Chapter III

Effect of Withdrawal of Chromium Treatment and Simultaneous Administration of Vitamin C and E on Chromium-Induced Reproductive Toxicity in the Testis
3.1 INTRODUCTION

Recent studies have implicated the importance of nutrition in protecting the living organisms against the toxic effects of ROS and toxic environmental chemicals (Lall et al., 1999). Antioxidants are known to prevent free radical induced damage by blocking the formation of radical species, scavenging them, or by enhancing the decomposition of ROS (see Young and Woodside, 2001). The data from the previous chapter showed the involvement of oxidative stress in chromium induced reproductive toxicity. Deficiency of vitamins A, C and E are known to induce reproductive impairments, which are alleviated by dietary replacements or supplements (Evans and Bishop, 1922; see Eskild and Hansson, 1994; see Luck et al., 1995). The role of vitamins in protecting cells against metal induced damage is well known (see Sugiyama, 1992; see Sokol, 1996). However, there is dearth of knowledge regarding the potential efficacy of antioxidants supplementation on chromium-induced reproductive impairment.

3.2 REVIEW OF LITERATURE

One of the most attractive approaches to protect tissues against toxic and carcinogenic injuries, and to prevent diseases involves the use of specific nutrients (see Pryor, 1991). The most widely studied protective agents are the antioxidant vitamins.

3.2.1 Effect of Vitamin C on Reproductive and Cytotoxicity of Heavy Metals

Dietary supplementation of vitamin C was shown to prevent cadmium, copper and mercury induced growth retardation in chick (Hill, 1979). Ascorbic acid was reported to protect Japanese quail against cadmium induced growth retardation, decreased bone
mass, anemia and hypogonadism in male (Fox, 1975). Ascorbic acid (100 mg/day for 5 weeks) was reported to suppress cadmium induced lipid peroxidation in the kidney, liver and serum of guinea pigs (Hudecova and Ginter, 1992) and the thyroid of Swiss male mice at a dose of 1 mg/kg b.wt for 15 days (Gupta and Kar, 1998). The protective effect of vitamin C supplementation on nickel-induced alteration in the activities of alkaline phosphatase, acid phosphatase, glucose-6-phosphatase, succinic dehydrogenase is also known (Chatterjee et al., 1979). Ameliorative effect of vitamin C on thallium induced nephrotoxicity in 10 and 55-days old rats, without altering the thallium content in the tissue was reported recently (Appenroth and Winnefeld, 1998).

A protective role of ascorbic acid against lead toxicity has been suggested as serum ascorbic acid showed an inverse relationship with elevated blood lead concentration in animals and humans (see Houston and Johnson, 2000). Vitamin C supplementation (1000 mg) was shown to decrease the concentration of lead in the blood of smokers (Dawson et al., 1999). Vitamin C (500 mg/L) was also shown to exert protective action against lead-induced ROS production in rat sperm and the resultant loss of sperm forward motility and oocyte penetrating capability (Hsu et al., 1998).

Chromium-induced increase in liver ornithine carbamyl transferase activity, and serum urea-nitrogen in mice were prevented by simultaneous injection of 100mg/kg ascorbic acid (Susa et al., 1989). Protective effect of Vitamin C against chromium-induced DNA lesions, lipid peroxidation, and cytotoxicity has also been studied well (see Sugiyama, 1992; Wise et al., 1993). Ascorbic acid was shown to protect CHO-AA8 cells from chromium-induced apoptotic cell death (Blankenship et al., 1997). Vitamin C prevented chromium-induced renal toxicity in young and puberal rats (Appenroth and

Vitamin C prevented the loss of sperm viability, membrane integrity and motility that occurs during storage of turkey semen (Donoghue and Donoghue, 1997). The protective effect of vitamin C on human sperm motility, viability and lipid peroxidation is evident only below 1000 μM concentration, above which vitamin C exerts a negative influence (Verma and Kanwar, 1998). Supplementation of ascorbic acid at doses higher than 200 mg/day was found to improve the suppressed sperm quality in heavy smokers (Dawson et al., 1992).

3.2.2 Effect of Vitamin E Supplementation on Reproductive and Cytotoxicity of Heavy Metals.

Ganther (1978, 1980) showed the efficacy of vitamin E in preventing mercury-induced toxicity in nervous tissue culture. Though α-tocopherol inhibited chromium induced chromosomal aberrations in CHO-AA8 or HLF cells, it could not protect cells from apoptotic cell death (Blankenship et al., 1997; Carlisle et al., 2000). Vitamin E abolished chromium and thallium-induced nephrotoxicity in 10 and 55 days old rats by lowering the tissue concentration of these metals (Appenroth and Winnefeld, 1998). α-tocopherol at a concentration of 36.2 μM effected a 50% decrease in the formation of 8-OHDG in calf thymus DNA in vitro (Qi et al., 2000). Supplementation of vitamin E (150 or 300 mg/kg diet) prevented lead-induced reduction in sperm motility and oocyte penetration and enhanced ROS production in rat sperm (Hsu et al., 1998).
Vitamin E deficient male rats suffered spermatogenic arrest, and females failed to retain zygotes, whereas vitamin E supplementation prevented these events (Evans and Bishop, 1922). Vitamin E supplementation to nine oligoasthenoteratospermic men for a period of 6 months improved sperm motility, and survival (Vezina et al., 1996). α-tocopherol (200 mg/day for 3 months) improved the fertilization rate of fertile normospermic male volunteers who previously had low fertilization rate, probably by reducing LPO, without causing any quantitative change in the subcellular organelles (Geva et al., 1996). Treatment of asthenospermic patients with vitamin E decreased sperm lipid peroxidation, and ROS production with or without improving sperm motility (Suleiman et al., 1996; Donnelly et al., 1999). Vitamin E was shown to improve turkey sperm survival, motility and membrane integrity in vitro (Donoghue and Donoghue, 1997). In rats treated with vitamin-E deficient diet from day 10 pp to day 48 pp, the predominant germ cells observed were step-7 spermatids, many of those were undergoing degeneration. Immature germ cells and multinucleated cells could be observed in testicular and epididymal lumen. Principal, narrow and apical cells of the epididymis of these rats showed poorly developed secretory and endocytotic apparatus. Normal histoarchitecture in testis and epididymis was restored with vitamin E readministration (Bensoussan et al., 1998). α-tocopherol (30 and 60 µM) was found to protect sperm DNA against X-ray induced damage (Hughes et al., 1998).
3.2.3 Glutathione and Other Antioxidants in Reproductive and Cytotoxicity of Heavy Metals

GSH administration ameliorated cadmium toxicity in isolated rat hepatocytes (Stacey, 1986). Cadmium-induced acute testicular toxicity and interstitial cell tumors in rats can be prevented by low-dose cadmium pretreatment, which increases intracellular GSH (Wahba et al., 1990). Protective effect of GSH monoesters against HgCl$_2$ induced renal toxicity and mortality is known (Naganuma et al., 1990; Houser et al., 1992). GSH supplementation improved progressive sperm motility, velocity, linearity, amplitude of lateral head displacement together with reduction in abnormal sperm morphology in patients with varicocele and germ-free genital tract inflammation (Lenzi et al., 1993). These authors have also shown increased serum and erythrocyte content of polyunsaturated fatty acids (PUFA), due to decreased lipid peroxidation in subjects treated with GSH (Lenzi et al., 1994).

