

Effect of Korean Red Ginseng on Sexual Dysfunction and Serum Lipid Level in Old Aged Men

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To investigate the effect of Korea Red Ginseng (KRG) on male sexual function and serum lipid level, the results of KRG treatments were compared to placebo group. A total of 35 patients with psychogenic impotence were assigned to medication group with KRG (n=28) and placebo (n=7). KRG and placebo were given to each group for 2 months. Changes in symptom such as frequency of coltus, morning erection, penile rigidity and tumescence in the group receiving KRG were significant higher than placebo group. The overall therapeutic efficacies on erectile function were 67% for KRG group and 28% for placebo group ($p < 0.08$). In the group treated with 9 tablets of KRG, there was tendency of better efficacy as compared to the group with KRG 6 tablets. The level of serum High Density Lipid-Cholesterol (HDL-Cholesterol) was significantly elevated ($p < 0.05$) while the other serum lipid such as total cholesterol, triglyceride and Low Density Lipid-Cholesterol (LDL-Cholesterol), were not changed after administration of KRG. This effect was observed significantly in the group in which KRG exerts its effect on sexual function ($p < 0.05$). The effect of KRG was pronounced when the level of cholesterol was high before starting KRG medication. 1 case of aggrevation of symptoms was reported. The Rigiscan parameters, maximal rigidity and tumescence, the tip and base of the penis, were not changed after administration of KRG. Neither the value of blood pressure nor the level of serum hormone, testosterone and prolactin, were statistically insignificant between pretreatment and posttreatment state. From the above results, the administration of Korea Red Ginseng has shown to have superior effects compared to the placebo. The tendency of good effect was increased when a lagre amount of KRG were administered. The mechanism of KRG in improving sexual function would be the result of elevating the level of seum HDL-Cholesterol in impotent patients. The effect of KRG was pronounced when the level of cholesterol was high before starting KRG medication. Therefore, the effect of KRG could be anticipated more accurately when the patients have high serum cholesterol level.

The Effect of Red Ginseng on the Immune Function of Gastrointestinal Carcinoma

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1. Backgrounds

This study was performed to evaluate the usefulness of red ginseng extract as adjuvant therapeutic agent improving immune function in immune compromising gastrointestinal carcinoma patient.

2. Material and Methods

We were treated 48 patients with two regimens after we were undertaken the curative resection for gastrointestinal carcinoma:

①only chemotherapy and immunotherapy (control group), ②chemotherapy and immunotherapy with 4500~5400 mg red ginseng for 6 months (study group). For investigating the immunologic alternations alongside the numerical changes in peripheral blood lymphocyte and their subsets in the gastrointestinal carcinoma patients, lymphocyte surface markers were determined by monoclonal antibodies on the preoperative day, and postoperative 5 days, 1 months, 3 months and 6 months in 28 controls and 20 red ginseng groups in gastrointestinal carcinoma patients which was recruited at Korea university hospital from march, 1995 to Desember, 1995.

The patient was measured and compared in both groups with the body weight, total protein and albumin, blood hematocrit and hemoglobin, total leukocyte, lymphocyte and lymphocyte subsets count in peripheral blood through planed schedules.

3. Results

The results were as follow:

- 1) The mean values for body weight increased in ginseng groups than in the control groups.
- 2) There were no significant differences of serum hematocrit, hemoglobin, total protein and albumin in both groups.
- 3) The total lymphocyte count was increased in the ginseng group than in control groups.
- 4) The T cell, B cell, suppressor/cytotoxic cel (CD8), helper/inducer cell (CD4), natural killer cell and activated T cell was increased in ginseng groups than in control groups.
- 5) There were increased significantly the T cell on postoperative 5 day, 3 months and 6 month, and B cell on postoperative 6 month in ginseng groups than in control groups.
- 6) There were increased significantly the suppressor/cytotoxic cell on postoperative 3 months and 6 months, helper/inducer cell on postoperative 5 days, and the natural killer cell and activated T cell on postoperative 6 months in ginseng groups than in control groups.

4. Conclusion

This data suggests that red ginseng may be useful as a longterm adjuvant therapeutic agent for improving the immune function and anticarcinogenic effect after curative operation for immune compromising gastrointestinal carcinoma patients.

