

Protective Effect of Korean *Panax ginseng* against Chromium VI Toxicity and Free Radicals Generation in Rats

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Abstract : Earlier studies have demonstrated that chromium (Cr) VI compounds have been shown to be more toxic and carcinogenic than other chromium compounds. The aim of the present work was to evaluate the antioxidant effects of red ginseng against chromium VI-induced toxicity and free radical generation. Sixty adult male rats were divided into six equal groups include: control group, group received Cr VI alone (50 mg/kg b.w.), group treated with Korean ginseng (K. ginseng) alone (20 mg/kg b.w), group treated with Cr VI for 15 days then received K. ginseng for other 15 days, group treated with Cr VI and K. ginseng at the same time for 15 days, and group treated with K. ginseng for 15 days then Cr VI for other 15 days. The results revealed that Cr VI caused significant increase in ALT, AST, ALP, G-GT, urea, creatinine, and acid phosphatase. Whereas, it caused significant decrease in TP, albumin, testosterone, GPX, and SOD indicating a stress for liver, kidney and testes. K. ginseng alone caused significant increase in GPX and SOD activities in healthy animals and this result suggests a prophylactic role for this herb in protection against the damaging impact induced by free radical species. Furthermore, the other biochemical parameters measured after K. ginseng administration were comparable to the control values. Treatment with Cr VI followed by K. ginseng, Cr VI and K. ginseng or K. ginseng followed by Cr VI resulted in significant improvement in all tested parameters towards the normal values of the controls. However, this improvement was pronounced in the group pre-treated with K. ginseng for 15 days before Cr VI administration. It could be concluded that K. ginseng exhibited a protective action against the toxic effects of Cr VI and it had the ability to scavenge free radicals resulted from Cr VI intoxication.

Key words : Chromium, antioxidant, GPX, SOD

INTRODUCTION

Recent evidence suggests that exposure to diverse environmental toxicants, including heavy metals, may involve a common cascade of events that involves the production of reactive oxygen species and oxidative tissue damage.⁴⁴⁾ In recent years, chromium (Cr) has been shown to play a role in glucose intolerance in type 2 diabetes and gestational diabetes.^{3,4)} Chromium is an industrial element widely used in chrome plating, steel alloys, cast steel, metal finishing, glassware-cleaning solutions, leather tanning, wood treatment and in the production of pigments. The physical and chemical properties, environmental sources, toxicokinetics, and pathophysiology of chromium have been reviewed by Barceloux.⁵⁾ Furthermore, Von Burg and Liu⁵²⁾ and Barceloux⁵⁾ have summarized the acute toxicity, chronic toxicity, neurotoxicity, dermatotoxicity, genotoxicity, carcinogenicity, immunotoxicity, and general environmental toxicity of chromium. Hepatic and renal toxicity have been reported either in workers or in experimental animals exposed to chromium

VI^{21,24,31)} Chromium VI compounds also induced DNA damage *in vivo*¹³⁾ and in cultured cells⁴⁷⁾ as well as inducing inhibition of the activity of enzymes in mammalian cells.⁴⁶⁾

The toxicological effects of chromium VI are generally attributed to cellular uptake, because chromium VI, in contrast to chromium III, is easily taken into the cells by sulfate anion transport system. Once inside the cells, chromium VI is reduced, via reactive intermediates such as chromium V and IV, to more stable chromium III by cellular reductants.¹⁵⁾ Thus, the formation of chromium III or other intermediate oxidation states, in particular chromium V, is believed to play the key role in the hazardous effects of chromium VI compounds. As a result of this reduction process the generation of reactive oxygen species can be manifested.⁴⁶⁾