CAT and SOD were shown to inhibit cadmium induced chromosomal aberrations, DNA breaks and growth inhibition in cultures of V7G cells and human fibroblasts (Ochi et al., 1983, 1987; Ochi and Ohsawa, 1985; Snyder, 1988). CAT is known to suppress chromium induced 8-OHHDG formation in calf-thymus DNA (Faux et al., 1992). CAT has been shown to prevent the decrease in bovine sperm motility in cultures containing aminoacids such as phenylalanine, tyrosine, and aromatic amino acids (Lapointe and Sirard, 1998). CAT is also known to enhance the oocyte penetration capacity of bovine sperm in vitro (Blondin et al., 1997). N-acetylcysteine pretreatment was reported to have a protective effect against renal and lung toxicity of mercury (Girardi and Elias, 1991;
Livardjani et al., 1991). Lipoic acid and lipoate have been shown to protect the liver against cadmium toxicity (Muller, 1989; Muller and Menzel, 1990; Bludovska et al., 1999). A protective effect of vitamin D against cadmium toxicity on rat osteosarcoma cells is reported (Angle et al., 1990). Griveau and LeLannou (1994) showed that addition of GSH, CAT and SOD prevented the fall in the motility of sperm prepared by Percoll gradient centrifugation. While SOD was able to improve only acrosome reaction, CAT was ineffective in both the cases (Griveau and LeLannou, 1994).

Scope of the Present Investigation

The previous two chapters of the thesis have established that chromium induced male reproductive toxicity is mediated by a hostile oxygen stress in the testis of rats. The foregoing survey of literature reveals the beneficial effect of antioxidant supplementation in ameliorating metal toxicity and also in improving sperm function. However, there is little data available regarding the potential beneficial action of antioxidant supplementation against chromium-induced reproductive toxicity or oxygen toxicity in reproductive tract organs. It is also essential to understand whether chromium-induced reproductive toxicity is permanent or reversible. If reversible, it is to be understood whether prevention of the subjects getting exposed to the toxic metal is enough or it needs any additional measures. Having this background in mind, the third chapter of the thesis was designed and executed to test the hypotheses “supplementation of antioxidant vitamins C and E could prevent chromium-induced reproductive and oxygen toxicity in testis of rats”, and “chromium induced changes in testis are reversible, when the affected subjects are rehabilitated under a chromium-exposure-free condition”. 
3.3 MATERIALS AND METHODS

3.3.1 Experimental Design

3.3.1.1 Animals

The details provided under chapter I are applicable to this chapter as well.

3.3.1.2 Schedules of Chromium and Vitamin Supplementation

Rats treated with 400 ppm hexavalent chromium, in the form of potassium dichromate, through drinking water for 30 days from day 91 post-partum (pp) to day 120 pp were given simultaneous vitamin C or E treatment. Another set of rats was withdrawn of chromium treatment for a period equal to the period of exposure. The dose of chromium was selected on the basis of the data on reproductive and oxygen toxicities in the testicular tissue in treated rats, as elaborated in the first chapter of the thesis, which revealed 400 ppm to be the best effective dose.

The animals were divided into the following groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>120 days old control rats, given drinking water alone</td>
</tr>
<tr>
<td>Group II</td>
<td>Rats given 400 ppm chromium from day 91 to day 120 pp</td>
</tr>
<tr>
<td>Group III</td>
<td>400 ppm chromium treated rats given simultaneous vitamin C administration through drinking water (0.125 g or 0.25 g or 0.5 g/L).</td>
</tr>
</tbody>
</table>
Group IV : 400 ppm chromium treated rats given simultaneous vitamin E injections (12.5 mg or 25 mg or 50 mg/kg b.wt/day for 5 days a week, i.p).

Group V : 150 days old control rats

Group VI : Rats given 400 ppm chromium from day 91 pp to day 150 pp.

Group VII : 400 ppm chromium treated rats withdrawn of treatment and maintained in a chromium exposure-free condition for a further period of 30 days from day 121 pp to day 150 pp.

Each group consisted of six animals. Rats were killed at the end of the experimentation, trunk blood collected, plasma separated and used for hormone assays. Epididymal tissue was separated, cleaned and used for sperm count and motility. One testis from each animal was fixed in 7% formalin for histological studies, while the other was used for interstitial fluid collection.

3.3.2 Analytical Methods

All the parameters studied in chapter I and II were studied in animals given simultaneous vitamin treatment and those subjected to withdrawal regimen.
3.3.3 Statistical Analysis

The data from rats given simultaneous vitamin treatments were subjected to one-way analysis of variance with Duncan's multiple comparison tests for analysis of statistical significance between the mean. The data from withdrawal groups were compared with the respective control and analysed for statistical significance using unpaired Students 't' test.

3.4 RESULTS AND DISCUSSION

3.4.1 Effect of withdrawal of chromium treatment on chromium-induced reproductive toxicity

Results

The concentration of plasma and testicular tissue total chromium remained elevated despite a decrease when compared to chromium treated rats, in rats withdrawn of chromium treatment and maintained in a chromium-exposure free condition for a period of one-month (Table 3.1). Withdrawal of chromium treatment resulted in restoration of normal body and testicular weight, and normal epididymal sperm count and motility (Table 3.1).
Table 3.1: Effect of withdrawal of chromium treatment on chromium-induced changes on body weight, testicular weight, epididymal sperm count, and forward motility in adult male rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>400 ppm chromium</th>
<th>Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum chromium (µg/mL)</td>
<td>0.028 ± 0.008</td>
<td>4.32 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.144 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Testicular chromium (µg/g tissue)</td>
<td>0.0174 ± 0.006</td>
<td>1.99 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.654 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body weight (% increase)</td>
<td>19.23 ± 0.90</td>
<td>-10.23 ± 0.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.54 ± 0.94</td>
</tr>
<tr>
<td>Epididymal sperm forward motility (µM travelled/sec)</td>
<td>266.56 ± 10.11</td>
<td>118.52 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>258.64 ± 8.1</td>
</tr>
<tr>
<td>Caput sperm concentration (10&lt;sup&gt;6&lt;/sup&gt; sperm/region)</td>
<td>39.26 ± 2.20</td>
<td>21.56 ± 0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.26 ± 1.11</td>
</tr>
<tr>
<td>Cauda sperm concentration (10&lt;sup&gt;6&lt;/sup&gt; sperm/region)</td>
<td>68.26 ± 0.97</td>
<td>35.23 ± 0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.67 ± 0.53</td>
</tr>
<tr>
<td>Testicular weight (g)</td>
<td>1.33 ± 0.04</td>
<td>1.35 ± 0.05</td>
<td>1.32 ± 0.03</td>
</tr>
</tbody>
</table>

Each value is mean ± SEM of 6 animals.  
<sup>a</sup> represents statistical significance at p < 0.05 compared to control.

