*Korean J. Ginseng Sci.*
Vol. 19, No. 1, 31~38(1995)

# Antioxidant Action of Ginseng : An hypothesis

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(Received November 28, 1994)

**Abstract**□Antioxidant effect of Korean ginseng (*Panax ginseng* C.A. Meyer) was investigated in rats. Long-term administration of ginseng water extract protected the activity of liver cytosolic SOD, catalase and glutathione peroxidase from being significantly decreased with advancing age ($p<0.05$). It was more effective toward glutathione peroxidase than other antioxidant enzymes. However, the level of sulfhydryl compounds and its related enzymes such as glutathione reductase and glutathione-S-transferase was not significantly changed by the administration of ginseng. Liver microsomal formation of reactive oxygen species such as superoxide and hydrogen peroxide did not show a significant difference between two groups although it was slightly decreased with age, but lipid peroxidizability of microsomal membrane induced by a prooxidant was slightly lower in ginseng-treated rats. Interestingly, antioxidant capacity of plasma from ginseng treated rats on autooxidation of ox-brain homogenates was much higher than that of normal ones. However, resistance of RBC membrane against oxidative stress showed a similar tendency. The content of serum TBA reactive substances lowered consistently in the rats treated with ginseng at all corresponding age and a significant difference between two groups was found at 24 months of age ($p<0.05$). Ginseng extract protected lipid peroxidation in brain and liver. This protection was more effective in the stressed rats imposed by immobilization than normal ones. In conclusion, ginseng water extract protected the age related deterioration of major antioxidant enzymes, and this effect was more striking with increasing duration of treatment. This comprehensive antioxidant action of ginseng seems to be bya certain action of ginseng other than a direct antioxidant action, which might be a long term normalizing effect through the harmony of various components.

**Key words**□ginseng, antioxidants, SOD, TBARS, immobilization stress.

## Introduction

Antioxidant action of Korean ginseng (*Panax ginseng* C.A. Meyer) has been known as one of the putative pharmacological efficacies. Anti-aging effect of ginseng which was alluded in many previous literatures might be based on this action. Because free radical theory of biological aging is accepted as one of the most plausible hypothesis and many studies evidenced that free radicals are involved in the incidence of major degenerative diseases.[1-3] Various beneficial effect was observed in animals and human administrated with well known antioxidants.[4,5]

So far, various components possessing antioxidant activity have been identified in ginseng roots.[6] However, we can find that there are several serious problems in evaluating antioxidant action of ginseng on the based of traditional concept. Firstly, in most cases, antioxidant activity was observed in short term oxidative stress models or *in vitro* assay systems, and a single component or subfraction obtained by solvent extraction was used.[7-10] Although such approaches have some advantages in expediency, these are irrelevant to the traditional concepts in verifying ginseng efficacy. In traditional oriental

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medicine, ginseng roots are used as a decoction rather than a single extract. Long-term treatment rather than short-term with the individualization of the prescription according to the pateint's physical conditiion was usually adopted. In this case, by-products through gastrointestinal digestion or metabolites of ginseng components formed by metabolism via organs such as liver would also be an ingredient for the antioxidant action.

Secondly, ginseng has been known to have various components having antioxidant activity such as maltol and some phenolic compounds.[11,12] Many people have believed that antioxidant action of ginseng was due to such compounds. But, the antioxidant potency of these components against the peroxidative reaction of lipids is not high, and their contents are relatively low.[11] Therefore, it is difficult to expect the effective antioxidative action from them.

Another point is the difference in the composition between organic solvent extract and water extract which is used in the real practice. Nevertheless, many investigators still have their interest on the antioxidant activity of ginseng and are seeking it by using more scientific methods. Chung *et al.*[13,14] demonstrated that some components of ginseng activate the activity of antioxidant enzymes. These results suggest that ginseng has an effect to modulate the antioxidant capacity although the mechanism is yet to be elucidated.

