The protective effect of *Panax ginseng* against chromium picolonate induced testicular changes

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Chromium occurs in the environment mainly in two states, tri (Cr(III)) and hexavalent (Cr(VI)). Chromium(III) (e.g. chromium picolonate) is essential for proper insulin function and is required for normal protein, fat and carbohydrate metabolism, and is acknowledged as a dietary supplement. In this study, we investigated the possible antioxidative properties of *Panax ginseng* against chromium picolonate (CrPic)-induced male reproductive toxicity at low and high doses. Ninety Sprague-Dawley rats were divided into six groups included the control groups, *P. ginseng* group (200 mg/kg body weight), the groups treated with Cr-picolinate (0.8 and 1.5 mg/100 g body weight) alone or in combination with *P. ginseng* for 90 days. Testicular tissue and blood samples were taken for determination of epididymal sperm analysis, daily sperm production, testicular chromium, mitochondrial lipid peroxidation (LPO), hydrogen peroxide generation, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), reduced glutathione (GSH) and ascorbic acid (Vitamin C), DNA fragmentation and plasma male sex hormones. The results indicated that the low and high doses of CrPic induced a significant alteration in all measured parameters. *P. ginseng* supplementation succeeded to restore these changes to great extent especially in low dose CrPic. It could be concluded that consumption of CrPic for a long time induced several hazards to testes. Supplementation with extra amounts of *P. ginseng* may be useful to restrain the CrPic induced testicular changes.

**Key words:** *Panax ginseng*, chromium picolonate, testicular changes.

**INTRODUCTION**

The decrease in human semen quality over the past several years is considered to be the result of deteriorating environmental conditions due to increased pollution (Jurewicz et al., 2009). However, the mechanism of reproductive toxicity of chromium is either poorly understood or unknown (Ong et al., 2002). Chromium picolinate (CrPic; chromium (III) tris (picolinate)) is primarily used by the general public for reduction of body weight and by athletes for improvement of body composition (Mirasol, 2000). Chronic CrPic supplementation results in distribution...
of chromium into a variety of tissues including the epididymal fat and testes (Hepburn and Vincent, 2003). A number of studies investigated the potential toxicity effects of CrPic, but the results are controversial. Golubnitschaja and Yeghiazaryan (2012) reported that, there have been isolated reports of deleterious effects of CrPic supplementation in rats, including decreased antioxidative enzyme activity, lipid peroxidation, and oxidative DNA damage.

*Panax ginseng*, a traditional multipurpose herb in Asia, has become the world’s most popular herbal supplements in recent years. *Ginseng* has a variety of beneficial biological processes that include anti-carcinogenic, anti-diabetic and anti-inflammatory effects, as well as cardiovascular- and neuro-protection (Jung et al., 2005). These properties of the *ginseng* are thought to provide many beneficial effects against organ damages (Huang et al., 2005). It has been used to important roles in maintaining oxidative status, by possessing either direct or indirect antioxidant functions (Chang et al., 2007).

The aim of the present study is to evaluate the possible antioxidative properties of *P. ginseng* against CrPic-induced male reproductive toxicity at low and high doses.

### MATERIALS AND METHODS

#### Chemicals

Chromium-picolinate was obtained from Amoun Pharmaceutical Co. (El-Obour City, Cairo, Egypt). *P. ginseng* (Korean *ginseng*) was purchased from Pharco Pharmaceuticals, Alexandria, Egypt and was present in the form of syrup containing the dried roots of the *P. ginseng*; each 1 ml contains 10 mg pyrogallol, sodium azide, glutathione reductase, GSH, 5,5-dithiobis (2-nitrobenzoic acid), and 2,4-dinitrophenylhydrazine were purchased from Sigma-Aldrich Chemical Company, St. Louis, MO, USA. All other chemicals are of analytical grade.

#### Animals

The study was carried out on 90 young male albino rats (Sprague-Dawley) (8 to 12 weeks old) weighing 106 to 129 g and obtained from the Animals House Laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt. Rats were maintained on the standard laboratory chow diet (El-Nasr Chemical Company, Cairo, Egypt) and water *ad libitum*. After an acclimation period of 1 week, the animals were distributed into six groups (15 rats/group) and housed individually in stainless steel cages in a temperature-controlled (23±1°C) and artificially illuminated (12 h dark/light cycle) room free from any source of chemical contamination. This study was approved by the ethical committee of Tanta, Faculty of Pharmacy, Egypt.

#### Experimental design

Animals within different treatment groups (15 rats each) were treated for 90 days as follows: (I) untreated control group fed on the standard diet; (II) untreated control group fed on the standard diet plus oral *P. ginseng* (200 mg/kg body weight) (Ramesh et al., 2012); (III) treated orally with Cr-picolinate (0.8 mg/100 g body weight; low-dose group) (Mahmoud et al., 2006); (IV) treated orally with Cr-picolinate (1.5 mg/100 g body weight; high-dose group) (Mahmoud et al., 2006); (V) treated orally with Cr-picolinate at the low dose plus *P. ginseng* (200 mg/kg body weight) (Ramesh et al., 2012); (VI) treated orally with Cr-picolinate at the high dose plus *P. ginseng*.