Animals subjected to withdrawal regimen exhibited a normal concentration of plasma LH and plasma and TIF steroid hormones. Though plasma FSH in these rats withdrawn of chromium treatment was significantly higher than chromium treated rats, it remained less than the control value, even after one-month of chromium-exposure free condition. The concentration of plasma and TIF ABP was restored to normal level in animals withdrawn of chromium treatment and maintained in a chromium-exposure-free condition for one-month period (Table 3.2).
Table 3.2:  Effect of withdrawal of chromium treatment on chromium-induced changes on plasma and TIF hormones and binding proteins in adult male rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>400 ppm chromium</th>
<th>Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma FSH (ng/mL)</td>
<td>24.26 ± 0.53</td>
<td>5.43 ± 0.02⁰</td>
<td>20.85 ± 0.90⁰</td>
</tr>
<tr>
<td>Plasma LH (ng/mL)</td>
<td>2.21 ± 0.05</td>
<td>0.54 ± 0.53⁰</td>
<td>1.96 ± 0.13</td>
</tr>
<tr>
<td>Plasma testosterone (ng/mL)</td>
<td>1.66 ± 0.04</td>
<td>0.51 ± 0.01⁰</td>
<td>1.47 ± 0.06</td>
</tr>
<tr>
<td>Plasma estradiol (pg/mL)</td>
<td>30.27 ± 2.32</td>
<td>22.56 ± 1.08⁰</td>
<td>28.35 ± 0.83</td>
</tr>
<tr>
<td>Plasma DHT (pg/mL)</td>
<td>28.76 ± 0.82</td>
<td>12.31 ± 0.05⁰</td>
<td>26.54 ± 0.53</td>
</tr>
<tr>
<td>TIF testosterone (ng/mL)</td>
<td>56.27 ± 1.6</td>
<td>30.31 ± 2.56⁰</td>
<td>59.26 ± 1.35</td>
</tr>
<tr>
<td>TIF estradiol (pg/mL)</td>
<td>2.85 ± 0.02</td>
<td>1.13 ± 0.05⁰</td>
<td>2.92 ± 0.02</td>
</tr>
<tr>
<td>TIF DHT (ng/mL)</td>
<td>23.21 ± 2.21</td>
<td>30.23 ± 1.21⁰</td>
<td>21.26 ± 0.47</td>
</tr>
<tr>
<td>Plasma ABP (pmoles/mL)</td>
<td>0.37 ± 0.01</td>
<td>0.14 ± 0.004⁰</td>
<td>0.36 ± 0.01</td>
</tr>
<tr>
<td>TIF ABP (nmoles/mL)</td>
<td>3.85 ± 0.04</td>
<td>1.2 ± 0.02⁰</td>
<td>3.92 ± 0.03</td>
</tr>
</tbody>
</table>

Each value is mean ± SEM of 6 animals.

⁰ represents statistical significance at p < 0.05 compared to control.

Normality in seminiferous tubular and tubular lumen diameters was restored in animals withdrawn of chromium treatment and maintained in a chromium-exposure-free period for a period of one-month. These rats also exhibited normal testicular somatic and germ cell numbers (Table 3.3)
Table 3.3: Effect of withdrawal of chromium treatment on chromium-induced changes on testicular histomorphometry in adult male rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>400 ppm chromium</th>
<th>Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminiferous tubular diameter (μM)</td>
<td>279.76 ± 6.01</td>
<td>125.23 ± 3.26a</td>
<td>289.31 ± 3.43</td>
</tr>
<tr>
<td>Seminiferous tubular lumen diameter (μM)</td>
<td>102.35 ± 2.46</td>
<td>45.23 ± 3.21a</td>
<td>109.26 ± 1.82</td>
</tr>
<tr>
<td>Leydig cells (10⁶ cells/testis)</td>
<td>24.210 ± 0.61</td>
<td>7.83 ± 0.09a</td>
<td>26.36 ± 0.39</td>
</tr>
<tr>
<td>Sertoli cells (10⁶ cells/testis)</td>
<td>36.247 ± 1.78</td>
<td>7.6 ± 0.05a</td>
<td>39.625 ± 1.36</td>
</tr>
<tr>
<td>Myoid cells (10⁶ cells/testis)</td>
<td>7.342 ± 0.08</td>
<td>4.23 ± 0.11a</td>
<td>7.214 ± 0.09</td>
</tr>
<tr>
<td>Spermatogonia (10⁶ cells/testis)</td>
<td>25.78 ± 0.28</td>
<td>9.53 ± 0.46a</td>
<td>23.8 ± 0.21</td>
</tr>
<tr>
<td>Spermatocytes (10⁶ cells/testis)</td>
<td>27.98 ± 0.28</td>
<td>10.43 ± 0.23a</td>
<td>28.57 ± 0.21</td>
</tr>
<tr>
<td>Round spermatids (10⁶ cells/testis)</td>
<td>51.32 ± 1.24</td>
<td>16.53 ± 0.42a</td>
<td>49.65 ± 0.87</td>
</tr>
<tr>
<td>Elongated spermatids (10⁶ cells/testis)</td>
<td>15.59 ± 0.98</td>
<td>5.13 ± 0.08a</td>
<td>16.18 ± 0.15</td>
</tr>
</tbody>
</table>

Each value is mean ± SEM of 6 animals.

a represents statistical significance at p < 0.05 compared to control.

Testicular lipid peroxidation was reverted to untreated control level in rats subjected to withdrawal of chromium treatment. This was accompanied by the normal quantity of ROS production in the testis of these rats. The activities of testicular SOD and CAT were also reverted to normality in animals withdrawn of chromium treatment and maintained in a chromium-exposure-free condition for a period of one-month. Activities of testicular GSH dependent/metabolizing enzymes were also reverted to
control levels in chromium treated animals, which were maintained in a chromium-exposure-free period for duration of one-month (Table 3.4).

