotene spermatocytes (pLSc), mid-pachytene spermatocytes (mPSc) and step 7 spermatids (7Sd) [17]. Theoretically, the mPSc to 7Sd ratio should be 1:4 [18]. The percentage of effective 7Sd degeneration was calculated from this ratio. The percentage of 7Sd degeneration in Cr treated rats was deducted from vehicle-treated rats, which showed the effective percentage of spermatid degeneration after Cr treatment.

The sperm count was determined by counting in a haemocytometer [19]. Sperm samples were collected from the cauda epididymis. To minimize the error count was repeated at least five times for each rat.

The level of lipid peroxidation (LPO) in the testicular homogenate was measured based on the formation of thiobarbituric acid-reactive substances (TBARS) [20]. Malondialdehyde (MDA) forms adducts with thiobarbituric acid, which is measured spectrophotometrically (UV-1240 Shimadzu, Japan) at 532 nm. MDA, a product of LPO, was used as a standard. An extinction coefficient of 156,000 M−1 cm−1 was applied for calculation. The assay of testicular superoxide dismutase (SOD) activity consists of purely chemical reaction sequence, which generates superoxide from molecular oxygen in the presence of EDTA, manganese(II) chloride, and mercaptoethanol. NAD(P)H oxidation is linked to the availability of superoxide anions in the medium [21]. The decrease in absorbance at 340 nm was monitored for 20 min over a 5 min interval at 25 °C in a spectrophotometer (UV-1240 Shimadzu, Japan). In this assay system, 1 unit of SOD activity is defined as the amount of enzyme required to inhibit the rate of NADPH oxidation of the control by 50%. Testicular catalase activity was assayed biochemically [22]. In the ultraviolet range H2O2 shows a continual increase in absorption with decreasing wavelength. The decomposition of H2O2 can be correctly followed by monitoring the decrease in absorbance at 240 nm for 60 s in spectrophotometer. The change in absorbance is the measure of the catalase activity and was expressed as nmole/mg protein/s. The extinction coefficient of H2O2 at 240 nm is 40 M−1 cm−1.

Testicular and adrenal Aβ-3β-hydroxysteroid dehydrogenase (HSD) enzyme activity was estimated according to the method of Talalay [23]. Testicular 17β-hydroxysteroid dehydrogenase (HSD) enzyme activity was assayed by the method of Jarabak et al. [24]. Tissues were homogenized, maintaining chilling conditions (4 °C) in 20% spectroscopic-grade glycerol containing 5 mM of potassium phosphate and 1 mM of EDTA at a tissue concentration of 100 mg/ml homogenizing mixture in a Potter-Elvehjem glass homogenizer. The enzyme activity was measured at 340 nm against a blank without NAD. One unit of enzyme activity is equivalent to a change in absorbance of 0.001 min−1.

ELISA of serum testosterone was assayed using ELISA kit obtained from Equipar Diagnostici, SRL, Italy (code no. 74010) following the procedure as mentioned in the kit. The absorbance was read against blanking well at 450 nm within 30 min in ELISA reader (Merck). The sensitivity of the testosterone assay was 5 pg/ml and inter and intra-run precision had a coefficient of variation of 3.9 and 6.2%, respectively.

Serum levels of FSH and LH were assayed by RIA [25] using reagents supplied by Rat Pituitary Distribution and NIDDK (Bathesda, MD, USA). Carrier freees 125I for hormone iodination was obtained from Bhabha Atomic Research Center (BARC), Mumbai, India. Pure rat FSH (NIDDK-r FSH-I-11) and LH (NIDDK-r LH-I-11) were iodinated using chloramines-T (Sigma Chemical Company, St. Louis, MO, USA) as the oxidizing agent following standard procedure [26]. The second antibody was goat anti-rabbit γ-globulin purchased from Indo-Medicine (Friendswood, TX, USA). The intra-assay variation for FSH and LH was 5.0 and 4.5%, respectively. All samples were run in one assay to avoid inter-assay variation.