There is a growing evidence in the literature that *Panax ginseng*, the well known traditional herbal remedy used in Chinese medicine for thousands of years, possesses an array of interesting pharmacological effects, such as cardioprotection, vasorelaxant, antistress and stimulating

action on the central nervous system.¹⁸⁾ Zuin⁵⁸⁾ reported that the administration of ginseng extract clearly recovered chronic liver damage induced by certain drugs including alcohol. The recovery of liver damage by ginseng extract has also been reported in other clinical studies as well. Jeong²⁶⁾ reported that oral administration of ginseng extract resulted in improvement of body and organ weights as well as hematological and serum clinical parameters. They also reported that the elevation of GPT and GOT activities, liver vacuolization and lymphoid cell aggregation induced by carbon tetrachloride (CCl₄) were clearly recovered by the administration of ginseng extract. In another study, Kim²⁸⁾ stated that ginseng has a potent protection against CCl₄-induced lipid peroxidation and it showed inhibitory effects on cytochrome P₄₅₀-associated monooxygenase activities. Further study suggested that ginseng and/or its ginsenosides could induce the antioxidant enzymes which are essential for maintaining cell viability by lowering the level of oxygen radical generated from intracellular metabolism.¹⁰⁾ The objective of the present study is to evaluate the protective role of *Panax ginseng* root against chromium VI-induced toxicity on liver, kidney and testis. Likewise to assess the potent antioxidant effect of *Panax ginseng* as free radical scavenger in rat model.

MATERIALS AND METHODS

Chemicals

Potassium dichromate (K₂Cr₂O₇) was purchased from Kanto Chemical Co. (Tokyo, Japan) and *Panax ginseng* roots were obtained from EPICO Co., 10th Ramadan, Egypt, and was imported from South Korea.

Kits

Transaminases (ALT and AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (G-GT), urea, creatinine, total protein (TP), albumin, acid phosphatase, glutathione peroxidase (GPX) and superoxide dismutase (SOD) were purchased from Randox Laboratories, U.K. whereas, testosterone kit was purchased from Biochem ImmunoSystems Co., Italy.

Experimental Animals

A total number of 60 adult male Sprague-Dawley rats (120-150 g, purchased from Animal House Colony, NRC, Dokki, Cairo, Egypt) were maintained on a standard laboratory diet (protein: 16.04; fat: 3.63; fiber: 4.1 g/kg and metabolisable energy 12.08 MJ), and housed in a room

free from any source of chemical contamination, artificially illuminated and thermally controlled, at the Animal House Lab., National Research Center, Dokki, Cairo, Egypt. After an acclimation period of one week, the animals were divided into six equal groups (10 rats/group) and housed in filter-top polycarbonate cages.

Experimental Design

Animals within different treatment groups were treated orally as follows: group 1, untreated control; group 2, treated with potassium dichromate (Cr VI) dissolved in water (50 mg/kg b.w.)⁴³⁾ for 15 days; group 3, treated with *K. ginseng* suspended in water (20 mg/kg b.w.)⁷⁾ for 15 days; group 4, treated with Cr VI for 15 days then followed by *K. ginseng* for other 15 days; group 5, treated with Cr VI plus *K. ginseng* at the same time; and group 6, treated with *K. ginseng* for 15 days then followed by Cr VI for other 15 days. At the end of the treatment period, two blood samples were collected from all animals from retro-orbital venous plexus⁴²⁾ after being fasted for 12 hours for conducting different biochemical analyses. The first blood sample from each animal, within each treated group, was placed in heparinized plastic tube and assayed in the same day of collection for the determination of glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities, whereas, the second blood sample for each animal was left to clot and centrifuged at 5000 rpm under cooling for 10 min to separate the serum for the other biochemical analyses.

The activities of ALT and AST in serum were determined according to the method recommended by The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology.⁵¹⁾ Alkaline phosphatase activity in serum was determined according to Bowers and McComb.⁸⁾ Serum G-GT activity was determined according to the method described by Szasz.⁵⁰⁾ Serum urea level was determined according to Fawcett and Scott¹⁹⁾ and creatinine level was determined according to Bartles.⁶⁾ Serum total protein content was determined according to Weichselbaum.⁵⁴⁾ Albumin content in serum was determined according to Doumas¹⁷⁾ Serum acid phosphatase activity was determined according to the method described by Fishman and Lerner²⁰⁾ and testosterone level was estimated according to the enzyme immunoassay procedure of Parker.³⁹⁾ Erythrocyte GPX activity was determined using Ransel kit according to Paglia and Valentine.³⁸⁾ The activity of SOD was determined using Ransod kit according to the method described by Suttle.⁴⁹⁾ In brief, this method employs xanthine and xanthine oxidase (XOD) to generate