Table 3.4: Effect of withdrawal of chromium treatment on chromium-induced changes on testicular lipid peroxidation, free radicals and antioxidant enzymes in adult male rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>400 ppm chromium</th>
<th>Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxidation (nmole MDA formed/min/mg protein)</td>
<td>2.45 ± 0.06</td>
<td>6.54 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.24 ± 0.13</td>
</tr>
<tr>
<td>Hydrogen peroxide (umole formed/min/mg protein)</td>
<td>2.51 ± 0.07</td>
<td>18.95 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.29 ± 0.10</td>
</tr>
<tr>
<td>Hydroxyl radicals (umole formed/min/mg protein)</td>
<td>0.57 ± 0.02</td>
<td>12.65 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.61 ± 0.01</td>
</tr>
<tr>
<td>SOD (Units/mg protein)</td>
<td>7.26 ± 0.14</td>
<td>4.98 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.94 ± 0.10</td>
</tr>
<tr>
<td>CAT (Units/mg protein)</td>
<td>18.52 ± 0.91</td>
<td>36.52 ± 1.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.64 ± 0.55</td>
</tr>
<tr>
<td>GPx (Units/mg protein)</td>
<td>8.74 ± 0.28</td>
<td>3.86 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.37 ± 0.21</td>
</tr>
<tr>
<td>GR (Units/mg protein)</td>
<td>9.56 ± 0.23</td>
<td>4.29 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.79 ± 0.06</td>
</tr>
<tr>
<td>γ-GT (Units/mg protein)</td>
<td>1.26 ± 0.07</td>
<td>0.34 ± 0.009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.19 ± 0.09</td>
</tr>
<tr>
<td>G6PDH (Units/mg protein)</td>
<td>1.46 ± 0.02</td>
<td>0.22 ± 0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.38 ± 0.07</td>
</tr>
<tr>
<td>GST (Units/mg protein)</td>
<td>31.26 ± 0.49</td>
<td>9.43 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.56 ± 0.81</td>
</tr>
</tbody>
</table>

Each value is mean ± SEM of 6 animals.

<sup>a</sup> represents statistical significance at p < 0.05 compared to control.

The concentrations of testicular non-enzymic antioxidant GSH, vitamin A, C and E were restored to control level in chromium treated rats, which were maintained in a chromium-free water for a further period of one-month (Table 3.5).
Table 3.5: Effect of withdrawal of chromium treatment on chromium-induced changes on testicular non-enzymic antioxidants in adult male rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>400 ppm chromium</th>
<th>Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (μg/mg tissue)</td>
<td>14.32 ± 0.41</td>
<td>6.49 ± 0.12a</td>
<td>13.25 ± 0.37</td>
</tr>
<tr>
<td>Vitamin C (μg/mg tissue)</td>
<td>1.04 ± 0.03</td>
<td>0.65 ± 0.05a</td>
<td>1.16 ± 0.04</td>
</tr>
<tr>
<td>Vitamin E (μg/mg tissue)</td>
<td>59.26 ± 1.76</td>
<td>40.53 ± 1.59a</td>
<td>56.23 ± 0.53</td>
</tr>
<tr>
<td>Vitamin A (μg/mg tissue)</td>
<td>13.21 ± 0.21</td>
<td>4.26 ± 0.11a</td>
<td>12.24 ± 0.28</td>
</tr>
</tbody>
</table>

Each value is mean ± SEM of 6 animals.
a represents statistical significance at p < 0.05 compared to control

Discussion

The data from animals subjected to withdrawal regime reveals that chromium induced testicular impairments are transient and are reversible, under a chromium-free condition. A similar reversible effect of chromium on epididymal sperm count and motility has been reported by Ernst and Bonde (1992). These authors reported complete reversal of chromium (0.5 mg/kg b. wt for 5 days a week, for 8 weeks) induced suppression of epididymal sperm count, motility and testicular atrophy, when treated animals were kept for a further 8 weeks period under chromium-exposure-free condition. In the present study restoration of normal testicular function in rats exposed to chromium for one month could be achieved within 30 days of chromium-free period. Data on testicular chromium reveals that the testis retains a significant portion of the metal, even after one month of chromium-exposure free periods. The urinary half-life of chromium is 15-44 months under normal physiological conditions (Tossavainen et al., 1980; Kerger et al., 1997). A recent study on a plasma cutter of stainless steel industry revealed the half
life of chromium to be 40 months and 129 months in serum and urine respectively, indicating accumulation of chromium in the body and slower elimination after the exposure period (Petersen et al., 2000). Therefore, the reversal of normal sperm count and motility, and the number of testicular somatic and germ cells in rats withdrawn of chromium treatment, despite a perceptible accumulation of chromium in the testis points out normalization of testicular morphology, irrespective of the presence of chromium. Since the hexavalent form of chromium, which is highly toxic, gets converted to trivalent form quickly within the cell (see DeFlora et al., 1990), it is possible that most of the accumulated chromium is the trivalent form which is least toxic. Probably, data on bioaccumulation and half-life of various chromium species at tissue level will help to understand the problem better. Due to practical difficulties, the same could not be carried out in the present study.

Sokol (1989) reported reversibility of lead (0.6% for 30 days) induced suppression of serum testosterone, sperm count and daily sperm production, after a 30-days recovery period. Sokol (1989) also showed an influence of the age of exposed animals wherein he could observe normalization of lead-induced toxicities in prepuberal animals (treated from day 27 pp), but normalization could not be attained in pubertal animals (treated form day 52 pp). It is not known at this juncture, whether such an age-dependent effect exists for chromium also. However, the data from the present study clearly reveal that impairments of testicular structure and function due to chromium-exposure at adult age could be restored after a 30 days period of recovery.

One interesting aspect seen in the animals withdrawn of chromium treatment is the normal Sertoli cell number and function in these animals despite a decrease in plasma
FSH, the classical regulator of Sertoli cell function. Probably, normality of Sertoli cell function was restored by the action of testosterone, another major hormone involved in Sertoli cell regulation. Animals withdrawn of chromium showed normal titres of testosterone both in blood plasma and TIF testosterone. Studies on the status of the testicular receptors for these hormones could help to understand more about the problem. However, it is quite perplexing to note revival of normal Sertoli cell number in animals, which were subjected to chromium treatment for one month at adulthood and withdrawn of chromium for another one month. Since it is well-known that Sertoli cells cease to proliferate by 15 ± 2 days of postnatal age and their number is maintained constant till adulthood (Bortolussi et al., 1990), the mechanism by which Sertoli cells are replenished in these animals incites a great interest, which warrants further exploration. Probably, there is a transient change to Sertoli cells of rats exposed to chromium, which gets repaired under a conducive environment.