*In vivo* status of antioxidant and prooxidant balance are settled by various factors. Therefore, we attempted the study to manifest antioxidative action of ginseng on the basis of traditional conception in the method of its preparation and administration. We chronically administrated the water extract of ginseng to rats with amount of common dose for human together with drinking water and investigated the change of antioxidant capacity in the rats with advancing ages. An the effect of ginseng on the stressed animal imposed by immobilization was also investigated. Based on the results obtained, we propose here an hypothesis on the antioxidant action of ginseng.

## Materials and Methods

### 1. Chemicals

Ferricytochrome c, NADH, NADPH, reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase, xanthine, xanthine oxidase, glucose-6-phosphate dehydrogenase, hydrogen peroxide, 5,5′-dithiobis-2(2-nitrobenzoic acid) (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), tris [hydroxymethyl] aminomethane, N-[2-hydroxyethyl] piperazine-N′-[2-ethansulfonic acid (HEPES), cumene hydroperoxide, potassium ferricyanide, sodium deoxycholate, sodium dodesylsulfate, trichloroacetic acid were puchased from Sigma Chemical Co. (St. Louis MO). 2,2′-Azobis (2-amidinopropane) hydrochloride (AAPH) was obtained from Polyscience Co. (Warrington, PA). Other chemicals used were of analytical purity grade.

### 2. Preparation of ginseng water extract

The extract was made from ginseng powder (roots of 6-year-old, 30~40 mesh) by soaking it for 4 hrs in 5 volumes of hot water. Temperature of the water was maintained at 70℃ to prevent saponins and other phenolic compounds from being destoryed by heat.[12] This procedure was repeated more than twice. The extract obtained was combined and concentrated to 16% water content. This preparation commonly contains 26 kinds of saponins including panaxadiols and triols.

### 3. Animals and their treatment

Male Sprague-Dawley rats were housed singly in polycarbonate cages and reared in the conventional system under condition of a 12 hr light as 200~300 Lux and dark cycle, 20±2℃ in temperature, 40~60% in humidity, and 15 times an hour in changing air. At 6 weeks of age rats were divided into two group : normal group was supplied with only water and the ginseng-treated group was administered ginseng water extract together drinking water (25 mg/kg/day). Water and the ginseng solution were replaced every day and this treatment had been continued until the animal was sacrificed. Seven rats in each group were sacrified at 3, 6, 12 and 24 months of age, respectively, and blood was obtained by cardiac puncture. Liver tissues were homogenized in 4 volume of 0.1 M HEPES buffer (pH 7.4) after washing with saline. Cytosols were prepared frm liver homogenates bydifferential cent-

rifugation. For the determination of plasma antioxidant capacity and the resistance of RBC membrane against oxidative stress, only rats aged for 21 months were used. All materials including serum, plasma, cytosols and microsomes were stored at −70℃ until use.

**4. Biochemical assay**

Superoxide dismutase (SOD) activity was measured by monitoring the inhibition of cytochrome c oxidation at 550 nm by using xanthine oxidase to generate superoxide radical according to the procedure of McCord *et al.*[16] Catalase activity was assayed by the method of Aebi[17] based on the direct measurement of decomposition of hydrogen peroxide at 240 nm spectrophotometrically. Glutathione peroxidase activity was measured with the coupled-enzyme system using cumene hydroperoxide as a substrate.[18] Glutathione reductase activity was determined by measuring NADPH oxidation at 340 nm.[19] Glutathione-S-transferase activity was assayed by the method of Habig *et al.*[20] using CDNB as a substrate.

Content of total sulfhydryl groups was measured at 412 nm according to the procedure of Sedlak and Lindsay[21] using DTNB. Protein concentration was determined by the method of Lowry *et al.*[22] with bovine serum albumin as a standard. Microsomal superoxide and hydrogen peroxide formation was measured by the methods of Aust *et al.*[23] and Alfred *et al.*[24] respectively. Lipid peroxidizability of microsomal membrane by AAPH was determined bythe method of Lee and Yu.[25] TBA reactive substances in serum were determined by the method of Suematsu *et al.*[26] Plasma antioxidant capacity of rats was measured by the method of Stockes *et al.*[27] using ox brain homogenates. The resistance of RBC membrane against oxidative stress was measured according to the method of Niki *et al.*[28]

Results are expressed as mean± SD. The data were analyzed by student's t-test using T-Test software.