Chemical Structures of Korean Red Ginseng Polysaccharides

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Neutral and acidic polysaccharides from white ginseng have been reported to have antitumor and immunopotentiating activities, respectively. It was also reported that the chemical compositions of the polysaccharides from white and red ginsengs quite differed from each other. However, the red ginseng polysaccharides have not been studied on the respects of their immunopotentiating activity. This project involves the purification and characterization of the red ginseng polysaccharides having the immunopotentiating activity, and the establishment of the method for large scale production of the immunopotentiating polysaccharides. This report describes the results obtained during the first year.

- 1) Ginseng marc (marc 1) obtained after the extraction of red ginseng with 95% ethanol contained the neutral and acidic polysaccharides. Both polysaccharides could be separated from each other

only by ion exchange chromatography on DEAE-cellulose.

- 2) Ginseng marc (marc 2) after the extraction of marc 1 with 65% ethanol absolutely contained the acidic polysaccharides, suggesting the acidic polysaccharides was separated from the neutral ones without ion exchange chromatography.
- 3) The three kinds of acidic polysaccharide fractions were prepared as following: ①Extraction of marc 2 with water gave PS2N, ②Extraction of marc 2 with 0.1 N NaOH gave PS2K, and ③Extraction of marc 2 with 0.1 N NaOH and then neutralization gave PS2H. The most high yield was found in PS2K. Ion exchange chromatography on DEAE-cellulose and gel filtration on Sephacryl S-500 revealed that the acidic polysaccharides having the average molecular weight of 2,00 K and 580 K were found in PS2K and PS2H, and that the 10 K acidic polysaccharide was found in all the 3 preparations. ④The immunopotentiating activity was found in the acidic polysaccharides. Among the 3 preparations, PS2N showed the most potent activity.

These results revealed that the immunopotentiating polysaccharides in red ginseng was the 10 K acidic polysaccharides. In the next research period we will characterize the 10 K acidic polysaccharides and elucidate its chemical structure.

Anticarcinogenic and Nicotine Detoxifying Effects of Red Ginseng

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Anticarcinogenic effect of Red Ginseng was comparatively studied in smokers for 4 weeks. Blood and urine samples were collected from nonsmokers (N=5), smokers controls (N=4), and smokers (N=12) who daily took RDA doses of Vit. C, Vit. E, β -carotene and Red Ginseng.

Quantitation of Benzo(a)pyrene-diolepoxide-I-tetrol (BPDE-I-tetrol) showed that β -carotene reduced the urinary extraction BPDE-I-tetrol by 45%, Red Ginseng by 41%, Vit. E by 34%, and Vit. C by 25% 4 weeks after treatment.

On the other hand, the formation of carbonyl contents, a form of oxidative protein damages, was 35% inhibited by Vit. E and 11~13% by other agents.

These data suggest that inhibitory effect of Red Ginseng on the urinary excretion of BPDE-I-tetrol may be chemopreventive in smokers.

Preclinical Evaluation of Polysaccharides Extracted from Korea Red-ginseng as an Antineoplastic Immunostimulator

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As a part of our ongoing effort to develop new antineoplastic immunostimulator from natural

sources, bioassay-directed fractionation on polysaccharide of Korea red ginseng was carried out by observing the proliferation of murine spleen cells and the generation of activated killer (AK) cells. The acidic polysaccharide fractions in proportion to their acidity proliferated spleen cells and generated activated killer cells *in vitro*. These activated killer cells killed both NK cell sensitive and insensitive tumor target cells without major histocompatibility (MHC) restriction. Adherent macrophages and CD4⁺ helper T cells were involved in the generation of the AK cells. The acidic polysaccharide from Korean red ginseng synergized with recombinant IL-2 (rIL-2) to induce increased AK activity and most dramatic synergy was seen at lower rIL-2 concentration than 3 U/ml. The optimal doses of the acidic polysaccharide from Korean red ginseng for the proliferation of spleen cells and for the generation of AK cell was 1 mg/ml and 100 µg/ml, respectively; this means that the mechanisms for the both activities may be different from each other.