In the previous two chapters, we have seen an association between the reproductive toxicity of chromium and oxidative stress in the testis of chromium treated rats. Data on the testicular pro-oxidant and antioxidants in the testis of rats subjected to withdrawal of chromium treatment attest the above association. Testicular concentration of free radicals, nonenzymic antioxidants and activities of antioxidant enzymes registered normality in rats withdrawn of chromium treatment, despite an elevated concentration of testicular total chromium. Therefore, it is clear that the retention of chromium in the testis of these rats is of the least toxic form i.e. chromium (III). This is the reason that testicular structure and function are at normal level, irrespective of the fact that there is chromium accumulation in the testis of these rats. In the case of rats exposed continually to
chromium water (chapter I and II), there may be a fresh spurt of ROS production accompanied by a decrease in antioxidant defense due to chromium (VI), whenever rats consumed chromium-water.

Thus, it becomes obvious that chromium induced reproductive toxicity is reversible and is associated with reversibility of the optimum oxygen balance in the testis.

3.4.2 Effect of simultaneous administration of vitamin C or E on chromium-induced reproductive toxicity

Results

3.4.2.1 Body Weight

Simultaneous supplementation of vitamin C or vitamin E with 400 ppm chromium treatment prevented the slump in body weight due to chromium exposure. Supplementation with lower doses of vitamin C (125 or 250 mg/mL) or vitamin E prevented the decrease in body weight but the weight of these rats did not reach the level of untreated controls. However, rats treated with 500 mg vitamin C or 50 mg vitamin E maintained the body weight at par with untreated controls (Fig.3.1).
FIG. 3.1: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE BODY WEIGHT OF ADULT MALE RATS

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
3.4.2.2 Testicular Weight

No change in testicular weight could be seen in rats given chromium plus vitamins treatment (Fig. 3.2), when compared to untreated control.

FIG. 3.2: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE TESTICULAR WEIGHT OF ADULT MALE RATS

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Difference between none of the mean value is statistically significant.
3.4.2.3 Epididymal Sperm Forward Motility

A perceptible increase in epididymal sperm forward motility as compared to rats given chromium alone was evident in rats given simultaneous treatment with low doses of vitamin C, though normality was not achieved. In rats given the maximum effective dose of vitamin C (500 mg), sperm forward motility was comparable to untreated control rats. Unlike in vitamin C treatment, all the three doses of vitamin E supplementation sustained normal motility of epididymal sperm in chromium treated rats (Fig. 3.3).

![Figure 3.3: Effect of simultaneous vitamin C or E treatment on chromium-induced changes on the sperm forward motility of adult male rats](image-url)

- Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
3.4.2.4. *Epididymal Sperm Concentration*

Chromium induced decrease in caput epididymal sperm concentration was blocked by simultaneous supplementation with vitamin C or E, irrespective of the doses of these vitamins (Fig. 3.4).

![Graph showing the effect of chromium on sperm concentration](image-url)
As in the caput region, in cauda epididymis also, all the three doses of vitamin C and E prevented chromium induced decrease in sperm concentration, and maintained parity with untreated control rats (Fig. 3.5).

**FIG. 3.5: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE CONCENTRATION OF SPERM IN THE CAUDA EPIDIDYMIS OF ADULT MALE RATS**

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
3.4.2.5 Plasma Gonadotrophins

The concentration of plasma FSH in vitamin C/E co-treated animals exhibited a dose dependent increase when compared to chromium alone treated rats. While the increase in FSH in rats treated with 125 or 250 mg/mL vitamin C was not sufficient to attain normality, normality of the same was achieved in animals given chromium and 0.5 mg/L vitamin C. Animals given 12.5 mg vitamin E had FSH levels higher than chromium alone treated rats but was still less than untreated controls. While 25 mg vitamin E treated animals had normal plasma FSH, those given 50 mg vitamin E had FSH level higher than that observed in untreated control animals (Fig. 3.6).

![Fig. 3.6: Effect of simultaneous vitamin C or E treatment on chromium-induced changes on the plasma FSH of adult male rats](image)

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
Plasma LH titre in animals given vitamin C or E together with chromium remained at par with control rats, without any obvious dose dependent effect among the different doses of vitamin C or E (Fig. 3.7).
3.4.2.6 Plasma and TIF Steroid Hormones

Plasma testosterone in rats supplemented with low doses of vitamin C (125 mg/L, and 250 mg/L) and vitamin E (12.5 and 25 mg) along with chromium exhibited a dose-dependent increase when compared with chromium alone treated rats but the level was significantly less than untreated controls. Normal plasma testosterone titre was maintained in animals treated with 500 mg/L vitamin C or 50 mg vitamin E along with chromium (Fig. 3.8).

**FIG. 3.8: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES IN PLASMA TESTOSTERONE OF ADULT MALE RATS**

Each bar represents the mean and the vertical line above denotes the SEM. n = 8. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
The concentration of TIF testosterone was elevated in animals given 125 or 250 mg/L vitamin C with chromium, when compared to chromium alone treated animals. Nevertheless, the level was less than untreated control rats. The concentration of TIF testosterone remained less than control in rats given 25 mg vitamin E with chromium, though the level was elevated when compared to chromium alone treated animals. Normal TIF testosterone titre was noticed in animals given 25 or 50 mg vitamin E with chromium (Fig. 3.9).

**FIG. 3.9: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON TIF TESTOSTERONE IN ADULT MALE RATS**

![Bar chart showing the effect of simultaneous vitamin C or E treatment on chromium-induced changes on TIF testosterone in adult male rats.](image)

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
Plasma DHT showed a trend similar to that of plasma testosterone. Chromium treated rats given simultaneously 125 or 250 mg/L vitamin C, and those animals treated with 12.5 or 25 mg vitamin E had DHT levels higher than chromium alone treated animals but was lesser than untreated control rats. While the animals treated with 50 mg vitamin E had plasma DHT levels comparable to control rats, animals treated with 500 mg/L vitamin C had elevated plasma DHT (Fig. 3.10).

**FIG. 3.10: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE TESTICULAR WEIGHT OF ADULT MALE RATS**

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
The concentration of TIF DHT was elevated in rats administered 125 or 250 mg/L vitamin C and 12.5 or 25 mg vitamin E together with chromium, as compared to chromium alone treated rats. Yet the level remained low, when compared to untreated control rats. Normal TIF DHT level was observed in animals treated with 500 mg/L vitamin C or 50 mg vitamin E (Fig. 3.11).
Plasma estradiol concentration remained at control level in animals given different doses of either vitamin C or E along with chromium (Fig. 3.12).
The concentration of TIF estradiol was also maintained at normal level in all groups of rats treated with either vitamin C or E along with chromium (Fig. 3.13).