Serum corticosterone was determined by spectrophotometric method [27,28]. The fluorescence was measured at 463 nm (excitation), 518 nm (emission) by setting the instrument at a spectrofluorometric reading 80 with a standard corticosterone solution having 1.6 μg/ml concentrations. A minimum of 1.6 μg corticosterone/100 ml serum can be measured by this method.

Proteins were estimated biochemically using bovine serum albumin (BSA) as the standard protein [29].

Results were expressed as mean ± standard deviation (S.D.). One-way analysis of variance (ANOVA) test was first carried out to test for any differences between the mean values of all groups. If differences between groups were established, the values of the treated groups were compared with those of the control
3. Results

Cr administration at different doses and durations did not alter the food and water intake in animals. There was no mortality or any visible toxic manifestation in the Cr treated animals. The body and organ weights after Cr treatment at various concentrations were displayed in Table 1. Body weight gain was less in medium and high dose Cr treated rats for different durations than their respective control group of animals. Testicular weight in low and medium dose Cr treated groups remained same but was decreased significantly \((p<0.05)\) in high dose Cr treated group for 26 days than control. Weight of ventral prostate, coagulating gland, seminal vesicle and cauda epididymis were decreased significantly after medium and high dose Cr treatment. No significant changes in testis and accessory sex organ weights (except ventral prostate) were observed in rats after low dose \((0.2 \text{ mg/kg bw/day})\) Cr treatment in comparison to the respective control groups (Table 1).

Epididymal sperm count was decreased significantly after Cr treatment at medium \((0.4 \text{ mg/kg bw/day})\) and high \((0.6 \text{ mg/kg bw/day})\) doses than control for both treatment schedules (Table 3).

Histological study (Plate 1) demonstrated testicular structures of control and Cr treated animals. Control animals showed normal cellular arrangement in the seminiferous tubule. On the other hand, seminiferous tubular shrinking was observed in 0.4 mg/kg bw (for 26 days) and 0.6 mg/kg bw (for 13 and 26 days) Cr treated testis. Sloughing of germ cells from seminiferous epithelium was observed in the testis of high dose Cr treated rats for 26 days. Degenerative changes in the testis with disintegration of spermatocytes, resulting in spermatogenic arrest with tubular necrosis and degenerating Leydig cells were observed in medium and high dose Cr treated testis.

The quantitative study of germ cells at stage VII (Table 2) revealed that medium and high dose Cr treatment had reduced the number of step 7 spermatid significantly in comparison to low dose \((0.2 \text{ mg/kg bw/day})\) Cr treatment. Medium dose Cr treatment for 26 days had significantly reduced the number of AStg, pLSc, mPSc and 7Sd as compared with control. Standard error of mPSc:7Sd is 1:4. This ratio was 1:1.30 and 1:3.44 in ASg, pLSc, mPSc and 7Sd as compared with control. Standard ratio of mPSc:7Sd is 1:4. This ratio was 1:1.30 and 1:3.44 in respective controls. The effective spermatid degeneration as calculated from the above ratios were decreased significantly after medium and high dose Cr treatment for 26 days than the control group.

Table 3 shows the activities of two key steroidogenic enzyme of testis, namely \(\Delta^23\beta\)-HSD and \(17\beta\)-HSD were decreased significantly in medium and high dose Cr treated group than control while enzyme activities remained almost unaltered after low dose Cr treatment in comparison to their respective controls. Serum testosterone and LH levels were significantly decreased in medium and high dose treated group than control. The decrease was more marked in high dose 26 days Cr treated group. However, serum FSH level was increased in medium and high dose