#### Necropsy

The animals were fasted overnight, weighed, and sacrificed 24 h after the last dose by intraperitoneal injection of 60 mg/kg sodium pentobarbital. Testes and epididymides were removed and cleaned from adhering fat and connective tissues. The weights of these tissues were recorded in grams as well as g/100 g body weight. The cauda epididymides from each animal were used for sperm count and motility. The body weight of the animals was recorded on the day of initiation of the treatment and also on the day of sacrifice.

#### Epididymal sperm analysis

Epididymal sperm count and evaluation of the motility of epididymal sperm were done by the method of Belsey et al. (1980). The epididymal fluid was obtained by mincing the cauda epididymis in physiological saline (0.9% NaCl in distilled water) at 37°C. A 10 ml of diluted epididymal fluid was placed in Neubauer haemocytometer and total, motile, and non-motile sperm were counted. The number of motile and non-motile sperm was determined microscopically within 5 min following their isolation from cauda epididymis at 37°C. Non-motile sperm numbers were determined, followed by counting of total sperm. The ratio of live and dead spermatozoa was determined using 1% trypan blue reagent (Talbot and Chacon, 1981). Briefly, one drop of diluted epididymal sperm suspension was mixed with one drop of 1% trypan blue solution on a microscope slide and covered with a cover slip. After incubation at 37°C for 15 min, the slides were observed under a microscope. Unstained spermatozoa were taken as viable and stained were counted as dead. Sperm viability was expressed as percentage of unstained sperm of the total sperm counted. The hypo-osmotic swelling (HOS) test was performed by combining 0.1 ml of sperm with 1.0 ml of a 150 mOsmol/L NaCl as a hypo-osmotic solution, following the method described by Jeyendran et al. (1992). After incubation of the mixture for 30 min at 37°C, sperm were observed for coiled tails under phase-contrast microscope (Olympus BX41; Olympus Optical Co. Ltd, Japan). The data were expressed as millions/ml for sperm count and for other sperm parameters, the data were expressed as percentage of total sperm.

#### Daily sperm production

Daily sperm production was determined in the testis of adult rats by the method of Blazak et al. (1993). Briefly, the testes were decapsulated and homogenized in 50 ml of ice cold 0.9% NaCl solution containing 0.01% Triton X-100 using a glass Teflon homogenizer. The homogenate was allowed to settle for 1 min and then was gently mixed and a 10 ml aliquot was collected into a glass vial and stored on ice. After thorough mixing of each sample, the number of sperm heads was counted in four chambers of an improved Neubauer-type haemocytometer. The number of sperm produced per gram of testicular tissue per day was calculated.

#### Determination of testicular chromium

0.2 g of testicular tissues was weighed for each group, placed in new 20 x 125-mm borosilicate glass culture tubes with polypropylene screw caps, and digested in a low-trace-metal
reagent grade HNO\textsubscript{3}/HClO\textsubscript{4} mixture until complete dissolution and destruction of organic matter occurred. Samples were then diluted up to 20 ml final volume with deionized water (final HClO\textsubscript{4} content was 10% [v/v]) and were analyzed in triplicate for total Cr by inductively coupled plasma–mass spectrometry (ICP-MS) (Sutherland et al., 2000).

Isolation of testicular mitochondrial fraction

Mitochondrial fraction was isolated from the other testis by the differential centrifugation method as previously described (Latchoumycandane et al., 2002). Briefly, a 20% (w/v) homogenate was prepared in ice-cold 0.25 M sucrose solution. The homogenate was centrifuged at 1000 g for 10 min at 4°C to obtain the nuclear pellet. Mitochondrial pellet was obtained by centrifuging the post-nuclear supernatant at 10,000 g for 10 min at 4°C. The fraction was washed three times with ice cold 1.15% potassium chloride solution and finally suspended in 0.25 M sucrose solution (10 mg protein/ml). The mitochondrial fraction was used for the subsequent biochemical studies. Protein concentrations were determined using a BCA kit (Pierce, Rockford, USA) that employed bovine serum albumin as a standard.

Assay of oxidative status

Lipid peroxidation (LPO)

Malondialdehyde (MDA), formed as an end product of the peroxidation of lipids, served as an index of the intensity of oxidative stress. MDA reacts with thiobarbituric acid to generate a coloured product that can be measured optically at 532 nm. A break down product of LPO, thiobarbituric acid reactive substance was measured by the method of Buege and Aust (1976). Briefly, the stock solution contained equal volumes of trichloroacetic acid 15% (w/v) in 0.25 N HCl and 2-thiobarbituric acid 0.37% (w/v) in 0.25 N HCl. One volume of the test sample (mitochondrial fraction) and two volumes of stock reagent were mixed in a screw-capped centrifuge tube, vortexed and heated for 15 min on a boiling water bath. After cooling on ice, the precipitate was removed by centrifugation at 1000 g for 15 min and absorbance of the supernatant was measured at 532 nm against blank containing all the reagents except test sample. The value is expressed in µmol of malondialdehyde equivalent formed/min/mg protein.