**FIG. 3.13: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON TIF ESTRADIOL OF ADULT MALE RATS**

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
3.4.2.7 Plasma and Testicular Interstitial Fluid Androgen Binding Protein (ABP)

Plasma ABP concentration exhibited a dose dependent increase in rats supplemented 125 and 150 mg/L vitamin C along with chromium, when compared to chromium alone treated rats. However, the level remained low when compared to untreated control rats. Normality in plasma ABP concentration could be noticed in 500 mg/L vitamin C treated animals. In the case of vitamin E treatment, animals treated with 12.5 and 25 mg exhibited an elevation in plasma ABP when compared to chromium alone treated rats, without any dose dependent variation. Nevertheless, the concentration remained less than control animals. Normal concentration of plasma ABP was noticed, in animals given 50 mg vitamin E along with chromium (Fig. 3.14).

![FIG. 3.14: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON PLASMA ABP OF ADULT MALE RATS](image)

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
ABP concentration in the TIF of animals given 125 mg/L vitamin C did not show any change as compared to chromium alone treated animals. Rats given 250 mg/L vitamin C along with chromium showed a perceptible increase in TIF ABP but it remained significantly low, when compared with untreated controls. Normal TIF ABP was sustained in animals given 500 mg/L vitamin C together with chromium. In the case vitamin E treatment, the level of TIF ABP was elevated in animals given 12.5 mg vitamin E along with chromium, when compared to chromium alone treated rats but remained low when compared to untreated control rats. 25 and 50 mg vitamin E co-administered with chromium maintained normal level of TIF ABP (Fig. 3.15).

![Graph](image-url)

**Fig. 3.15: Effect of Simultaneous Vitamin C or E Treatment on Chromium-Induced Changes on TIF ABP in Adult Male Rats**

- Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
3.4.2.8 *Seminiferous Tubular and Tubular Lumen Diameter*

Seminiferous tubular diameter remained at normal level in animals given either vitamin C or vitamin E together with chromium, except in animals given 12.5 mg vitamin E, which had tubular diameter higher than chromium alone treated rats but lesser than untreated control (Fig.3.16).

**FIG. 3.16: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON TESTICULAR SEMINIFEROUS TUBULAR DIAMETER IN ADULT MALE RATS**

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
The diameter of seminiferous tubular lumen was increased in animals given low doses of either vitamin C (25 or 50 mg/L) or vitamin E (12.5 or 25 mg) when compared to chromium alone treated rats. However, it remained less than that observed in untreated control rats. While normal diameter of seminiferous tubular lumen was observed in rats given 500 mg/L vitamin C along with chromium, it was perceptibly increased in rats given 50 mg vitamin E, when compared with untreated control rats (Fig. 3.17).
Co-administration of vitamin C or E together with chromium prevented the slump in Leydig cell number due to chromium treatment. The number of Leydig cell was comparable to untreated control in rats belonging to all groups except that of 125 mg/mL vitamin C treated rats, which showed a slight but significant decrease from that of untreated control rats (Fig. 3.18).

![Fig. 3.18: Effect of simultaneous vitamin C or E treatment on chromium-induced changes on the Leydig cell number in adult male rats](image)

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
Co-administration of vitamin C or vitamin E with chromium maintained Sertoli cell number in a significantly higher level than chromium alone treated rats. Nevertheless, the number of Sertoli cells in animals given low doses of vitamin C (125 or 250 mg/L) or vitamin E (12.5 or 25 mg) remained less than the untreated control level and normality was evident in rats given either 500 mg/L vitamin C or 50 mg vitamin E (Fig. 3.19).

FIG. 3.19: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE SERTOLI CELL NUMBER IN ADULT MALE RATS

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
The number of myoid cells increased in animals given 125 mg/L vitamin C with 400 ppm chromium, as compared to chromium alone treated rats, yet normality in myoid cell number was not maintained in these animals. Normal myoid cell number was maintained in animals given either 250 or 500 mg/L vitamin C together with chromium. In the case of vitamin E treatment, normal myoid cell number was sustained in animals given 50 mg vitamin E, while 12.5 or 25 mg vitamin E treated rats failed to maintain normality, though they exhibited a dose dependent increase as compared to chromium alone treated rats (Fig. 3.20).

**FIG. 3.20: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE MYOID CELL NUMBER IN ADULT MALE RATS**

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
The spermatogonial and spermatocytes numbers were maintained at normal levels in rats given higher doses of either vitamin C (250 or 500 mg/mL) or vitamin E (25 or 50 mg) along with chromium. Animals given the lowest dose of either vitamin C (125 mg/L) or vitamin E (12.5 mg) along with chromium showed an increase in these germ cells but the number was significantly less than that of untreated control rats (Fig. 3.21).

![Figure 3.21: Effect of simultaneous vitamin C or E treatment on chromium-induced changes on the spermatogonial and spermatocytes number in the testis of adult male rats](image)

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
The round spermatid number in rats co-administered vitamins with chromium showed a significant increase when compared with chromium alone treated rats. Normal level was maintained in most of the groups except in animals given the lowest doses of vitamins (125 mg/L vitamin C or 12.5 mg vitamin E), which showed round spermatid number higher than chromium treated rats but lesser than untreated rats (Fig. 3.22).

**Fig. 3.22: Effect of simultaneous vitamin C or E treatment on chromium-induced changes on the round spermatid number in adult male rats**

- **Control**
- 400 ppm Cr
- 400 ppm Cr + Vitamin C 125 mg/L
- 400 ppm Cr + Vitamin C 500 mg/L
- 400 ppm Cr + Vitamin E 25 mg/kg b.wt
- 400 ppm Cr + Vitamin E 12.5 mg/kg b.wt
- 400 ppm Cr + Vitamin E 50 mg/kg b.wt

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
The number of elongated spermatids was maintained at par with control animals when the rats were given simultaneous supplementation of vitamin C or E with chromium (Fig. 3.23).
3.4.2.10 Lipid Peroxidation (LPO)

Testicular LPO in rats given simultaneous vitamin E and C along with chromium decreased in a dose dependent manner, when compared with rats given chromium alone. While animals given 125 or 250 mg/L vitamin C and 12.5 mg vitamin E still had an elevated level of testicular LPO when compared with untreated controls, normality was maintained in animals given 500 mg/L vitamin C and 25 or 50 mg vitamin E along with chromium (Fig. 3.24).

FIG. 3.24: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON TESTICULAR LIPID PEROXIDATION IN ADULT MALE RATS

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
3.4.2.11 Reactive Oxygen Species

Chromium induced increase in the production of hydrogen peroxide was suppressed in a dose dependent manner in animals given simultaneous supplementation of vitamin C or E together with chromium. However, normality was maintained in animals given the highest dose of vitamin C (500 mg/L) or vitamin E (50 mg) (Fig. 3.25) with along chromium.