**5. Immobilization stress**

Immobilization stress of rats (Albino rats) was carried out for 2 hrs by using a universal restrainer. Then liver and brain were carefully removed after cervical dislocation. And antioxidant activity of ginseng water extract was tested in ascorbate[29] and NADPH-dependent[30] lipid peroxidation in liver and brain microsomes.

## Results

To compare the antioxidant status of the cytosols from normal and ginseng-treated rats with age, the level of major antioxidant enzymes, sulfhydryl com-

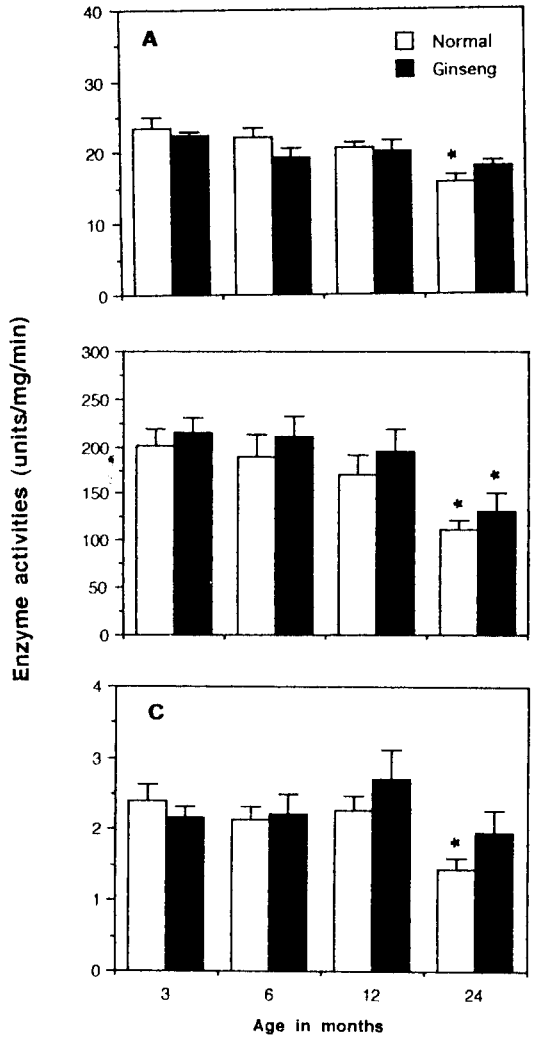


**Fig. 1.** The effect of ginseng-treatment on the activity of superoxide dismutase (A), catalase (B) and glutathione peroxidase (C) in rat liver. *Significant different from 3 month ($p<0.05$).

**Table 1.** Age-related changes in the level of free-SH total-SH, GSH reductase and GSH-S-transferase in the liver of normal and the ginseng-administered rats

| | | Age (months) | | | |
|---|---|---|---|---|---|
| | | 3 | 6 | 12 | 24 |
| Free-SH[a] | N | 1.3± 0.3 | 3.3± 0.5 | 4.3± 0.5 | 3.4± 0.6 |
| | G | 1.4± 0.3 | 4.4± 1.0 | 4.1± 0.6 | 4.0± 0.5 |
| Total-SH[a] | N | 15.0± 1.0 | 14.9± 1.6 | 15.2± 2.0 | 14.7± 0.9 |
| | G | 16.9± 1.2 | 16.2± 1.8 | 14.5± 2.4 | 15.0± 1.1 |
| GSH reductase[b] | N | 33.2± 4.2 | 26.1± 5.3 | 31.7± 6.8 | 25.1± 6.3 |
| | G | 32.7± 2.6 | 28.1± 4.2 | 24.4± 6.9 | 31.6± 4.1 |
| GSH-S-transferase[b] | N | 1.16± 0.02 | 1.01± 0.01 | 0.96± 0.07 | 0.44± 0.04* |
| | G | 1.15± 0.07 | 1.00± 0.11 | 1.06± 0.13 | 0.44± 0.05* |

[a] μmole/g tissue.
[b] μmole/mg protein/min.
*Significantly different from 3 month of age ($p<0.01$).