Protein carbonyl contents

Protein carbonyl content was determined by the most common and reliable method (Levine et al., 1990) based on the reaction of carbonyl groups with 2,4-dinitrophenylhydrazine to form a 2,4-dinitrophenylhydrazone. The levels of protein carbonyls were expressed as nmol/mg protein.

Hydrogen peroxide generation assay

Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) generation was assayed according to Picc and Keisari (1981). Briefly, incubation mixture contained 1.641 ml of phosphate buffer (50 mM, pH 7.6), 54 µl of horse radish peroxidase (8.5 units/ml), 30 µl of phenol red (0.28 mM), 165 µl of dextrose (5.5 mM) and 100 µl of mitochondrial fraction was done at 35°C for 30 min. The reaction was terminated by the addition of 60 µl of 10 N NaOH and the absorbance was read at 610 nm against blank on a spectrophotometer. The quantity of H\textsubscript{2}O\textsubscript{2} produced was expressed as nmol of H\textsubscript{2}O\textsubscript{2} generated/min/mg protein at 35°C.

Enzymatic antioxidant assay

Superoxide dismutase (SOD)

SOD was assayed by the method of Marklund and Marklund (1974). Briefly, the assay mixture contained 2.4 ml of 50 mM Tris–HCl buffer containing 1 mM EDTA (pH 7.6), 300 µl of 0.2 mM pyrogallol and 300 µl enzyme source. The decrease in absorbance was measured immediately at 420 nm against blank at 10 s intervals for 3 min on a spectrophotometer. The activity of enzyme was expressed in nmol pyrogallol oxidized/min/mg protein.

Catalase (CAT)

CAT was assayed as previously mentioned (Claiborne, 1985). Briefly, the assay mixture contained 2.40 ml of phosphate buffer (50 mM, pH 7.0), 10 µl of 19 mM hydrogen peroxide and 50 µl enzyme source. The decrease in absorbance was measured immediately at 240 nm against blank at 10 s intervals for 3 min on a spectrophotometer. The activity of enzyme was expressed in µmol of hydrogen peroxide consumed/min/mg protein.

Glutathione peroxidase (GPx)

GPx was assayed by the method of Paglia and Valentine (1967). Briefly, the assay mixture contained 1.59 ml of phosphate buffer (100 mM, pH 7.6), 100 µl of 10 mM EDTA, 100 µl of sodium azide, 50 µl of glutathione reductase, 100 µl of reduced glutathione, 100 µl of 200 mM NADPH, 10 µl of hydrogen peroxide and 10 µl enzyme source. The oxidation of NADPH was measured immediately at 340 nm against blank at 10 s intervals for 3 min on a spectrophotometer. The activity of enzyme was expressed in nmol of NADPH oxidized/min/mg protein.

Glutathione reductase (GR)

The activity of GR was assayed by the method of Carlberg and Mannervik (1975). Briefly, the assay mixture contained 1.75 ml of phosphate buffer (100 mM, pH 7.6), 100 µl of 200 mM NADPH, 100 µl of 10 mM EDTA, 50 µl of 20 mM oxidized glutathione and 50 µl enzyme source. The oxidation of NADPH was measured immediately at 340 nm against blank at 10 s intervals for 3 min on a spectrophotometer. The activity of enzyme was expressed in nmol of NADPH oxidized/min/mg protein.

Non-enzymatic antioxidant assay

Reduced glutathione (GSH)

GSH was determined by the method of Ellman (1959). Briefly, mitochondrial GSH content was estimated through suspending 0.1 ml mitochondrial homogenate with 1.7 ml 0.1 M potassium phosphate buffer (pH 8) followed by the addition of 0.1 ml Ellman’s reagent (5,5-dithiobis(2-nitrobenzoic acid). After 5 min, the absorbance was measured spectrophotometrically at 412 nm against a blank. The GSH values are expressed as nanomoles per milligram of mitochondrial protein.

Ascorbic acid (Vitamin C)

Vitamin C was assayed by the method of Omaye et al. (1979). Vitamin C was oxidized by copper to form dehydroascorbic acid which reacts with 2,4-dinitrophenylhydrazine to form the derivative bis-2,4-dinitrophenylhydrazone. This compound in strong sulphuric
acid undergoes a rearrangement to form a product which was measured at 520 nm. A mildly reducing medium with thiourea was used to prevent non-asorbin chromogen interference. The level of vitamin C was expressed as μg/mg protein.