The Effect of *Panax ginseng* on the Rabbit and Rat Corpus Cavernosal Smooth Muscle

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Ginseng, a medicinal herb, has been used in maintaining physical vitality throughout the far-eastern countries including Korea and China. The red ginseng is a complex mixture of natural products from the ginseng and there are a lot more reports, which are effects on diabetes mellitus, effects on atherosclerosis and hypertension, anti-stress, anti-aging, improving effects on blood circulation, effect on cancer, *et al.* In this study we investigated the action of ginseng on the corpus cavernosum using rabbit and rat.

Rabbit muscle strips submaximally precontracted with phenylephrine (5×10^{-6} M) and treated with increasing concentrations of ginseng (1, 5, 10, 20, 30, 40 mg/ml) showed tension decrease concentration-dependently (1 mg/ml : 1.7%, 5 mg/ml : 10.2%, 10 mg/ml : 22.7%, 20 mg/ml : 44.0%, 30 mg/ml : 65.2%, 40 mg/ml : 95.6%). Relaxations to ginseng were inhibited significantly by endothelial disruption, by pretreatment with methylene blue, pyrogallol, L-NNA and atropine. Pretreatment of muscle strips with ginseng caused concentration-related inhibition of a phenylephrine induced contraction, and in calcium-free high potassium depolarizing solution, decreased basal tension as well as inhibited contraction induced by CaCl₂. Ginseng also produced the reduction of responses to depolarizing medium (20, 40, 60 mM KCl).

Intracavernous ginseng at doses of 10 mg and 100 mg caused an increase in the intracavernous pressure of 19 cm H₂O and 42 cm H₂O, respectively.

After 2 month administration of ginseng, the relaxation action of acetylcholine and ginseng was significantly increased and long-term administration of ginseng significantly increased the intracavernous pressure response to electrostimulation.

With these results we can confirm the relaxation effect of ginseng at a dose dependent on the cavernosal smooth muscle and suggest that its action is mediated by multiple action mechanisms that in-

clude increasing the release of nitric oxide from the corporal sinusoids, increasing intracellular calcium sequestration, and a hyperpolarizing action. Long-term administration of ginseng also enhances the erectile capacity.

Studies on the Differentiation Mechanisms of Ginsenosides in Tumor Cells

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We have previously identified that the ginsenosides, extracts of *Panax ginseng* C.A. Meyer, have the ability to induce the differentiation in cultured F9 teratocarcinoma stem cells. Among the various purified ginsenosides, Rh₁ and Rh₂ caused the differentiation of F9 cells most effectively. Since ginsenosides and steroid hormone are very similar in their gross molecular shapes, we studied the possibility of the involvement of nuclear receptor(s) in the differentiation process induced by the ginsenosides. To identify the possible nuclear receptor(s) induced by ginsenosides, we performed RT-PCR with degenerated primers of highly conserved domain of nuclear receptors. As a result, we identified several PCR products which have a high level of sequences similarity of known nuclear receptors and the expression of them was regulated by ginsenoside Rh₁ and Rh₂. Since one of identified clones (c3-4) has highly similar sequence homology with GR, we tested the possible involvement of GR in the mechanism of differentiation by performing cotransfection assay with GR expression plasmid and GRE-luciferase reporter vector. In the presence of ginsenoside Rh₁ or Rh₂, the luciferase activity of the GRE reporter was stimulated and the effect was augmented in combination with dbcAMP. In addition, G-Rh₁ and G-Rh₂ bound to a GR assessed by whole-cell binding assay, even though the specific binding affinity was weaker compared to dexamethasone.

To identify novel gene(s) which is induced by ginsenosides, we performed DDRT-PCR and identified several bands which are differently displayed during the differentiation induced by ginsenoside Rh₁.

The changes in protein kinase C (PKC) isoform pattern were examined immunologically during differentiation of HL60 cells by ginsenoside Rh₂. In untreated HL60 cells, cytosolic PKC α and β isoforms were detected, but neither cytosolic PKC γ nor all three isoforms in membrane fraction from both untreated and ginsenoside Rh₂-treated HL60 cells were detected. When HL60 cells were treated with 25 μ M ginsenoside Rh₂ for 3, 5, 7 days, no significant change was observed in PKC α level, however 55 kDa protein of similar mass to catalytic domain appeared following treatment with ginsenoside Rh₂. The β and γ isoforms were increased by prolonged treatment and at least 5 days after treatment with ginsenoside Rh₂, respectively. These results suggest that the increase in the isoforms levels may coordinately contribute to differentiation of HL60 cells by ginsenoside Rh₂ and proteolysis of α isoform may be ginsenoside Rh₂-specific process.