**Table 2.** The formation of superoxide and hydrogen peroxide in liver microsomes of aging rats

| Age (months) | Superoxide | | Hydrogen peroxide | |
|---|---|---|---|---|
| | Normal | Ginseng | Normal | Ginseng |
| 3 | 40.1± 5.0 | 44.0± 4.5 | 1.98± 0.08 | 1.94± 0.16 |
| 6 | 35.5± 5.5 | 39.1± 4.0 | 1.87± 0.31 | 1.76± 0.14 |
| 12 | 25.5± 5.1 | 24.5± 6.1 | 1.31± 0.19 | 1.34± 0.49 |
| 24 | 18.2± 5.2 | 20.2± 6.1 | 0.87± 0.14 | 0.96± 0.07 |

Units : μmoles/mg/min.

pounds and glutathione related enzymes activities was measured. As shown in Fig. 1-A, the activity of cytosolic SOD which scavenges superoxide anions was significantly decreased in normal group age-dependently. However, it was not significantly decreased in the ginseng-treated rats with age ($p<0.05$). Catalase has been known as one of important antioxidant enzyme which catalyzed the conversion of hydrogen peroxide to water. This enzyme activity was also higher in ginseng-treated group than in normal one at all corresponding ages as shown in Fig. 1-B ($p<0.05$). Glutathione peroxidase scavenges hydroperoxide such as lipid hydroperoxides. The enzyme activity was also decreased at 24 months of age in normal rats, however, it was not changed and was maintained with high activity until senescence in ginseng treated rats (Fig. 1-C).

**Table 3.** Lipid peroxidizability of microsomal membrane induced by AAPH (Unit : $A_{532}$)

| Age (months) | Normal | Ginseng |
|---|---|---|
| 3 | 0.25± 0.02 | 0.24± 0.02 |
| 6 | 0.24± 0.03 | 0.23± 0.02 |
| 12 | 0.22± 0.01 | 0.20± 0.09 |
| 24 | 0.20± 0.02 | 0.18± 0.02 |

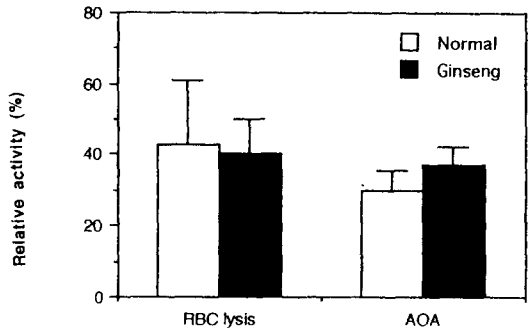


**Fig. 2.** Resistance of RBC membrane against oxidative stress induced by AAPH and antioxidant activity (AOA) of plasma from normal and ginseng-treated rats.

Table 1 shows age-related change in the level of free or total sulfhydryl compounds, glutathione reductase and glutathione-S-transferase in the liver of normal and ginseng treated rats. Activities of these enzymes are not significantly changed between two groups or with age except that glutathione-S-transferase activity was remarkably decreased at 24 months of age in both group ($p<0.01$).

Table 2 shows results of liver microsomal formation of superoxide and hydrogen peroxides. Formation of these reactive oxygen species (ROS) was slightly decreased with age, but there was no significant difference between two groups at the corresponding age. To compare the rigidity of microsomal membrane, lipid peroxidizabilityof the membrane by AAPH was measured. The inducibility was decreased with age, and was slightly lower in the ginseng-treated rats than in normal ones (Table 3).