DNA fragmentation assays for apoptosis protocol

A distinctive feature of apoptosis at the biochemical level is DNA fragmentation. This method was used as a semi-quantitative method for measuring apoptosis. Apoptotic changes in testes were evaluated calorimetrically by DNA fragmentation and by agarose gel electrophoresis according to the procedure of Perandones et al. (1993). Testis samples were homogenized in 700 μl hypotonic lysis buffer and centrifuged for 15 min at 11,000 rpm. The supernatants (SN) containing small DNA fragments were separated; one-half the volume was used for gel electrophoresis and the other half, together with the pellet containing large pieces of DNA were used for quantification of fragmented DNA by the diphenyl amine (DPA) assay. The samples were treated with equal volumes of absolute isopropyl alcohol and 0.5 M NaCl to precipitate DNA. The samples were then kept at -20°C overnight and centrifuged at 11,000 rpm for 15 min. The pellets were then washed with 500 μl of 70% ethanol and allowed to dry at room temperature. Extracted DNA was reconstituted in 12 μl of Tris-EDTA buffer and 3 μl loading buffer. The samples were incubated at 37°C for 20 min, then electrophoresed on 1% agarose gels containing 0.71 μg/ml ethidium bromide. At the end of the runs, gels were examined using UV trans-illumination. The DPA assay reaction was modified by Perandones et al. (1993) from Burton (1956). Briefly, perchloric acid (0.5 M) was added to the pellets containing native DNA (reconstituted in 400 μl of the hypotonic lysis buffer) and to the supernatants containing fragmented DNA followed by the addition of 2 volumes of DPA solution. The samples were kept at 4°C for 48 h. The colorimetric reaction was then measured spectrophotometrically at 575 nm. The percentage of DNA fragmentation was calculated in testis.

Determination of male sex hormones

Levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the plasma were measured using automated immunofluorescent assay-based commercial kits and a Brahms Kryptor immunoassay analyzer (Brahms LH Kryptor 820.050 and Brahms FSH Kryptor 818.050, respectively). Testosterone levels were measured using a chemiluminescence immunoassay-based commercial kit (Access testosterone 33560) and an access immunoassay analyzer (Beckman Coulter).

Protein estimation

Proteins were measured by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as the standard protein.

Statistical analysis

Data are expressed as mean±standard deviation (SD) for fifteen animals per dose and analyzed statistically using one-way analysis of variance (ANOVA), followed by Tukey’s test. Probability values less than 0.05 were considered to be statistically significant against control. The SPSS Version 16.0 (SPSS, Inc., Chicago) package was used for all statistical analyses.

RESULTS

The body and organ weights after CrPic administration at 0.8 and 1.5 mg/100 g body weight (low and high dose, respectively) concentrations were displayed as shown in Table 1. Body weight gain showed significant decrease in low and high dose CrPic administered animals and also in those administered high dose CrPic plus *P. ginseng* (18, 30.9 and 15.3%, respectively) compared to control groups. It showed no significant change in body weight gain in animals exposed to low dose CrPic plus *P. ginseng* as compared to the control groups. Body weight gain showed significant increase in low and high dose CrPic plus *P. ginseng* groups as compared to their corresponding untreated groups. Testes (absolute and relative weights) and epididymis (absolute and relative weights) showed significant decrease in high dose CrPic administered animals and also in those administered high dose CrPic plus *P. ginseng* (23.3, 30.7, 27, 32.4 and 9.3, 8.6, 16.9, 15.5% respectively) as compared to the control groups. On the other hand, testes (absolute and relative weights) and epididymis (absolute and relative weights) showed no significant change in animals administered low dose CrPic and low dose CrPic plus *P. ginseng* as compared to the control groups. Testes (absolute and relative weights) and epididymis (absolute and relative weights) showed significant decrease in high dose CrPic plus *P. ginseng* administered animals as compared to their corresponding untreated group, while those administered low dose CrPic plus *P. ginseng* showed no significant changes in these weights when compared with their corresponding untreated group (Table 1).

Animals administered low and high doses of CrPic showed a significant decrease in sperm count (16.2 and 25.6%, respectively), motility (20.4 and 32.9%, respectively) and daily sperm production (14.3 and 26.9%, respectively) when compared with the control groups. The addition of *P. ginseng* showed normalization in sperm count, motility and daily sperm production in low dose CrPic administered animals and significant decrease in animals administered high dose CrPic (17.3, 26.7 and 20%, respectively) when compared with the control groups. Sperm count, motility and daily sperm production showed significant decrease in high dose CrPic plus *P. ginseng* administered animals and no significant changes in low dose CrPic plus *P. ginseng* administered animals when compared with their corresponding untreated groups (Table 1).

Daily abnormal sperm production showed significant increase in animals administered high dose CrPic (20.3%) and no significant change in animals administered low dose CrPic, low dose CrPic plus *P. ginseng* and high dose CrPic plus *P. ginseng* as compared to the control groups. Daily abnormal sperm production showed no significant change in low and high dose CrPic administered animals when compared with their corresponding treated groups with *P. ginseng* (Table 1).