Table 1. Effect of chromium with or without K. ginseng on different biochemical parameters in rats (means \pm SE)

Parameter	Control	Cr	Gin	Cr then Gin	Cr and Gin	Gin then Cr
ALT U/L	30.75 \pm 1.44 ^a	111.75 \pm 4.80 ^b	36.75 \pm 1.79 ^a	81.5 \pm 5.72 ^c	97.25 \pm 1.11 ^d	54.5 \pm 3.52 ^e
AST U/L	33.75 \pm 2.49 ^a	128.0 \pm 3.63 ^b	38.75 \pm 1.31 ^a	85.5 \pm 3.57 ^c	102.75 \pm 1.60 ^d	65.0 \pm 3.34 ^e
ALP U/L	228.0 \pm 4.24 ^a	602.0 \pm 7.79 ^b	296.75 \pm 14.56 ^c	544.25 \pm 17.75 ^d	435.5 \pm 11.15 ^e	430.75 \pm 10.68 ^e
G-GT U/L	8.0 \pm 0.54 ^a	38.75 \pm 2.29 ^b	12.5 \pm 1.04 ^c	36.75 \pm 2.69 ^d	34.0 \pm 1.29 ^d	25.5 \pm 1.55 ^e
Urea mg/dl	31.28 \pm 2.20 ^a	81.85 \pm 2.52 ^b	33.5 \pm 0.68 ^a	55.66 \pm 12.56 ^c	61.4 \pm 2.88 ^d	51.95 \pm 1.13 ^c
Creatinine mg/dl	0.75 \pm 0.05 ^a	3.98 \pm 0.32 ^b	0.73 \pm 0.04 ^a	1.58 \pm 0.07 ^c	1.32 \pm 0.01 ^c	1.18 \pm 0.12 ^d
T.P g/dl	6.02 \pm 0.40 ^a	2.3 \pm 0.16 ^b	6.6 \pm 0.30 ^a	4.75 \pm 0.17 ^c	4.9 \pm 0.25 ^c	5.6 \pm 0.18 ^a
Albumin g/dl	3.46 \pm 0.14 ^a	1.85 \pm 0.35 ^b	3.56 \pm 0.13 ^a	2.55 \pm 0.18 ^c	2.6 \pm 0.11 ^c	2.88 \pm 0.09 ^a

Within each raw means with the same letter are not significantly different (P>0.05)

superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride(INT) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction.

Statistical analysis

All data were statistically analyzed using the General Linear Models Procedure of the Statistical Analysis System.⁴¹⁾ The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio.⁵³⁾ All statements of significance were based on probability of P \leq 0.05.

RESULTS

The present results revealed that treatment with chromium VI (50 mg/kg b.w.) for 15 days caused significant increase in serum ALT, AST, ALP, G-GT activities as well as urea and creatinine levels, whereas, it caused significant decrease in TP and albumin levels. On the other hand, K. ginseng alone caused significant increase in serum ALP and G-GT activities, while it caused insignificant change in serum AST, ALT, urea, creatinine, TP and albumin as compared to the control values (Table 1). It is of interest to mention that treatment with K. ginseng for 15 days before, together with or 15 days after chromium VI administration caused significant improvement in all tested parameters. It can be noted from Table (1) that administration of chromium VI for 15 days then followed by K. ginseng for other 15 days resulted in significant improvement in all tested parameters and this effect was more pronounced on serum ALT, AST, and urea levels. Co-administration of chromium and K. ginseng for 15 days caused significant improvement in all tested parameters, but the effect was less pronounced on serum ALT and AST activities. On the other hand, pre-administration of K. ginseng for 15 days before chromium administration was the most effective treatment in improving the bio-

chemical parameters towards the normal values of the control (Table 1).