Effects of Rb₁, Rb₂, Rc, Rd, and Rg₁ on Antinociception Induced by Opioids Administered Intracerebroventricularly

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척수상부로 투여된 morphine, beta-endorphin, DPDPE, 그리고 U50,488H는 각각 다른 opioid 수용체를 자극하여 다른 내재성 하행성 동통억제계를 활성화 시킴으로 진통작용을 유발시킨다. 본 연구에서는 척수강으로 투여된 Rb₁, Rb₂, Rc, Rd, 그리고 Rg₁이 척수상으로 투여된 morphine, beta-endorphin, DPDPE, U50,488H에 의한 진통작용에 아무런 영향을 미치지 아니하였다. 척수로 투여된 Rd는 뇌실내로 투여된 morphine, DPDPE, U50,488H에 의한 진통작용을 효과적으로 감소시키었지만 beta-endorphin에 의한 진통작용에는 아무런 영향을 미치지 아니하였다. 척수로 투여된 Rg₁는 뇌실내로 투여된 morphine, DPDPE에 의한 진통작용을 효과적으로 감소시키었지만 beta-endorphin, U50,488H에 의한 진통작용에는 아무런 영향을 미치지 아니하였다. 척수로 투여된 Rb₁는 뇌실내로 투여된 U50,488H에 의한 진통작용만 선택적으로 감소시키었다. morphine, beta-endorphin, DPDPE에 의한 진통작용에는 아무런 영향을 미치지 아니하였다. 척수로 투여된 Rb₂는 뇌실내로 투여된 morphine과 U50,488H에 의한 진통작용을 감소시키었지만 beta-endorphin과 DPDPE에 의한 진통작용에는 아무런 영향을 미치지 아니하였다. 척수로 투여된 Rc는 뇌실내로 투여된 morphine, beta-endorphin, U50,488H에 의한 진통작용을 효과적으로 감소시키었지만 DPDPE에 의한 진통작용에는 아무런 영향을 미치지 아니하였다. 이와 같은 결과는 각각 다른 ginsenosides는 척수강으로 투여될 때에 다른 종류의 opioid 수용체를 자극하는 opioid 효현제들의 진통작용을 다른 mode로 block하는 작용이 있음을 시사한다.

이상의 연구를 통하여 척수상부로 투여된 β -endorphin, morphine, DPDPE 및 U50,488H의 진통작용에 대한 ginsenosides의 영향을 검색함으로써 진통작용 기전에 있어서 opioid system과 홍삼의 상호관계를 자세히 이해하는데 기여하리라고 사료된다. 더 나아가서 임상적으로도 응용되리라 사료된다.

Effect of Red Ginseng Saponins on Intestine Contractility

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Isolated rabbit jejunal segments were used to study the effects of ginseng total saponins (GTS), protopanaxatriol saponins (PT) and protopanaxadiol saponins (PD) on intestinal contractility.

GTS, PT, and PD caused a dose-dependent decrease in intestinal spontaneous movements, and PT was the most efficacious of them.

Pretreatment with tetraethylammonium chloride (3~10 mM) antagonized the effect of GTS, PT, and PD. Furthermore, 4-aminopyridine (1 mM) also inhibited the effect of GTS, PT, and PD.

However, the effect of GTS, PT, and PD were not blocked by pretreatment with phenotolamine (10^{-6} M), yohimbine (10^{-6} M), dl-propranolol (10^{-6} M), naloxone (10^{-6} ~ 10^{-5} M), N_ω-nitro-L-arginine methyl ester (10^{-4} M), methylene blue (10^{-5} M), and N-ethylmaleimide (10^{-4} M).

The results suggest that GTS, PT, and PD inhibited the spontaneous movements in isolated rabbit je-

junum by causing hyperpolarization through an activation of K^+ channels directly.