The hypo-osmotic swelling (HOS) test values showed
Table 1. Protective effect of *Panax ginseng* against Chromium-picolinate induced male reproductive system toxicity on the body weight and weights of the testis and epididymis of adult male rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th><em>Panax ginseng</em> (200 mg/kg/day)</th>
<th>Chromium picolinate (0.8 mg/100 g b.w.)</th>
<th>Chromium picolinate (0.8 mg/100 g b.w.) plus <em>Panax ginseng</em> (200 mg/kg/day)</th>
<th>Chromium picolinate (1.5 mg/100 g b.w.) plus <em>Panax ginseng</em> (200 mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (b.w.) (g)</td>
<td>203.07±10.07</td>
<td>203.40±9.52</td>
<td>166.60±7.28</td>
<td>ab</td>
<td>198.40±9.07</td>
</tr>
<tr>
<td>Testes</td>
<td></td>
<td></td>
<td></td>
<td>ab</td>
<td>140.27±13.13</td>
</tr>
<tr>
<td>Absolute weights (g)</td>
<td>2.15±0.16</td>
<td>2.17±0.16</td>
<td>2.00±0.25</td>
<td>ab</td>
<td>1.65±0.29</td>
</tr>
<tr>
<td>Relative weights (g/100 g b.w.)</td>
<td>1.40±0.13</td>
<td>1.43±0.18</td>
<td>1.20±0.39</td>
<td>ab</td>
<td>0.97±0.32</td>
</tr>
<tr>
<td>Epididymis</td>
<td></td>
<td></td>
<td></td>
<td>ab</td>
<td>1.28±0.10</td>
</tr>
<tr>
<td>Absolute weights (g)</td>
<td>0.89±0.42</td>
<td>0.89±0.42</td>
<td>0.87±0.09</td>
<td>ab</td>
<td>0.65±0.14</td>
</tr>
<tr>
<td>Relative weights (g/100 g b.w.)</td>
<td>0.71±0.15</td>
<td>0.72±0.11</td>
<td>0.65±0.16</td>
<td>ab</td>
<td>0.48±0.13</td>
</tr>
<tr>
<td>Epididymal spermatozoa</td>
<td></td>
<td></td>
<td></td>
<td>ab</td>
<td>0.60±0.13</td>
</tr>
<tr>
<td>Sperm count (10^6/g epididymis)</td>
<td>217.86±5.59</td>
<td>218.03±6.69</td>
<td>182.47±10.03</td>
<td>ab</td>
<td>212.40±12.09</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>78.87±4.79</td>
<td>79.07±4.56</td>
<td>62.80±3.67</td>
<td>78.20±4.28</td>
<td>52.93±7.21</td>
</tr>
<tr>
<td>DSP (10^6/g testis/day)</td>
<td>29.27±2.58</td>
<td>29.33±2.26</td>
<td>25.07±3.43</td>
<td>28.73±1.91</td>
<td>21.40±2.13</td>
</tr>
<tr>
<td>DASP</td>
<td>1.82±0.47</td>
<td>1.83±0.37</td>
<td>1.97±0.36</td>
<td>1.94±0.11</td>
<td>2.19±0.39</td>
</tr>
<tr>
<td>HOS</td>
<td>62.37±2.52</td>
<td>64.43±3.02</td>
<td>51.37±2.52</td>
<td>ab</td>
<td>36.13±5.59</td>
</tr>
</tbody>
</table>

Data shown are mean±SD from 15 rats per group. Data significantly different from untreated control group fed on the standard diet (group I) and untreated control group fed on the standard diet plus oral *Panax ginseng* (group II) control groups at p<0.05 are indicated by a and b respectively. Data of groups treated orally with Cr-picolinate (0.8 mg/kg b.w.; the low dose) (Group III) and (1.5 mg/100 g b.w.; high-dose group) (Group V) significantly different from their corresponding treated groups (Group IV and VI) with *Panax ginseng* at p<0.05 are indicated by c. DSP: Daily sperm production; DASP: daily abnormal sperm production; HOS: hypo-osmotic swelling test.

significant decrease in animals administered low and high dose CrPic (17.8, 20.4, 40.7 and 43.9%, respectively) as compared to the control groups. It showed significant decrease in high dose CrPic group treated with *P. ginseng* and no significant change in low dose CrPic group treated with *P. ginseng* when compared with the control groups. High dose CrPic group treated with *P. ginseng* showed significant decrease whereas low dose CrPic group treated with *P. ginseng* showed no significant change in the hypo-osmotic swelling (HOS) test values as compared to their corresponding untreated groups (Table 1).

Testicular chromium concentrations showed significant increase in animals administered CrPic low and high either alone or treated with *P. ginseng* (150, 134.9, 804.8, 826.8, 114.3, 119.5, 804.8 and 826.8%) as compared to the control groups. Testicular chromium concentrations showed no significant change in high and low doses CrPic plus *P. ginseng* administered animals as compared to their untreated groups (Figure 1). CrPic at low and high doses showed a significant increase in H$_2$O$_2$ production (20, 17.34, 49.77% and 47.78%, respectively), LPO (18.1, 14.17, 54.7 and 49.58%, respectively) and protein carbonyl contents (108.5, 115.8, 284.7 and 108.5%, respectively) in the mitochondrial fraction of testis in a dose-related manner as compared to the control groups. However, *P. ginseng* was shown to be effective in reducing H$_2$O$_2$ production, protein carbonyl contents and LPO. They showed no significant change in animals administered low
dose CrPic treated by *P. ginseng* and showed significant increase in animals administered high dose CrPic treated with *P. ginseng* as compared to the control groups. Hydrogen peroxide production, protein carbonyl contents and LPO in the mitochondrial fraction of testis showed significant decrease in low and high doses CrPic plus *Panax ginseng* compared to low and high dose CrPic administered animals (Figure 2).