Fig. 2 shows the resistance of RBC membrane against oxidative stress induced by AAPH, a peroxyl radical generator, and plasma antioxidant capacity. In this case, RBC obtained from 21 months

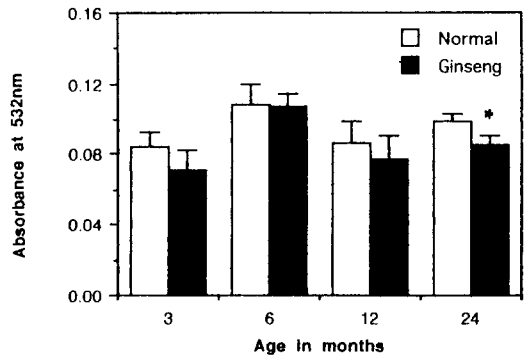


**Fig. 3.** The content of TBARS in serum from normal and the ginseng-treated rats. *Significantly different from normal at the same age ($p<0.05$).

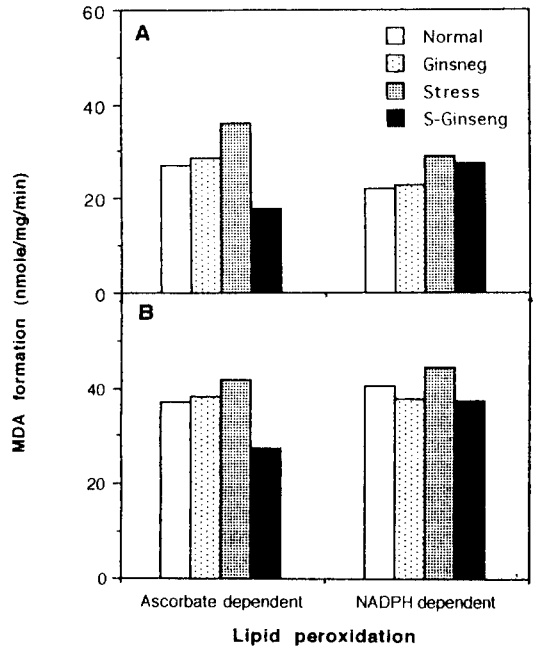


**Fig. 4.** The influence of ginseng extract on lipid peroxidation in brain (A) and liver (B) microsomes from normal and the stress imposed rats with immobilization. The activity was measured in pooled microsomes from 5 animals in each group.

old rats were used. Hemolysis value of washed RBC obtained from normal rats was 42.4± 5.0% under our suggested condition, and ginseng treated rats showed similar tendency. However, antioxidant capacity of plasma from ginseng-treated rats was slightly higher than that from normal ones.

TBA reactive substances (TBARS) in serum can be an useful index for the oxidative damage of biomacromolecules, because the oxidized proteins or by-products of lipid peroxidation form complex with TBA which shows absorbance at 532 nm. As shown in Fig. 3, no significant change was noticed with advancing age in both groups. But, it was consistently lower in ginseng-treated rats than in normal ones during their life time, and significant difference was noticed at 24 months of age ($p<0.05$).

Fig. 4 shows that the ascorbate- and NADPH-dependent lipid peroxidation in brain microsomes increase correspondingly by about 28% and 23% during immobilization stress. In stressed animals, ginseng decreases ascorbate dependent lipid peroxidation by 47% and has a small decreasing effect on NADPH dependent lipid peroxidation. Interestingly, the extract has no effect no lipid peroxidation in intact (not stressed) animals. It showed a similar effect on lipid peroxidation in liver microsomes.

## Discussion

Preservation of the cytosolic defense system against the threat of deleterious oxidative processes and maintenence in balance of *in vivo* antioxidant and prooxidant are essential for the anti-aging of organism. Therefore, we investigated the status of antioxidant defense system in normal and ginseng-treated rats to evaluate the antioxidant action of ginseng. Antioxidant activity of ginseng has been extensively studied, mostly focusing on the direct action of some components such as fat-soluble fractions including polyphenols.[5,9,14] But, some components of ginseng can also be converted to different components through digestion in gastrointestinal systems, which can act as an antioxidant.

