*Proceedings of the 5th International Ginseng Symposium, Seoul, 1988*

# The Protective Effect of Red Ginseng $S_I$-fraction against Dichromate-Induced Acute Renal Failure

Ki Jung Na, Kyu Sang Kang, and Eun Kim

Korea Ginseng and Tobacco Research Institute, 302 Shinsung-dong , Yousung-ku, Teajon 302-345 Korea

## Abstract

The nephrotoxicity of dichromate was reduced upon the administration of red ginseng $S_I$-fraction. Rabbits treated with dichromate showed acute renal failure and the majority of animals died within ten days. Mortality and physioligical status were significantly improved by the administration of ginseng. The effect of red ginseng $S_I$-fraction was studied by hematological analysis, urinalysis, and histophathological examination. It was found that red ginseng $S_I$-fraction has a potent effect on glomerulotubular imbalance induced by dichromate. In addition, $S_I$-fraction normalized the rate of glycogenolysis, glycolysis, and the rate of lactate production, which had been accelerated by intravenous dichromate injection. Red ginseng $S_I$-fraction was superior to ascorbic acid, CaEDTA, and furocemide in the protection and recovery from dichromate-induced acute renal failure.

## Introduction

It is well known that ginseng (*Panax ginseng* C.A. Meyer) has diverse pharmacological effects on the human body[1]. Saponins have been identified as the main components in pharmacological activities of ginseng[2,3]. The mode of pharmacological activities is believed to be stimulation of RNA and protein synthesis, and stimulation of glucose and lipid metabolism through numerous animal investigations[2,3,4].

The kidneys are vital organs which perform two major functions; first, they excrete most of the end-products of bodily metabolism and second, they control the concentrations of most of the constituents of the body fluids. And the kidneys are target organs for the toxic actions of various drugs and chemicals[5,6].

However, many attempts have not been made to investigate the pharmacological properties of ginseng on the renal functions. In this study, we have investigated the protective effect of red ginseng $S_I$-fraction against the acute renal failure induced by dichromate which is known as a nephrotoxin[7,8]

## Materials and Methods

Male Sprague-Dawley rats (200± 10 g) and male New Zealand white rabbits (2.5± 0.1 kg) were used in this study. The sodium dichromate (12.5 mg/kg, in saline, adjusted to pH 7.4 with NaOH) was injected intravenously to the rats and rabbits. Red ginseng $S_I$-fraction (6.5 mg/Kg, prepared according to the Kim's method[9]) was dissolved in distilled water and administered orally 30 min prior to sodium dichromate treatment. Ascorbic acid (1700 mg/kg), CaEDTA (100 mg/kg), and furocemide (2 mg/kg), obtained from Sigma (St Louis, Mo, USA) were dissolved in distilled water and administered orally 30 min prior to sodium dichromate treatment. The corresponding control animals received physiological saline.

In the experiments where attempts were made to document the onset of the nephrotoxicity, the rabbits were placed in metabolism cage in which they had free access to drinking water and food. Seven control days were allowed to treatment. Urine was collected on a daily basis. Urine pH and volume were measured quantitatively, and various substances (glucose, protein, ketones, blood, bilirubin, and nitrites) were qualitatively using Combur$^9$-Test-U(Boehringer). Aliquot was saved for 24h excretion of urea, creatinine, and chromium analysis.

To investigate the ginseng effect on carbohydrate metabolism, blood samples were obtained from heart of thiopental sodium-anesthetized rats at the requisite time. Blood urea nitrogen(BUN) was measured using UN-V$^R$ reagent (Kyokuto, Japan). Creatinine was measured by reaction with picrate, using CRE(E)$^R$ reagent(Kainos, Japan). Glucose was determined colorimetrically with V-Glucase$^R$ reagent(Nissui, Japan) utilizing the glucose-oxidase reaction. Lactate was determined using Sigma reagent. Chromium was analyzed colorimetrically according to the Goodersons's methods[10].

Kidney sections were fixed in buffered-formaline solution and paraffin sections were prepared and stained with hematoxyline and eosine.

Serum protein electrophoresis was carried out in cellulose acetate membrane using Gelman apparatus.

The data obtained were analyzed statistically by Student's t test. The 0.05 level of probability was used as the criterion of significance.