Administration of CrPic at low and high doses a showed significant reduction in SOD (9.8, 9, 36.3% and 36.9%), CAT (27.0, 26.1, 40.3 and 39.5%), GPx (10.1, 9.4, 22.7 and 22.1%), GR (9.3, 8.9, 27.4 and 27.0%), GSH (19.1, 18.7, 60.9 and 60.7%) and vitamin C (36.5, 36.4, 52 and 52.1%), respectively in mitochondrial fraction of testis as compared to the control groups. *P. ginseng* improved the activity levels of SOD, CAT, GPx, GR, GSH and vitamin C levels. They showed no significant change in animals administered low dose CrPic plus *P. ginseng* and significant decrease in animals administered high dose CrPic plus *P. ginseng* as compared to the control groups. SOD (24.7 and 24%, respectively), CAT (20.7 and 19.7%, respectively), GPx (14.7 and 14%, respectively), GR (13.1 and 12.7%, respectively), GSH (41.2 and 40.9%, respectively) and vitamin C (36.5 and 36.4%, respectively) in mitochondrial fraction of testis showed significant decrease in high dose CrPic plus *P. ginseng* administered animals, whereas those administered low dose CrPic plus *P. ginseng* showed no significant change as compared to their untreated groups (Figure 2).

CrPic at low and high doses induced marked DNA fragmentation in testis (65.2, 76.4, 357.7 and 388.7%, respectively) as compared to the control groups. Animals treated with CrPic plus *P. ginseng* at high dose showed significant increase (287.1 and 206.6%, respectively), while at low dose showed no significant change in DNA fragmentation as compared to the control groups. DNA fragmentation in testis showed significant decrease in high dose CrPic administered animals and also in those administered high dose CrPic plus *P. ginseng* as compared to those administered low dose CrPic and low dose CrPic plus *P. ginseng* administered animals (Figure 2).

Serum testosterone and LH levels showed significant increase (12.7, 13.9, 30, 30.9, 20.3, 15.7%, 33.6 and 29.8%, respectively), while FSH level showed significant increase in low and high doses of CrPic animals (19.9, 15.7, 33.6 and 29.8%, respectively) as compared to the control groups. *P. ginseng* treatment in high dose CrPic animals showed a significant decrease in serum testosterone and LH levels (17.5, 18.6, 18.8 and 14%, respectively), while at low dose showed no significant change as compared to the control groups. FSH level showed significant increase in high doses of CrPic animals treated by *P. ginseng* (35.8 and 39%, respectively) as compared to the control groups. Serum testosterone and LH levels showed significant decrease, while FSH level showed significant increase in low and high dose CrPic plus *P. ginseng* administered animals, whereas FSH levels showed significant decrease in these groups as compared to their corresponding untreated groups (Table 2).

**DISCUSSION**

Trivalent chromium supplements such as chromium picolinate and niacin-bound chromium, Cr(III) is consumed by the general population through its presence in many foods. In addition, there is widespread consumption of Cr(III) present in dietary supplements, such as CrPic, that are marketed primarily for weight loss and antidiabetic effects. Humans typically ingest 20 to 45 μg Cr(III) per day in the diet (IOM, 2001), while typical daily doses of supplements may contain 200 to 1000 μg Cr(III) (Komrowski et al., 2008).

It is known that Cr(III) can accumulate in the body. Ingestion of CrPic supplements was found to produce serum levels of chromium that were equivalent to serum levels measured in workers occupationally exposed to chromium, and to produce urinary chromium levels higher than those in people environmentally exposed to chromium. Chromium has also been shown to accumulate in rat liver, kidney, spleen, lung, gastrocnemius, testes and heart after ingestion of CrPic in the diet. The structure and coordination chemistry of CrPic may make it more toxic than other forms of Cr(III) (Stearns et al., 2002).

This study is focused to determine the comparative effects of low and high doses of CrPic-induced male reproductive toxicity and to evaluate the possible antioxidative properties of *ginseng* extract against these effects.

Trivalent chromium compounds cause toxicity at higher concentrations and/or depending on the ligands attached to it (Barceloux, 1999).