<table>
<thead>
<tr>
<th>Parameters</th>
<th>13 days treated groups</th>
<th>26 days treated groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td>Initial body weight (gm)</td>
<td>204.36 ± 5.19</td>
<td>202.46 ± 3.19</td>
</tr>
<tr>
<td>Final body weight (gm)</td>
<td>220.07 ± 11.36</td>
<td>216.87 ± 10.23</td>
</tr>
<tr>
<td>Increase in body weight (%)</td>
<td>12.70%</td>
<td>11.36%</td>
</tr>
<tr>
<td>Testis (pair) weight (gm)</td>
<td>2.79 ± 0.05</td>
<td>2.69 ± 0.05</td>
</tr>
<tr>
<td>Testis (pair) weight in gm% bw</td>
<td>1.21 ± 0.04*</td>
<td>1.21 ± 0.02*</td>
</tr>
<tr>
<td>Seminal vesicle weight (mg)</td>
<td>362.31 ± 11.02</td>
<td>352.59 ± 9.23</td>
</tr>
<tr>
<td>Seminal vesicle weight in mg% bw</td>
<td>156.33 ± 5.27*</td>
<td>155.06 ± 4.14*</td>
</tr>
<tr>
<td>Coagulating gland weight (mg)</td>
<td>113.21 ± 10.54</td>
<td>107.22 ± 9.23</td>
</tr>
<tr>
<td>Coagulating gland weight in mg% bw</td>
<td>48.80 ± 5.44*</td>
<td>47.24 ± 4.39*</td>
</tr>
<tr>
<td>Ventral prostate weight (mg)</td>
<td>497.06 ± 10.13</td>
<td>484.67 ± 9.72</td>
</tr>
<tr>
<td>Ventral prostate weight in mg% bw</td>
<td>199.95 ± 10.13</td>
<td>184.06 ± 9.72</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. *Values bearing different superscript within the row indicate significant difference \((p<0.05)\).
Cr exposed group than control. No such changes were observed after low dose Cr exposure for 13 and 26 days duration when compared with the respective control groups (Table 3).

Testicular antioxidant enzymes SOD and catalase activities were decreased significantly \( p<0.05 \) in medium and high dose Cr treated group while no such change was observed in antioxidant enzyme activities in low dose group for different durations than the respective control (Fig. 1).

Low dose (0.2 mg/kg bw) Cr treatment showed no effect on testicular lipid peroxidation however lipid peroxidation was increased significantly after Cr treatment at medium and high dose (0.4 mg and 0.6 mg/kg bw/day) than controls (Fig. 1).

Adrenal weight, adrenal \( \Delta^5 \)3\( \beta \)-HSD activity and serum corticosterone level were increased significantly \( p<0.05 \) in medium and high dose Cr treated group (Table 4). Adrenal weight and other functional parameters were normal in at low dose Cr exposure when compared with the control animals.

4. Discussion

The study demonstrated that Cr exposure at medium and high doses as used in this investigation had reduced the net gain in body weight in adult albino rats when compared with the control animals. The results are similar with the earlier observations where decrease in body weight due to gain in lean body mass and decrease in body fat noted in humans exposed to chromium picolinate [31]. Testicular weight was decreased significantly only after high dose Cr exposure for 26 days. Weight of accessory sex organ viz. ventral prostate,
Table 2
Changes in the sperm count and relative number of germ cells per tubular cross section at stage VII of spermatogenesis following chromium treatment at different doses and durations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>13 days treated groups</th>
<th>26 days treated groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Low</td>
</tr>
<tr>
<td>Epididymal sperm count (million cells/cauda epididymis)</td>
<td>135.53 ± 5.18*</td>
<td>132.33 ± 6.2*</td>
</tr>
<tr>
<td>ASg (spermatogonia A)</td>
<td>0.64 ± 0.04*</td>
<td>0.63 ± 0.05*</td>
</tr>
<tr>
<td>pLSc (preleptotene spermatocytes)</td>
<td>17.87 ± 0.75*</td>
<td>18.12 ± 0.68*</td>
</tr>
<tr>
<td>mPSc (mid-pachytene spermatocytes)</td>
<td>19.55 ± 0.95*</td>
<td>19.11 ± 0.89*</td>
</tr>
<tr>
<td>7Sd (step 7 spermatid)</td>
<td>64.52 ± 1.82*</td>
<td>62.84 ± 1.25*</td>
</tr>
<tr>
<td>7Sd:7Sd (AF)</td>
<td>1:3.30</td>
<td>1:3.28</td>
</tr>
<tr>
<td>7Sd degeneration (%)</td>
<td>17.50</td>
<td>18.00</td>
</tr>
<tr>
<td>Effective 7Sd degeneration</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± S.D., n = 8. Values bearing different superscript within the row indicate significant differences (p<0.05).