## Results and Discussion

Figure 1 represents the protective effects of red ginseng $S_I$-fraction against the nephrotoxicity developed after a single i.v. injection of sodium dichromate (12.5 mg/kg) on male rabbits. Rabbits lost weight, stopped eating and became listless and lethargic after the injection of sodium dichromate. Although some animals survived for as long as 20 days, many died within 10 days. At this dose it was very unusual for an animal to recover. There was a prompt reduction in 24h urine volume. Most rabbits had a 3- to 5-day period of oliguria that was usually followed by a variable period of diuresis.

The duration and severity of the oliguric phages were variable.

The dichromate decreased the daily creatinine excretion 50% in the 3-to 5-day period. Here there was a tendency toward an increase in the daily creatinine excretion during diuretic phages, but the urine creatinine concentration was very diluted.

Administration of red ginseng protected against dichromate-induced nephrotoxicity and improved the physiological

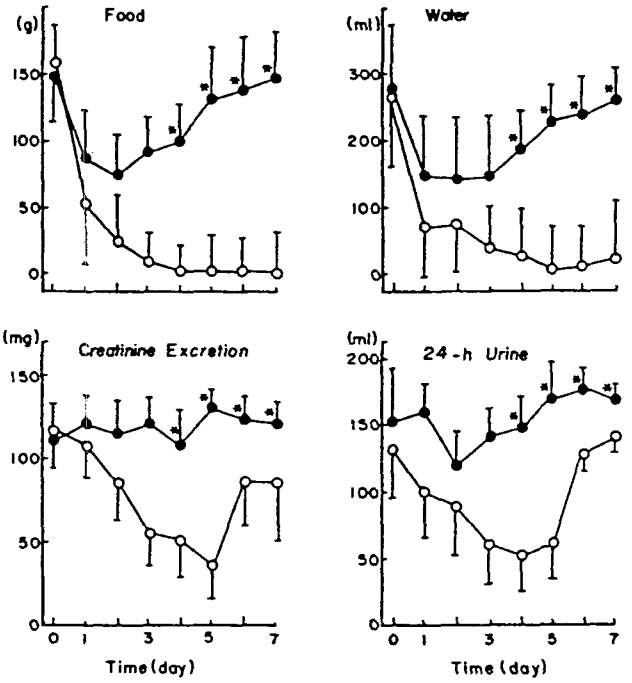


Figure 1. The protective effect of red ginseng $S_1$-fraction against sodium dichromate nephrotoxicity. Each point is mean, and bar the S.E. of ten animals.
○ : sodium dichromate (12.5 mg/kg, i.v.)
● : sodium dichromate (12.5 mg/kg, i.v.) + $S_1$-fraction (6.5 mg/kg, p.o.)

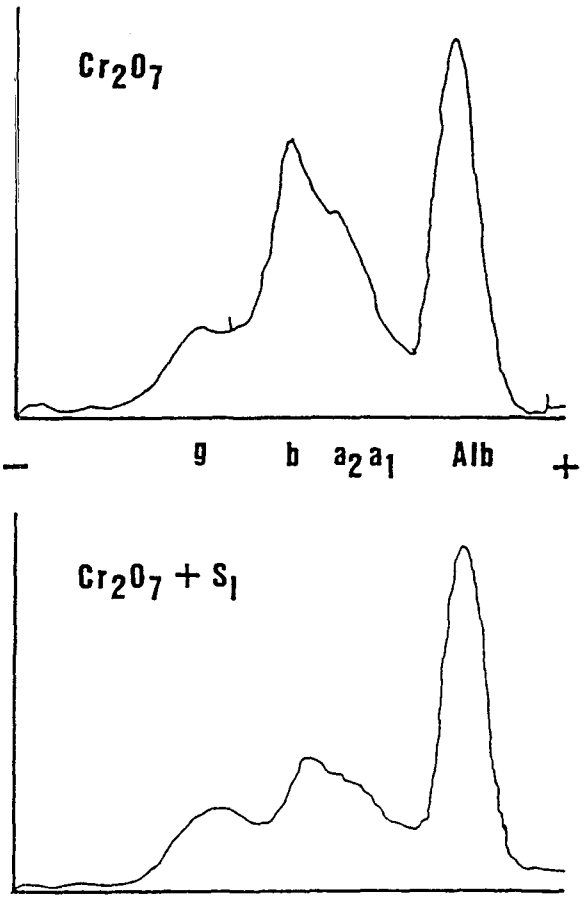


Figure 2. Major serum protein electrophoretic patterns. The serum 7 days after dichromate intoxication was separated on cellulose acetate membrane. The fractions were visualized by staining with Ponceau S.

status significantly.