FIG. 3.25: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON HYDROGEN PEROXIDE PRODUCTION IN THE TESTIS OF ADULT MALE RATS

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
As in the case of hydrogen peroxide, testicular hydroxyl radical production also was suppressed in a dose dependent manner in animals given vitamin C and E along with chromium when compared with rats treated with chromium alone. Normality of *OH was maintained in rats given 250 or 500 mg/L vitamin C or 50 mg vitamin E (Fig. 3.26).

**Fig. 3.26: Effect of simultaneous vitamin C or E treatment on chromium-induced changes on hydroxyl radical production in the testis of adult male rats**

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
3.4.2.12 Antioxidant Enzymes

3.4.2.12.1 Superoxide Dismutase (SOD)

The activity of testicular SOD in chromium treated rats was elevated in a dose dependent manner when vitamin C or E was co-administered, compared with chromium alone treated rats. However, normal SOD activity was maintained only in rats treated with 500 mg/L vitamin C or 50 mg vitamin E along with chromium (Fig. 3.27).

FIG. 3.27: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE ACTIVITY OF TESTICULAR SUPEROXIDE DISMUTASE IN ADULT MALE RATS

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p <
3.4.2.12.2 **Catalase (CAT)**

The activity of testicular catalase, which was elevated in chromium alone treated rats was significantly inhibited by simultaneous administration of chromium and vitamin C or vitamin E. Nevertheless, normal catalase activity was maintained only in rats given 500 mg/L vitamin C or 50 mg vitamin E (Fig. 3.28).

![FIG. 3.28: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE ACTIVITY OF TESTICULAR CATALASE IN ADULT MALE RATS](image)

Each bar represents the mean and the vertical line above denotes the SEM, n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
3.4.2.12.3 *Glutathione Peroxidase (GPx)*

The activity of testicular GPx was maintained at control level in animals given higher doses of vitamin C or E together with chromium. In the case of animals supplemented with the lowest dose of vitamin C (125 mg/L) or vitamin E (12.5 mg), the level of testicular GPx remained higher than rats treated with chromium alone but there was a perceptible decrease from the level of untreated control rats (Fig. 3.29).

**FIG. 3.29: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE ACTIVITY OF TESTICULAR GLUTATHIONE PEROXIDASE IN ADULT MALE RATS**

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars same alphas denote statistically insignificant difference between the respective means, while those with different alphas indicate statistically significant difference between those means at p < 0.05 level.
3.4.2.12.4  *Glutathione Reductase* (GR)

The activity of testicular GR increased in an appreciable dose-dependent manner in rats given simultaneous supplementation of vitamin C or E along with chromium, when compared with chromium alone treated animals. However, normal activity of testicular GR was sustained only in animals given either 500 mg/L vitamin C or 50 mg vitamin E along with chromium (Fig. 3.30).

![FIG. 3.30: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE ACTIVITY OF TESTICULAR GLUTATHIONE REDUCTASE IN ADULT MALE RATS](image)

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
Co-administration of vitamin C or E with chromium prevented chromium induced diminution of testicular $\gamma$-GT activity. The activity of testicular $\gamma$-GT increased and exhibited a dose dependent increase. Despite an increase in the enzyme activity in rats supplemented with 125 or 250 mg/L vitamin C or 12.5 mg vitamin E along with chromium, failed to reach normality of the same. While normal activity of testicular $\gamma$-GT was maintained in animals, which received 25 mg vitamin E, the same was elevated above the untreated control values in animals given either 500 mg vitamin C or 50 mg vitamin E with chromium (Fig. 3.31).

![Graph showing the effect of simultaneous vitamin C or E treatment on chromium-induced changes on the activity of testicular $\gamma$-GLUTAMYL TRANSPEPTIDASE in adult male rats. Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.]
3.4.12.4.6 Glucose-6-phosphate dehydrogenase (G6PDH)

Simultaneous treatment of vitamin C with chromium prevented the inhibitory effect of the latter on testicular G6PDH. While the maximum dose of vitamin C alone maintained normality of G6PDH activity, all the three doses of vitamin E maintained the activity of the enzyme at par with untreated control (Fig. 3.32).

**FIG. 3.32: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE ACTIVITY OF TESTICULAR GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN ADULT MALE RATS**

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
3.4.2.12.7 *Glutathione-S-Transferase (GST)*

As in the case of testicular G6PDH, supplementation of vitamin C or E along with chromium prevented the inhibitory effect of chromium on testicular GST except in rats supplemented with 250 mg/mL vitamin C or 12.5 mg vitamin E. Nevertheless, the testicular GST activity in these rats was higher than that observed in chromium alone treated rats (Fig. 3.33).

**FIG. 3.33: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE ACTIVITY OF TESTICULAR GLUTATHIONE-S-TRANSFERASE IN ADULT MALE RATS**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Activity (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28</td>
</tr>
<tr>
<td>400 ppm Cr</td>
<td>21</td>
</tr>
<tr>
<td>400 ppm Cr + Vitamin C 125 mg/L</td>
<td>14</td>
</tr>
<tr>
<td>400 ppm Cr + Vitamin C 500 mg/L</td>
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<tr>
<td>400 ppm Cr + Vitamin E 25 mg/kg b.wt</td>
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<tr>
<td>400 ppm Cr + Vitamin E 50 mg/kg b.wt</td>
<td>14</td>
</tr>
</tbody>
</table>

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
3.4.2.13 Non-enzymatic Antioxidants

3.4.2.13.1 Reduced Glutathione (GSH)

The testicular GSH content was maintained at normal level in all animals given either vitamin C or vitamin E along with chromium, irrespective of the dose of vitamins given (Fig. 3.34).

![Graph showing effect of simultaneous vitamin C or E treatment on chromium-induced changes on the concentration of reduced glutathione in the testis of adult male rats.]

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
The concentration of vitamin C was increased in a dose dependent manner in animals given exogenous vitamin C along with chromium, as compared to controls. Animals given vitamin E together with chromium also exhibited an elevation in testicular vitamin C concentration, compared to control (Fig. 3.35).

FIG. 3.35: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE CONCENTRATION OF VITAMIN C IN THE TESTIS OF ADULT MALE RATS

Each bar represents the mean and the vertical line above denotes the SEM. n = 8. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
3.4.2.13.3 Vitamin E

The testicular concentration of vitamin E was maintained at normal level in animals given different doses of vitamin C along with chromium. In the case of vitamin E treatment, the lowest dose (12.5 mg) maintained normal concentration of vitamin E in the testis, while the same was elevated in animals treated 25 or 50 mg vitamin E, when compared with untreated controls (Fig. 3.36).

**FIG. 3.36:** EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE CONCENTRATION OF VITAMIN E IN THE TESTIS OF ADULT MALE RATS

Each bar represents the mean and the vertical line above denotes the SEM, n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
3.4.2.13.4 Vitamin A

The concentration of vitamin A in the testis was maintained at control level in animals given different doses of either vitamin C or vitamin E along with chromium (Fig. 3.37).