The study demonstrated that, CrPic exposure at low and high dose showed significant decrease body weight gain, testes (absolute and relative weights) and epididymis (absolute and relative weights) when compared with the control groups. 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 (Gilbert et al., 1996). Hasten et al. (1997) reported a reduction in the body fat of rats supplemented with different doses of CrPic for 12 weeks without any reduction in the amount of food ingested, and attributed this finding to a possible thermogenic effect induced by the diet supplemented with CrPic, although no interaction nor treatment effects were seen for growth rate, lean body mass, or tissue weights. This could explain the lower final weights recorded for the animals that received high doses of supplementary CrPic.

Testicular weight was decreased significantly only after
only after high dose Cr exposure. In corroboration with the previous study, testes (absolute and relative weights) and epididymis (absolute and relative weights) were decreased after Cr treatment at high dose as compared to the control groups (Chowdhury and Mitra, 1995).

It is well documented that during normal spermatogenesis, ROS are produced by the electron leakage outside the electron transfer chain (Hanukoglu et al., 1993). Oxidative damage induced by reactive oxygen species (ROS) causes tissue damage by a variety of mechanisms including DNA damage, lipid peroxidation (LPO), impaired membrane function, decreased membrane fluidity, altered structural integrity, inactivation of several membrane bound enzymes and depletion of thiols. (Gutteridge and Halliwell, 2000). Mahboob et al. (2002) reported an increase in lipid peroxidation levels in tissues were observed in all chromium picolinate-treated rats. SOD, GPx and GSH levels in the tissues were decreased in all the treated groups, while the hepatic CAT level decreased in the high dose group. Testicular LPO was markedly increased in experimental group treated with high dose Cr. However, low dose Cr treatment showed 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 antioxidant enzyme superoxide dismutase and catalase (Murugesan et al., 2005). Decline in the SOD and catalase activities after high dosage 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 (Holdcraft and Braun, 2004). 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 (Steinberger and Steinberger, 1975). Decreased serum testosterone level in high dose Cr treated groups might be due to impaired activities of 17β-hydroxysteroid dehydrogenase (HSD) enzymes (Chandra et al., 2007). Low serum testosterone and LH levels in 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 level was observed in patients with low sperm counts (Li et al., 2001). Significant effective spermatid degradation occurred after high dose Cr administration. The possible mechanism for the structural alteration in testis of Cr treated experimental animals may be due to the disruption of blood–testis barrier with consequent accumulation of Cr in the testes. The accumulation of Cr in sperm and epididymis as studied earlier (Murthy et al., 1991) further strengthen this possibility. A possible mechanism responsible morphological and functional alterations in testis could be due to the generation of ROS in response to Cr exposure (Acharya et al., 2006). The deterioration observed in the for testis is in corroboration with the increased testicular

### Table 2. Protective effect of Panax ginseng against Chromium-picolinate induced male reproductive system toxicity on serum testosterone, FSH and LH levels of adult male rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Panax ginseng (200 mg/kg/day)</th>
<th>Chromium picolinate (0.8 mg/100 g b.w.)</th>
<th>Chromium picolinate (0.8 mg/100 g b.w.) plus Panax ginseng (200 mg/kg/day)</th>
<th>Chromium picolinate (1.5 mg/100 g b.w.) plus Panax ginseng (200 mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum testosterone (ng/ml)</td>
<td>3.77±0.38</td>
<td>3.82±0.41</td>
<td>3.29±0.36abc</td>
<td>3.60±0.37c</td>
<td>2.64±0.42abc</td>
</tr>
<tr>
<td>Serum FSH (mIU/ml)</td>
<td>3.07±0.21</td>
<td>3.00±0.30</td>
<td>3.68±0.99abc</td>
<td>3.00±0.46c</td>
<td>5.10±1.01abc</td>
</tr>
<tr>
<td>Serum LH (mIU/ml)</td>
<td>1.28±0.07</td>
<td>1.21±0.05</td>
<td>1.02±0.12abc</td>
<td>1.23±0.08c</td>
<td>0.85±0.09abc</td>
</tr>
</tbody>
</table>

Data shown are mean±SD from 15 rats per group. Data significantly different from untreated control group fed on the standard diet (group I) and untreated control group fed on the standard diet plus oral Panax ginseng (group II) control groups at p<0.05 are indicated by a and b respectively. Data of groups treated orally with Cr-picolinate (0.8 mg/kg b.w.; the low dose) (Group III) and (1.5 mg/100 g b.w.; high-dose group) (Group V) significantly different from their corresponding treated groups (Group IV and VI) with Panax ginseng at p<0.05 are indicated by c. DSP: Daily sperm production; DASP: daily abnormal sperm production; HOS: hypo-osmotic swelling test.
lipid peroxidation along with decreased SOD, catalase activities and GSH enzyme. Beside membrane effects, LPO can damage DNA and protein, which ultimately lead to sperm degradation and infertility (Sikka, 1996). These data explain our results about sperm morphology and function.