The blood non-protein-nitrogen concentration was determined after the dichromate administration. Both the blood urea nitrogen (BUN) and creatinine concentration were gradually increased.

At 7-day after the dichromate administration, both BUN and serum creatinine concentartion were increased 6 to 8 folds respectively. However, red ginseng $S_1$-fraction treated group maintained nearly normal renal function (Table 1). The dichromate did not influence the blood glucose and lactate level.

Figure 2 shows the electrophoretic patterns of rabbit serum at 7-day after dichromate intoxication. Nephrotoxic syndrome is obvious in dichromate treated group. $\alpha_2$-Globulin is increased and the ratio of albumin to $\alpha_2$-globulin is significantly decreased. But the red ginseng treated group shows nearly normal electrophoretic pattern.

Fig 3 shows the kidney cortex from a rabbit 7 days after dichromate administration. Proximal tubular necrosis is evident. Pigments and casts are filled within distal and collecting tubules. However, in the red ginseng treated group the renal damage is not too extensive, and so the kidney structure and function usually return to nearly normal state within weeks of the onset of injury.

Figure 4 represents the dose-response relationship 30 min after i.v. injection of various doses of dichromate. As the dichromate dose was leveled up, the changes of metabolite were severe. Above all, the lactate concentration increased at very low dichromate dose. Administration of red ginseng $S_1$-fraction normalize those accelerated glycogenolysis, glycolysis, and lactation production rate. Insulin treatment also has effect on the disruption

Table 1. Effect of red ginseng $S_1$-fraction on the parameters of renal functions[a].

(mg/dl)

| | BUN[b] | Creatinine[b] | Glucose[b] | Lactate[b] |
|---|---|---|---|---|
| Control | 30 ± 5.4 | 2.0 ± 0.18 | 150 ± 15.3 | 45 ± 3.3 |
| N | 190 ± 9.7 | 16.2 ± 0.91 | 153 ± 20.1 | 60 ± 4.7 |
| N + $S_1$ | 41 ± 3.8[c] | 2.5 ± 0.52[c] | 154 ± 19.2 | 49 ± 4.3 |

a. Male New Zealand white rabbits were given red ginseng $S_1$-fraction($S_1$) prior to dichromate(N) intoxication ; control rabbits received saline. The rabbits were sacrificed 7 days after dichromate treatment.
b. Values represent the mean(± S.E) of duplicate determinations from 10 animals.
c. Significantly different from dichromate treated group ($p<0.05$)

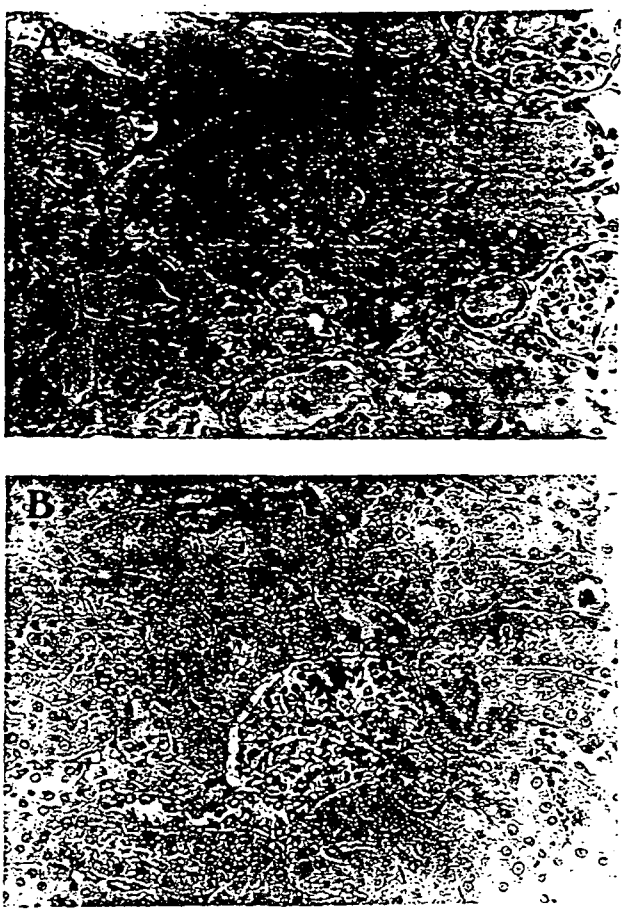

Figure 3. The dichromate-exposed rabbit kidney at 7 day. Kidney sections were taken 7 day after treatment. H & E. X 400.
a. Kidney sections from a rabbit treated with 12.5 mg/kg dichromate
b. Kidney sections from a rabbit treated with $S_I$-fraction prior to dichromate intoxication.

of carbohydrate metabolism. However insulin treatment was not beneficial to dichromate induced acute renal failure (data not shown).