The present investigation also clearly showed that chromium VI administration resulted in significant increase in serum acid phosphatase activity whereas this enzyme was comparable to the control value in the K. ginseng treated group. On the other hand, the combined treatment with K. ginseng and chromium caused an improvement in the acid phosphatase activity and the improvement was pronounced in the animals received K. ginseng for 15 days before chromium for other 15 days (Fig. 1). Regarding the effects of different treatments on serum level of testosterone as shown in Fig. 2, chromium VI administration caused significant decrease in serum testosterone level, while K. ginseng alone caused insignificant increase in the level of this hormone in serum. On the other hand, treatment with K. ginseng plus chromium caused significant improvement in serum testosterone level towards the control values and this improvement was pronounced in the group pre-treated with K. ginseng for 15 days before chromium administration for other 15 days.

The effect of different treatments on erythrocyte glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities is depicted in Figs. 3 and 4. The results revealed that chromium VI alone caused significant decrease in both of GPX and SOD activities, whereas, K. ginseng alone caused significant increase in the activity of both enzymes. Treatment with K. ginseng before, together with, or after chromium administration resulted in significant improvement in both of GPX and SOD activities and the improvement was pronounced in the animals pre-treated with K. ginseng for 15 days before chromium administration for other 15 days.

DISCUSSION

There is good evidence from the clinic and laboratory

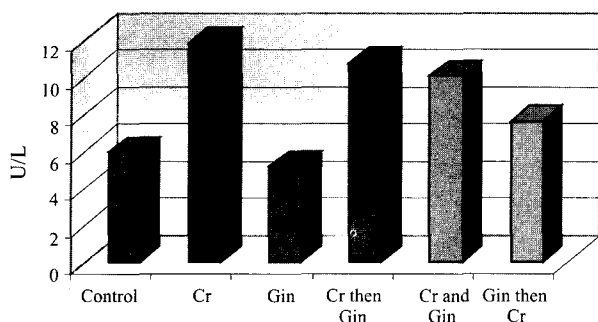


Fig. 1. Effect of chromium and ginseng on serum acid phosphatase activity in rats.

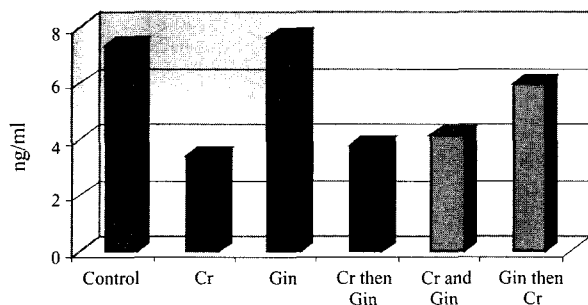


Fig. 2. Effect of chromium and ginseng on serum testosterone level in rats.

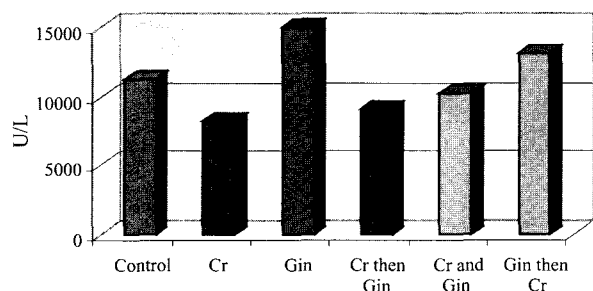


Fig. 3. Effect of ginseng on erythrocyte glutathione peroxidase activity in rats treated with chromium.