Cr induced hyperactivity of hypothalamo-pituitary-adrenal axis stimulates rapid release of corticotropin releasing hormone, corticotropin and glucocorticoids, respectively, from different part of this axis (Daliman et al., 1992), 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. The mechanism of reproductive impairment might be for the development of oxidative stress, disruption of hypothalamic-pituitary-testicular axis associated with hyperactivation of the stress signaling pathway through hypothalamicpituitary-adrenocortical axis resulting excess corticosterone secretion. In this paper, we have demonstrated the dose-dependent effects of chromium(III) picolinate on enhanced production of ROS including H₂O₂ production and LPO, and DNA fragmentation and significant reduction in SOD, CAT, GPx and GR activity in mitochondrial fraction of testis.

In accordance with our findings, Speetjens et al. (1999) postulated that chromium picolinate is capable of generating hydroxyl radicals, which in turn can cleave supercoiled DNA. These results are consistent with other studies, which demonstrate the ability of certain chromium complexes to nick DNA in the presence of peroxide and a reductant or in cells. Chromium picolinate has also been shown to generate hydroxyl radicals from hydrogen peroxide in a pathway independent of added reductant. Due to chromium’s ligand composition and the resulting redox potential, the complex can be reduced readily by abundant biological reductants and generate hydroxyl radicals via Haber-Weiss and Fenton reactions. The enhanced production of hydroxyl radicals results in appreciable DNA damage (Speetjens et al., 1999). Cr(III) compounds are genotoxic in certain test systems, and interaction of Cr(III) with DNA has been shown to result in the formation of DNA adducts, DNA-protein crosslinks, and DNA interstrand crosslinks (Andersson et al., 2007; Reynolds et al., 2007; Mozaffari et al., 2012).

ROS induce a significant reduction in semen quality by decreasing sperm count and motility. They can also increase sperm defects and impairment of antioxidant synthesis (Hatamoto et al., 2006). This study observed a decrease in the six parameters of sperm motility, VSL, VCL, VAP and ALH due to CrPic treatment. It can induce the formation of abnormal sperm cells; the increase in sperm abnormalities indicates that CrPic induced DNA damage in germ cells leading to altered sperm morphology. Sikka (2004) has reported that peroxidation of critical thiol groups in protein can alter the structure and function of spermatozoa. The decrease in sperm count is an important factor leading to male infertility (Meistrich and Brown, 1983).

Endogenous and exogenous antioxidants may protect cells and tissues from destructive effects of ROS and other free radicals. Previous studies reported that sperm disorders can be improved by exogenous antioxidants/ROS scavengers (Hosseini et al., 2012). Generally, it is known that the effects of P. ginseng are due to their numerous ginsenosides. Substances identified P. ginseng extract include a number of ginsenosides, polysaccharides, polyphenols, some minerals, etc (Geva et al., 1996). Ginseng or its extracts have been reported to exhibit free radical scavenging activities and can prevent lipid peroxidation (Hosseini et al., 2012). Polyphenols have antioxidant activity similar to vitamin C and E, which can enhance fertility by decreasing the level of free-radical damage to sperm cells (Geva et al., 1996).

Generation of ROS decomposes sperm plasma membrane and is therefore responsible for loss of sperm motility, which is presumably caused by a rapid loss of intracellular ATP leading to damage in sperm flagellum. Activity of Na⁺-K⁺-ATPase is highly sensitive to ROS, thus depletion of Na⁺-K⁺-ATPase can be a good reason for the reduction of sperm motility. The existence of morphologic abnormalities and decreased sperm viability has been associated with ROS production (Kim and Parthasarathy, 1998; Fischer et al., 2003). Also, defects in the flagella, changes in motility and morphology of spermatozoa, are likely associated with infertility. Sperm cells are more susceptible to peroxidative damage, because of high concentration of polyunsaturated fatty acids and low antioxidant capacity (Vernet et al., 2004). Membrane-associated polyunsaturated fatty acids such as sperm are readily attached by ROS. Peroxidation of membrane lipids can disrupt membrane fluidity and cell compartmentation, which can result in cell lysis (Fischer et al., 2003; Katoh et al., 2002). Reduction in sperm counts, the existence of morphologic abnormalities could be due to the generation of ROS by Cr(III).

Notably, P. ginseng was known to have protective and therapeutic effects against the testicular atrophy and other damages induced the most potent environmental pollutants toxic to reproductive organs (Kim et al., 1999). Salvati et al. (1996) also showed that men treated with ginseng have experienced an increase in spermatozoon number, improvement of motility, sperm viability, plasma testosterone, dihydrotestosterone (DHT), FSH, and LH concentrations. In rats fed with ginseng for 60 days, a significant increase of blood testosterone levels was found (Fahim et al., 1982). These spermatogenic effects of P. ginseng might be due to the combined effect of its many constituents, ginsenosides, polyphenol and minerals (Park et al., 2006). The results of this current study also suggest that the testicular changes induced by CrPic were significantly recovered by P. ginseng.
Conclusion

It could be concluded that consumption of CrPic for a long time induced several hazards to testes. Supplementation with extra amounts of *P. ginseng* may be useful to restrain the CrPic induced testicular changes.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Mahboob L, McNeil L, Tolliver T, Ogden L (2002). Effects of chromium