In addition to red ginseng $S_I$-fraction, ascorbic acid, CaEDTA were somewhat helpful to protect against dichromate nephrotoxicity. Even though insulin, and thiolar have a potency to decrease the rate of glycogenolysis, glycolysis, and the rate of lactate production, they could not prevent renal damage. The toxicity of the dichromate is apparently related to the powerful oxidizing action of the hexavalent compound. Antichrome agent such as ascorbic acid have been shown to be benificial in the experimental animal[11]. Red ginseng $S_I$-fraction has no reducing activity like ascorbic acid.

It has been reported that chelating agents such as N-acetylcystein and calcium EDTA increase the urinary excretion rates of chromium in the rat experiments[12].

Neither was red ginseng $S_I$-fraction able to produce an increase in chromium excretion, nor was it effective at decreasing the circulating chromium (Figure 5). Even though calcium EDTA increases the urinary chromium excretion, the prognosis is poor in our rabbit experiments.

Although not fully studied, dichromate toxicity appear to occur in two phases; and initial multisystem shock phase and a later phase with renal toxicity[13,14]. The

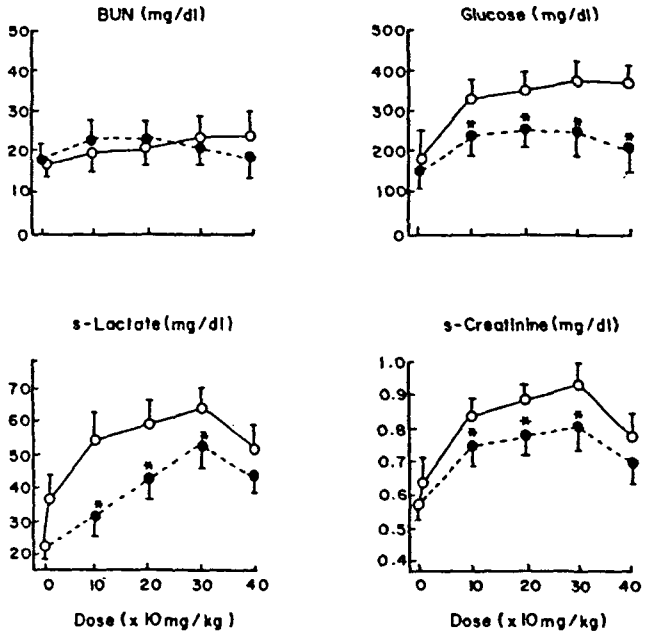


Figure 4. Effect of red ginseng $S_I$-fraction on the dichromate disrupted carbohydrate metabolism. Various doses of dichromate were injected i.v. into S.D. rats. Blood samples were obtained from heart of thiopental sodium anesthetized rat 30 min after treatment. Each bar represents the mean (± S.E) of determination from the five rats.