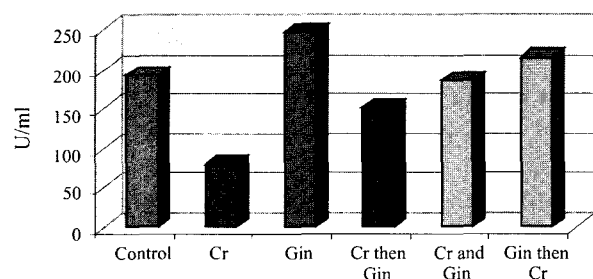


Fig. 4. Effect of ginseng on erythrocyte superoxide dismutase activity in rats treated with chromium.

that Cr VI is the ion responsible for most of the toxic actions, although much of the underlying molecular damage may be due to its intracellular reduction to the even

more highly reactive and short lived chemical species Cr III and Cr V.¹⁴⁾ Extensive studies *in vitro* and in model systems indicated that the reactive intermediate, Cr V is likely the candidate for the ultimate carcinogenic form of chromium compounds. Also *in vivo* studies demonstrated that Cr V thus formed can mediate the generation of free radicals. Cr V and its related free radicals are very likely to be involved in the mechanism of Cr VI-induced toxicity and carcinogenesis.³⁴⁾

In the current study, we evaluated the effect of *K. Panax ginseng* as a natural antioxidant against chromium VI toxicity and generation of oxygen radicals. The selected dose of chromium VI and *K. ginseng* were literature-based.^{7,43)} The present results illustrate that oral administration of chromium VI (50 mg/kg b.w.) for 15 days resulted in liver dysfunction as indicated by the significant increase in ALT and AST activities. Kumar and Barthwal²⁹⁾ demonstrated marked increase in the activity of ALT and AST as a result of liver impairment. The increased levels of ALT, AST, and ALP in animals treated with chromium VI in the current study may indicate degenerative changes in hepatic tissues and biliary system.^{27,29)} This result is consistent with the finding of Chorvaticova¹¹⁾ who demonstrated marked elevation in ALT activity in animals treated with dichromate compounds. However, it has been reported that addition of Cr VI (dichromate) to isolated rat hepatocytes resulted in rapid glutathione oxidation, reactive oxygen species (ROS) formation, lipid peroxidation, decreased mitochondrial membrane potential and lysosomal membrane rupture before occurrence of hepatocyte lysis.⁴⁰⁾ Moreover, Dey¹⁶⁾ observed that the intoxication with chromium VI causes significant decrease in the activity of liver alkaline phosphatase. They suggested that exposure to chromium induced alterations in the structure and function of liver plasma membrane. The elevated activity of G-GT in serum indicated severe injury of both liver lysosomes and mitochondria.²⁹⁾

The increased levels of urea and creatinine and decreased levels of total protein and albumin after chromium VI administration may indicate protein catabolism and/or kidney dysfunction.¹⁾ Kumar and Rana³⁰⁾ observed considerable kidney damage in chromium-poisoned rats resulting in significant functional impairment, necrosis and loss of enzymes activity from the renal tubules.

The present investigation clearly revealed that chromium VI administration caused significant decrease in serum testosterone level with concomitant increase in serum acid phosphatase activity in rats. This result indicates that chromium VI depressed the function of testes as a result of free radicals generation and the attack on DNA

and led to cross linkages within the molecule.²³⁾ In conformity with our observation, Chowdhury¹²⁾ reported that treatment with chromium resulted in decline of leydig cell population and nuclear diameter indicated the steroidogenic impairment and this effect was confirmed by the significant inhibition of 3 beta-delta 5-hydroxy steroid dehydrogenase that associated with low serum testosterone level. High serum acid phosphatase activity may occur as a result of ulcerative lesion in the prostate³²⁾ and/or prostate hyperplasia.²²⁾

The current study clearly indicated that chromium VI is able to induce damaging impact on liver, kidney and testis. Bragt and van Dura⁹⁾ found high concentration of chromium in the kidney and liver of rats after single dose administration of Na_2CrO_4 , ZnCrO_4 and PbCrO_4 that having equivalent hexavalent chromium content. In general, the toxic effect of chromium VI may be due, in part, to the production of oxidative stress on liver, kidney and testes as well as the generation of reactive oxygen species which produce a number of toxic reactions including DNA damage³⁷⁾ and lipid peroxidation.²⁵⁾ Our result demonstrated that treatment with chromium resulted in significant reduction in glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities as shown in Figs. 3 and 4. Previous studies have shown that these antioxidant enzymes were disrupted by chromium compounds.^{45, 46)} Similar result has been reported by Susa⁴⁸⁾ who observed that dichromate could decrease the activities of the antioxidant enzymes including glutathione reductase (GR), glutathione peroxidase (GPX) and superoxide dismutase (SOD).