FIG. 3.37: EFFECT OF SIMULTANEOUS VITAMIN C OR E TREATMENT ON CHROMIUM-INDUCED CHANGES ON THE CONCENTRATION OF VITAMIN A IN THE TESTIS OF ADULT MALE RATS

Each bar represents the mean and the vertical line above denotes the SEM. n = 6. Bars with same alphabets denote statistically insignificant difference between the respective means, while those with different alphabets indicate statistically significant difference between those means at p < 0.05 level.
3.4.2.14 Serum and Testicular Chromium Concentration

The serum and testicular concentration of chromium was quantified only in animals given the highest dose of vitamin C or E simultaneously with chromium. In both the cases, the concentration remained higher compared to control, though the same was significantly less than that of chromium alone treated rats (Fig. 3.38).
Discussion

The data from vitamin co-administration studies reveal the ameliorative efficacies of vitamin E and C in preventing the reproductive toxicity of chromium. Though the protective effect of vitamin C and E against chromium-induced DNA damages in tissues and isolated DNA, are well known (see Sugiyama, 1992; Qi et al., 2000; Carlisle et al., 2000), this is the first report on the effect of these vitamins against chromium-induced testicular derangements. The data on the effect of different doses of these two vitamins reveal that though normalization of all parameters studied could not be achieved with the lowest dose of vitamins (125 mg/L vitamin C or 12.5 mg vitamin E) tested (except in few cases), an improvement in the reproductive and oxidative status is quite obvious. While normalization of 50% of the parameters could be observed with the higher dose of vitamins (250 mg/L vitamin C or 25 mg vitamin E) employed, a complete normal picture at par with control could be visualized with the highest dose of vitamins C (500 mg/L) or vitamin E (50 mg). Efficacy of dietary supplements is high when the supplement provides a nutrient that is undersupplied to the cell and optimal function is achieved when the required nutrient reaches the specific concentration within the cell (see Zeisel, 2000). This property of the supplements may be the reason for the dose-dependent normalization of chromium-induced reproductive dysfunctions by different doses of vitamin C and E.

It is understood well that supplementation of vitamin C or E could improve sperm motility and viability in vitro. Data from the present study underscores the importance of vitamin C and E in quenching the toxic effect of chromium in vivo. Vitamin C imparts this protective effect mainly by virtue of its free radical quenching ability, whereas
vitamin E brings about its protective effect through its ability to prevent membrane lipid peroxidation. Hsu et al. (1998) recently reported the protective effect of simultaneous vitamin C and/or E treatment on lead-induced suppression in rat epididymal sperm motility and sperm-oocyte interaction due to suppression of ROS generation. The data on LPO and ROS generation in the testicular tissue of rats tested in the present study suggest the operation of a similar mechanism of vitamin C and E action in rats supplemented with exogenous vitamin C and E with chromium also. In addition, co-administration of sufficient vitamin concentration also maintained normal level of testicular antioxidant enzymes. Ascorbic acid supplementation to alloxan-diabetic rats blunted diabetes-induced oxidative stress associated with increased LPO and decreased SOD, CAT and GR, in the testicular tissue (El-Missiry, 1999). A protective effect of dietary ascorbic acid against cadmium-induced hypogonadism in human males has also been reported (Fox, 1975). Normalization of testicular structure and function along with normal oxidative balance in vitamin C/E co-administered rats attests the point that oxygen toxicity is one of the mechanisms underlying the reproductive toxicity of chromium.

The observed increase in testicular vitamin C and E in rats co-administered with respective vitamins reveal enhanced uptake of these vitamins by the testis. Increased testicular vitamin E concentration noticed in rats supplemented this vitamin is consistent with previous reports wherein vitamin E supplementation increased its own concentration in testis, liver and semen (Moilanen et al., 1993; Anderson Sr et al., 1997; Surai et al., 1997, 1998; Aruldhas et al., 2000). The observed increase in some parameters like plasma FSH, DHT, seminiferous tubular diameter and myoid cell number above the control level in rats supplemented with 500 mg/L vitamin C or vitamin E (50 mg/kg b.wt)
also indicate that these parameters are much more sensitive than other parameters studied to vitamin C and E. Probably, the stimulatory effect of these vitamins becomes obvious when chromium-induced oxidative stress has been countered. A stimulatory effect of vitamin E supplementation on phospholipid hydroperoxide glutathione peroxidase activity in reproductive tissues of rats was reported recently (Lei et al., 1997). Vitamin E supplementation has been shown not only to counter but also to improve reproductive traits in bulls treated gossypol (Velasquez-Pereira et al., 1998). Vitamin C directly interacts with chromium (VI) during its reduction to chromium (III) and both ascorbic acid and vitamin E are well-established free radical scavengers. Since these vitamins are administered together with chromium, the protective effects of these vitamins appear to be preventive rather than reversing chromium-induced reproductive toxicity. Vitamin E deficiency or supplementation of vitamin E to vitamin E-deficient rats, did not bring about any obvious change in serum LH or FSH titres in normal rats (Akazawa et al., 1986; Richards et al., 1999). Therefore, the stimulatory effect of this vitamin on LH/FSH titre observed in chromium treated rats of the present study may be the result of the protective effects of vitamin E on chromium-induced impairment in the synthesis/secretion of these gonadotropin rather than due to its direct effect.

The time of administration of vitamin C appears to have a crucial role in its effect against chromium toxicity. Administration of ascorbic acid before 30 minutes of sodium dichromate to within 2 hours after sodium dichromate treatment prevented chromate induced renal toxicity in rats, while administration of ascorbic acid after 2 hours of sodium dichromate treatment failed to prevent chromium-induced renal damage (see Bradberry and Vale, 1999). The data from the present study categorically reveals the
protective effect of simultaneous vitamin C administration against chromium-induced reproductive toxicity.

A differential mechanism of action of vitamin C and E in preventing thallium-induced renal damage has been reported. While vitamin E decreased thallium concentration in renal tissue, vitamin C prevented thallium-induced renal damage without altering the tissue concentration (Appenroth and Winnefeld, 1998). Data on testicular tissue concentration of chromium in vitamin co-administered rats reveal that both vitamin C and E decreases the accumulation of chromium in the testis. Probably these vitamins inhibit chromium uptake by the testis or enhance the metabolic clearance rate of chromium. Further studies on these aspects of vitamin C/E on chromium turnover may throw more light on the issue.

Thus, the data from rats given simultaneous vitamin C and E along with chromium supports the hypotheses that the chromium-induced reproductive toxicity is reversible and can be prevented by simultaneous antioxidant vitamins treatment.

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