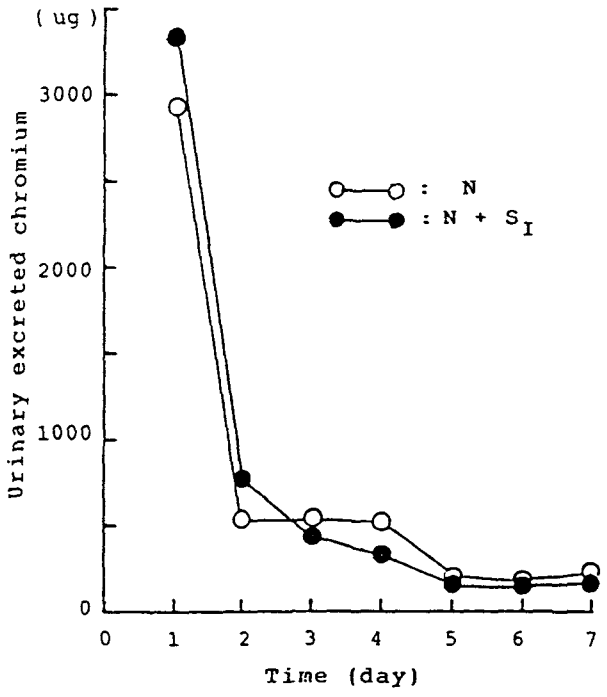


Figure. 5. Effect of red ginseng $S_I$-fraction on the urinary chromium excretion.
*N ; dichromate (12.5 mg/kg, i.v.)
$S_I$ ; red ginseng $S_I$-fraction (6.5 mg/kg, p.o)

disruption of carbohydrate metabolism is the characteristic property of dichromate intoxication, and it may be the first sign of an initial multisystem shock phase[15]. It is not the general poisoning stress.

It is suggested that red ginseng $S_I$-fraction has the powerful potency to calm the initial factor. This is very helpful to overcome the later renal toxicity, which may be related to hepatic dichromate metabolites[15].


## Acknowledgements

We should like to thank Dr. C. H. Lim for his discussion in the histophathological studies. And we are grateful to Mr. J. S. Kyung for technical assistance.


## References


1. Karzei K. : Pharmacological aspects of ginseng. A review. Proc. 1st Int. Ginseng Symp., 49(1974)
2. Oura, H : Biochemical and experimental medical studies on the effective ingredient of *Panax ginseng*. Kanpo KenKyu, 1 : 2(1968).
3. Oura, H. : Biochemical action of *Panax ginseng* principle. Proc. 1st Int. Ginseng Symp., 6(1974).
4. Oura, H : Stimulating effect of the roots of *Panax ginseng* C. A. Meyer on the incorporation of labeled precursors with rat liver R. N. A Chem. Pharm. Bull. 19. 453(1971).
5. Schreiner, G. E., and Maher, J. F. : Toxic nephropathy. Amer. J. Med. 38 : 409(1965)
6. Hepinstall, R. H. : Pathology of the Kidney. (1966) Little, Brown, Boston.
7. Berndt, W. O. : The effect of potassium dichromate on renal tubular transport processes. Toxicol. Appl. Pharmacol. 32 : 40(1975)
8. Hirsh, G. H. : Differential effects of nephrotoxic agents on renal organic ion transport and metabolism. J. Pharmacol. Exp. Therap. 186 : 593(1973)
9. Kim, E., and Na, K. J. : Ann. Report KGTRI (1987)
10. Gooderson, C. Y. and Salt, F. J. : Microdetermination of chromium in biological material. Lab. Pract. 17 : 921 (1968)
11. Samits, M. H. : A current review of the chromate problem, in Dermatology Digest. Winnetka, III, Medical Digest, pp.69-76(1964)
12. Banner, Jr. W., Koch M., Capin, D. M., Hopf, S. B., Chang, S., and Tong, T. G. Experimental chelation therapy in chromium, lead boron intoxication with N-acetylcystein and other compounds. Toxicol. Appl. Pharmacol. 83 : 141(1986)
13. Pedersen, R. S., and March, P. T. : Chromic acid poisoning treated with acute hemodialysis. Nephron 22 : 592(1978).
14. Kaufman, D. B., DiNicola, W., and McIntosh, R. : Acute potassium dichromate poisoning. Amer. J. Dis. Child 119 : 374(1970)
15. Kim, E. and Na, K. J. Unpublished data (1988).


## 중크롬산 유발 급성신부전증에 미치는 홍삼 $S_I$-분획의 효과


나기정 강규상 김 은

한국인삼연초연구소

대전직할시 유성구 신성동 302



홍삼 $S_I$-분획의 투여로 중크롬산에 의한 신장세포독성이 감소되었다. 토끼에 중크롬산을 투여하면 급성신부전이 유발되어 10일이내에 대부분이 죽었다. 반면 홍삼 $S_I$-분획을 투여하면 치사율이 상당히 낮아졌으며, 생리적 증상이 현저히 개선되었다. 홍삼 $S_I$-분획의 투여로 중크롬산에 의하여 생기는 glomerulotubular imbalance가 호전되었으며, 당원생성, 해당속도, 젖산 생성속도가 정상화되었다. 홍삼 $S_I$-분획의 효과는 비타민 C, EDTA, 휴로세마이드에 비하여 월등하였다.