Table 3
Changes in testicular Δ3 17β-hydroxysteroid dehydrogenase (HSD), 17β-HSD activity, epididymal sperm count, serum testosterone, FSH and LH level in rats after chromium treatment at different doses and durations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>13 days treated groups</th>
<th>26 days treated groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Low</td>
</tr>
<tr>
<td>Testicular Δ3 17β-HSD activity (ΔOD/mg protein/min)</td>
<td>0.130 ± 0.003*</td>
<td>0.131 ± 0.002*</td>
</tr>
<tr>
<td>Testicular 17β-HSD activity (ΔOD/mg protein/min)</td>
<td>0.020 ± 0.003*</td>
<td>0.019 ± 0.002*</td>
</tr>
<tr>
<td>Serum testosterone level (ng/ml)</td>
<td>3.70 ± 0.13*</td>
<td>3.68 ± 0.17*</td>
</tr>
<tr>
<td>Serum FSH level (ng/ml)</td>
<td>19.08 ± 1.18*</td>
<td>18.93 ± 0.85*</td>
</tr>
<tr>
<td>Serum LH level (ng/ml)</td>
<td>4.74 ± 0.64*</td>
<td>4.64 ± 0.46*</td>
</tr>
</tbody>
</table>

Values are mean ± S.D., n = 8. Values bearing different superscript within the row indicate significant differences (p<0.05).
Table 4
Changes in the adrenal gland weight, adrenal Δ5β-HSD dehydrogenase (HSD) activity and serum corticosterone level in rats after chromium treatment at different doses and durations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>13 days treated groups</th>
<th>26 days treated groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td>Adrenal gland weight (mg)</td>
<td>25.9 ± 1.34</td>
<td>25.3 ± 1.45</td>
</tr>
<tr>
<td>Adrenal gland weight (mg % of bw)</td>
<td>11.26 ± 0.66*</td>
<td>11.18 ± 0.72*</td>
</tr>
<tr>
<td>Adrenal Δ5β-HSD activity (OD/mg protein/min)</td>
<td>0.16 ± 0.08*</td>
<td>0.163 ± 0.006*</td>
</tr>
<tr>
<td>Serum corticosterone level (ng/dl)</td>
<td>3.19 ± 1.38</td>
<td>3.33 ± 0.12</td>
</tr>
</tbody>
</table>

Values are mean ± S.D., n = 8. Values bearing different superscript within the row indicate significant differences (p < 0.05).

Fig. 1. Changes in testicular lipid peroxidation, catalase and SOD activities in rats after chromium treatment at different doses and durations (values are mean ± S.D., n = 8). Bars bearing different superscript indicate significant differences (p < 0.05).

Incorporation of these findings with the previous study, altered testicular histarchitectural and structural integrity after Cr exposure is well documented in the present investigation. Although testicular weight remained unaltered, degenerative changes in spermatogenic cycle corresponded with the decreased serum testosterone level after Cr exposure at different doses. Testosterone is responsible for the growth, structural integrity and functional activities of accessory sex organs as well as it helps in the maintenance of spermatogenesis [32]. Cr exposure at medium and high doses was shown to reduce the activities of testicular steroidogenic enzymes viz. Δ5β-HSD and 17β-HSD. Therefore, decreased serum testosterone level in medium and high dose Cr treated groups might be due to impaired activities of these enzymes. Degenerative changes in spermatogenic cycle were very much conspicuous in medium and high dose Cr treated group. Spermatogenesis is more affected after high dose Cr exposure than moderate dose. The histopathological exam-

**Fig. 1.** Changes in testicular lipid peroxidation, catalase and SOD activities in rats after chromium treatment at different doses and durations (values are mean ± S.D., n = 8). Bars bearing different superscript indicate significant differences (p < 0.05).
ination of testis shows that there is visible disruption in germ cell arrangement near the wall of the seminiferous tubule. In high dose Cr exposed group, detachment of seminiferous cellular component from basement membrane was seen in some regions. Significant diminution of testicular DNA and RNA levels are found in rats treated orally with high dose potassium dichromate that demonstrate low cellular turnover rate [9]. In the present study, disruption in cellular association and reduced epididymal sperm count indicated degeneration in spermatogenesis after Cr exposure that correlates with the earlier observations.