Effect of Lipid-Soluble Components of Korean Red Ginseng on Proliferation and Surface Antigen Expression of Renal Cell Carcinoma Cell Lines

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We have investigated growth inhibitory effects of lipid soluble components of the Korean red ginseng. Petroleum ether extract of the Korean red ginseng (crude extract), its partially purified preparations (7:3 GX-1, and 7:3 GX-2), and two of its pure components, panaxydol and panaxynol were added to the cultures of three human renal cell carcinoma (RCC) cell lines, A498, Caki-1, and CURC II and 3H-thymidine incorporation was measured. On a silica gel TLC analysis, the crude extract contained 10 fractions (1~10), 7:3 GX-1 9 fractions (2~9), 7:3 GX-2 5 fractions (6~10), panaxydol 2 fraction (6~7), and panaxynol 2 fractions (4~5). Crude extract and 7:3 GX-1 inhibited the growth of A498, CURC II, and to a much less extent, Caki-1, in a dose dependent manner. Primary cultures of normal kidney epithelial cells were also used as control normal cells and the growth inhibition of normal kidney cells by the crude extract was comparable to that of Caki-1. On the other hand, 7:3 GX-2 was much potenter than the crude extract and the 7:3 GX-1 in inhibiting proliferation of RCC cell lines. Panaxydol was more effective in inhibiting the growth of RCC cell lines than crude extract and 7:3 GX-1, but less effective than 7:3 GX-2. However, the effect of panaxynol was about the same as crude extract or 7:3 GX-1. Therefore, it is suggested that using a combination of a few components of the 7:3 GX-2 is better than using a single component in inhibiting proliferation of RCC cell lines. Although what would be the best combination is a subject of a further investigation, 7:3 GX-2 is the most effective one among tested. IL-4 was added together with crude extract to the culture of RCC cell lines to investigate whether there is any synergistic or additive effect. IL-4, in fact, showed an additive effect in the growth inhibition of RCC cell lines by the crude extract. The additive effect was the most pronounced in Caki-1 which responded poorly to the crude extract. In conclusion, proliferation of the three human RCC cell lines was effectively inhibited by lipid soluble components of Korean red ginseng alone or in combination with IL-4, and among different preparations, the 7:3 GX-2 containing TLC fractions 6 to 10 was the most effective one.

Effect of Ginseng Components on Ryanodine Receptor-Ca²⁺ Channel Complex Protein in Sarcoplasmic Reticulum of Skeletal Muscle

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In this study, rat liver primary cultured cells were obtained by using the perfusion technique to investigate the mechanism of hypoglycemic action of *Panax ginseng* components on diabetic rats. These cells were incubated under 4 different media composition such as glucagon (control 1) containing media without insulin, glucagon+ginseng components [ginsenoside (mixture, Rb₁ and Rg₁), fat soluble fraction] containing media, glucagon+insulin containing media, and glucagon+insulin+ginsenoside (control 2) containing media for 12 h in the CO₂ incubator, and then homogenized and sonicated to examine the effect of ginseng components on the activities of Glucokinase, Glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, Acetyl CoA carboxylase and Glucose-6-phosphatase which were directly related to the glucose metabolism. The result from these assays was that the activities of all enzymes such as Glucokinase, Glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, Acetyl CoA carboxylase except Glucose-6-phosphatase were increased after the addition of ginseng components to the control 1 and control 2 at almost all the range of their concentrations. The activities of some enzyme were increased 2~4 folds than control 1 or control 2. This means that ginseng components can do well hypoglycemic action. However, ginsenoside Rb₁ and Rg₁ made increase of their activities rather than decrease. Hence, more detailed study of them must be made in the near future.

Random Amplified Polymorphic DNA (RAPD) 기술을 응용한 고려인삼과 외국인삼의 구별법 구명

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In contrast to most other crops, in ginseng, little effort has yet been exerted for breeding. Such a lack of breeding program in ginseng was mainly due to long generation time and difficulty of cultivation. Meanwhile, there was also rare data on genetic markers and its analysis. With the recent progress of molecular biology, new method such as Random Amplified Polymorphic DNA (RAPD) analysis with which the genetic variation could be identified by DNA polymorphism has been developed. RAPD analysis is a technique for *in vitro* amplification of specific DNA sequences by using Polymerase Chain Reaction (PCR).

There are a number of advantages of RAPD such as technical simplicity of RAPD assay, easiness of polymorphism detection with high level of resolution, uses of small amount of template DNA, and easy and fast manipulation.