The antioxidant compounds have been known to protect cells from various oxidative damage, and there is considerable evidence that the natural antioxidants are anticarcinogens.²⁾ Therefore, it is important to elucidate the effect of these antioxidants in chromium-induced toxicity.

In the present study, administration of *K. ginseng* to rats received chromium VI resulted in significant improvement in different biochemical parameters although it failed to normalize these parameters. Moreover, pre-treatment with *K. ginseng* for 15 days before chromium administration was found to induce the potent protective action. It is of interest to mention that *K. ginseng* displays a pronounced hepatoprotective effect, assessed through the transaminases (ALT, AST) activities following hepatotoxicity induced by carbon tetrachloride in rats.²⁶⁾ In the same way, administration of *K. ginseng* significantly reduced the activities of ALT and AST induced by the administration of dexamethasone in rats, with restoration of both adrenal and thyroid functions.³³⁾

Treatment of chromium VI-intoxicated rats with *K. ginseng* resulted in significant improvement in kidney function as indicated by the marked decrease in serum urea and creatinine levels with concomitant increase in serum total protein and albumin contents. This result was in conformity with those reported by Yokozawa⁵⁷⁾ who demonstrated that *K. ginseng* extract and its active component, saponin, could significantly reduce the blood urea nitrogen and creatinine levels and elevate the total protein and albumin concentrations in the blood of nephrectomized rats. More recent study asserted the nephroprotective effect of *K. ginseng* saponin against cisplatin-nephrotoxicity.³⁵⁾ They suggested that *K. ginseng* saponin reduced cisplatin-induced cytosolic free $[\text{Ca}^{2+}]$ ions overload and formation of DNA interstrand cross-link and DNA-protein cross-link. Another recent report demonstrated that ginsenoside could decrease the severity of renal injury induced by cisplatin, in which proximal urinaferous tubules represent the main site of injury.⁵⁶⁾ They stated that when ginsenoside was given orally prior to cisplatin injection, the activities of the antioxidant enzymes were higher in the treated rats than in the control ones. The levels of urea nitrogen and creatinine in serum were decreased in rats given ginsenoside. These findings reflected the protective action of ginsenoside against the renal dysfunction caused by cisplatin.⁵⁶⁾

Treatment of rats with *K. ginseng* conditioned the adverse effects of chromium on the testes. Although the testosterone level did not change significantly in the rats treated with *K. ginseng* alone, the combined treatment with both chromium and *K. ginseng* caused significant improvement in serum testosterone level but this treatment could not normalize it. Similar result was reported by Bashandy⁷⁾ who stated that ingestion of *K. ginseng* caused significant improvement in serum testosterone level, sperm counts, weight of testes and sexual organs of rats treated with mycotoxin (i.e. ochratoxin A). In general, *K. ginseng* exerted its protective activity against chromium either directly by inhibiting lipid peroxidation and scavenging free radicals³⁶⁾ or indirectly through enhancement of the activity of GPX and SOD, the enzymatic free radicals scavengers in the cells.⁵⁵⁾

CONCLUSION

The present results indicated that oral administration of chromium VI resulted in liver and kidney dysfunction as indicated by the elevation of serum ALT, AST, ALP, GGT, urea and creatinine as well as the reduction of total

protein and albumin levels. Chromium VI also impaired the testicular function as indicated by the reduction of testosterone level and the elevation of acid phosphatase activity in serum. It could be concluded also that chromium VI induced these harmful effects through its effects on the antioxidant capacity of the body as indicated by the reduction of GPX and SOD activities. K. ginseng exhibited protective efficacy against the toxic effects of chromium as indicated by the improvement of the different biochemical parameters under investigation. Moreover, pre-treatment with K. ginseng 15 days before chromium administration was the most effective regimen in K. ginseng protection.

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