It is well documented that during normal spermatogenesis reactive oxygen species are produced by the electron leakage outside the electron transfer chain [33]. These reactive species can initiate lipid peroxidation, increased LPO further leads to tissue damage [34], impaired membrane function, decreased membrane fluidity, altered structural integrity and inactivation of several membrane bound enzymes [35]. Earlier studies suggested that hexavalent Cr is an inducer of oxidative stress with the production of reactive oxygen species [36]. Testicular LPO was markedly increased in experimental group treated with medium and high dose Cr for 26 days. However, low dose Cr treatment for 13 and 26 days resulted no significant alteration in LPO when compared with their respective controls. Enhanced LPO in response to Cr exposure leads to cellular degeneration along with impairment in steroidogenic enzyme activities in testis of adult rats. Normally produced ROS are neutralized by cellular antioxidant defense mechanism, which includes the antioxidative enzyme superoxide dismutase and catalase [37]. Decline in the SOD and catalase activities after medium (0.4 mg/kg bw/day) and high (0.6 mg/kg bw/day) dosages of Cr exposure indicate increased production of reactive species beyond the physiological limit. Increased oxidative stress also influences the normal functioning of Leydig cell, which plays a pivotal role in decreased testosterone production [38]. Low serum testosterone and LH levels in medium and high dose Cr exposed group also signifies the impairment in Leydig cell function. Serum FSH level was high in medium and high dose Cr exposed group. Therefore, it can be speculated that decreased sperm count was associated with Cr induced alteration in testicular function, which involves impaired steroidogenesis and increased serum FSH level as high serum FSH and increased ACTH and high serum corticosterone level were observed after Cr exposure in adult albino rats after medium and high doses Cr exposure for 26 days. Increased adrenocortical activity in response to Cr exposure is concomitant with the stress induced hyper secretion of adrenal glucocorticoids via hypothalamic-pituitary-adrenal axis. Cr exposure induced oxidative stress to the experimental animals. The offspring of the chromium(III) treated fathers had significantly higher average serum corticosterone levels compared with offspring of vehicle-treated fathers [43]. Stress induced reproductive dysfunctions are generally mediated by gonadal and adrenocortical interactions. Stress responses activate hypothalamic-pituitary-adrenal axis (HPA) resulting in excess synthesis and release of glucocorticoids that act as defense against stressful situations [10]. During acute or chronic stress and adrenal hyperactivity, the medial paraventricular nucleus (PVN) release several peptides. Corticotropin releasing hormone (CRH) is one of the most important peptide among them. It stimulates the secretion of corticotropin (ACTH) from pituitary that in turn stimulates glucocorticoid synthesis and release from adrenal cortex [44]. There is a close anatomical relationship between CRH and gonadotropin releasing hormone (GnRH) nucleus [45]. High glucocorticoid secretion disrupts the HPG axis; inhibit luteinizing-hormone-releasing-hormone (LHRH) synthesis, plasma LH and sex steroid release [46,47]. Therefore, glucocorticoid mediates negative feedback potency against HPG axis [10]. Gonadectomized rats showed increased ACTH and corticosterone secretion in response to acute stress [48]. Thus, Cr induced hyperactivity of HPA axis stimulates rapid release of CRH, ACTH and glucocorticoids, respectively, from different part of this axis [49], resulting a fall in plasma LH and testosterone levels. Therefore, increased adrenocortical activities might be another distinctive cause for Cr induced impaired gonadal function and spermatogenesis.

It may be speculated from the present investigation that Cr might be toxic for male reproductive system at a much lower dose (0.4 mg/kg bw/day) and after a critical period (26 days) of exposure. The mechanism of reproductive impairment might be for the development of oxidative stress, disruption of hypothalamic-pituitary-testicular axis associated with hyper activation of the stress signaling pathway through hypothalamic-pituitary-adrenocortical axis resulting excess corticosterone secretion. The present study thus indicates that toxic effects of Cr on male reproductive system are not only mediated through its direct effect on testis by producing ROS induced oxidative stress but also interfering the HPG axis through adrenocortical hyperactivity.
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