The present study was attempted 1) to clarify the taxonomic relationships among *Panax* species using the RAPD analysis, 2) to explore the genetic diversity of *Panax ginseng* cultivars and lines, and 3) to develop RAPD markers for distinguishing the Korean and Chinese cultivated land varieties identification of *Panax ginseng*.

The results obtained were summarized as follows:

- 1) The number and intensity of bands were different among PCR products obtained by using different primers. As the result of RAPD which applied to ginseng cultivars using 20 primers, only 9 primers produced clear and reproducible band patterns. Selected 9 primers were A02, A04, A07, A09, A10, A11, A12, A13, and A20 of Operon kit series A.
- 2) For evaluation of geographical diversity among the *Panax* species, five *Panax* species were collected worldwide and analyzed using RAPD techniques. The size of the amplified DNA fragments ranged from 200 bp to 2,500 bp. Two hundreds ten bands of 218 stable amplification products obtained showed polymorphism, and the remaining products were mono-morphic. The five *Panax* species analyzed in this study showed a genetic similarity coefficient of 0.224 to 0.437 genetic distance (GD). The most close relationship was between Bamboo ginseng and American ginseng of 0.42 GD, and the Korean ginseng was least related among the 5 species.
- 3) To explore the relationships of genetic diversities among *Panax ginseng* lines, 9 lines collected from Korea, Japan and Russia were applied to RAPD analysis. The size of the amplified DNA fragments ranged from 170 bp to 2200 bp. One hundred sixty five bands of 178 stable amplification products obtained showed polymorphism. Similarity coefficients among 9 Korean ginseng lines were ranged from 0.289 to 0.858. The nine *Panax ginseng* lines analyzed were clustered into two groups, A and B. The group A is comprised of Cheongkyung, KG101 and Hwangasuk, and the group B is comprised of Jakyung strains (Jakyung 79742, Jakyung 81783, Jakyung 84791, Jinjakyung, Russia Jinjakyung and Mimaki).
- 4) As the result of PCR of 25 Korean ginseng land varieties collected from Korea, China and Japan, the size of amplified DNA fragments were distributed 0.2 to 2.5 Kb and produced the 165 polymorphic bands. Twenty five land varieties of the Korean ginseng were classified into two groups according to similarity coefficients. Group A includes most of land varieties collected from Korea and group B includes the land cultivars collected from China and Japan mostly.
- 5) A single RAPD band was enough to identify the land varieties collected from Jingyu, Fusong, Changbai, and Shizou in China and Nagano in Japan. Two RAPD bands with a single primer were needed to distinguish among the land cultivar collected from Gaeseong in north Korea and the Korean and cultivars. Three or 7 RAPD bands with one primer were needed to identify the land cultivars collected from Jian and Liaoning in China.

Biochemical and Molecular Biological Studies on Antioxidative Mechanisms through Induction of Antioxidative Enzyme and Protein of Crude Extract and Components of Red Ginseng

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Oxygen free radicals are highly reactive molecules with unpaired electrons, which are produced with in aerobic cells in the course of normal metabolic events. Normally, aerobic cells are protected from the damage of free radicals by antioxidative enzymes such as superoxide dismutase (SOD), catalase, glutathione (GSH), peroxidase, GSH S-transferase and GSH reductase which scavenge free radicals as well as nonenzymatic antioxidants such as ceruloplasmin, albumin and nonprotein-SH including GSH. We have investigated the effects of total saponin of red ginseng and components of red ginseng on the endogenous antioxidants in order to elucidate antioxidative mechanisms of red ginseng.

The treatment with total saponin of red ginseng significantly decreased the contents of malondialdehyde and total free radicals in the liver. On the other hand, total saponin of red ginseng significantly increased the activities of SOD, catalase and GSH reductase and nonprotein-SH level.

The effects of components (ginsenoside Rb₁, Rb₂, Rc, Rd, Re, Rg₁, Rf, Rh₁ and Rh₂) of red ginseng on the antioxidative enzyme activities were investigated in the liver. Especially, ginsenoside Rh₂ significantly increased catalase activity.

These results suggest that total saponin of red ginseng exerts an antioxidative effect by increasing endogenous antioxidants and ginsenoside Rh₂ is an important active component among total saponins of red ginseng.