In this study, we obtained a clear evidence that ginseng has an effect to preserve the activity of antioxidant enzymes from age-related deterioration. The activities of SOD, catalase and glutathione peroxidase, which are major antioxidant enzymes responsible for scavenging reactive oxygen species, were well maintained in the ginseng-treated rats until the senescence stage although. This is the

first report showing that the activity of antioxidant enzymes is well preserved by long term administration of water extract of ginseng. Such changes in the activity of enzymes imply a very important meaining in conection with *in vivo* free radical metabolism. If these enzymes were remarkably enhanced, it means that ginseng was the cause of the generation of oxygen free radicals. However, there was no significant difference in the radical production between two groups.

The data suggest that ginseng does not stimulate the generation of oxygen free radicals *in vivo*, but it retards the age-related deterioration of radical scavenging enzymes. Such effects might be resulted from homeostasis-maintaining action of ginseng when it was administered for long term.

Especially, catalase and glutathione peroxidase complement each other with respect to intercellular location. Catalse scavenges more effectively at high concentration of hydrogen peroxide, whereas glutathione peroxidase does it more at low concentration.[4] Glutathione peroxidase catalyzes preferably large molecular hydroperoxides such as lipid hydroperoxides formed by free radical attack to polyunsaturated fatty acids in membrans, whereas catalase is suitable for scavenging low molecular hydroperoxides such as hydrogen peroxide. [4] Therefore, the effective preservation of glutathione peroxidase activity in ginseng-treated rats can be thought as one of desirable phenomenon in free radical metabolism in connection with lipid peroxidation of biological membranes. The low level of TBARS in serum from ginseng-treated rats clearly evidence its antioxidant effect since TBARS in serum reflects the status of oxidative stress on the whole body. This seems to be closely related to the increase in glutathione peroxidase activity because it is more contributed to the elimination of toxic lipid hydroperoxides.

Thiols like GSH are very important and potent reducing agents in cells. Long term administration of ginseng to rats did not have an affect on the level of free of total sulfhydryl compounds, glutathione reductase and glutathione-S-transferase. These results suggest that ginseng is not involved in the metabolism of cellular thiols.

Microsomal mixed function oxidase system is an important source of *in vivo* free radical generation.[31] Age-related decline of this enzyme activity can cause to reduce the detoxification capacity.[32] The rate of superoxide and hydrogen peroxide generation in microsomes by the stimulation with NADPH was slightly decreased with increasing age. Such an age-dependent reduction in ROS formation was well in accord with the observation in our previous study.[33] Microsomal lipid peroxidizability also showed a similar pattern. This phenomenon might be closely related to the decrease in content of microsomal cytochrome P-450 in the aged-rats. However, the rate of lipid peroxidation in microsomal membranes induced by AAPH was low consistently in the ginseng-treated rats although the rate of ROS formation was not significantly different. It is unlikely that antioxidant enzymes were increased in ginseng-treated rats. These results indicate that hepatic cellular membranes from ginseng-treated rats is more stable against free radical attack than that of normal ones.

We compared the rigidity of RBC membrane against oxidative stress and plasma antioxidant capacity. The resistance of RBC membrane against oxidative stress was not significantly changed by long-term administration of ginseng. In general, the protection of cell membrane from damage by oxidative stress is accomplished by antioxidants incoporated in the membrane such as vitamine E and or intracellular antioxidants. Membrane fluidity also can influence on the membrane resistance. We did not check the level of intracellular antioxidants and membrane fluidity. However, antioxidant capacity of plasma was higher in ginseng-treated rats than in normal ones as showing the same result of our previous study.[5]

Many hypotheses are possible in connection with the enhancement of *in vivo* antioxidant capacity by administration of ginseng. The first possible explanation is that it might be due to the stimulation of gene expression of antioxidant enzymes because ginseng has components to stimulate the synthesis of proteins and DNA.[34]

The enhanced activity of antioxidant enzymes and plasma antioxidant capacity may contribute to

scavenge ROS or other free radicals formed through lipid peroxidation.

Secondly, *in vivo* generation of free radicals other than oxygen free radicals is reduced by certain actions of ginseng such as regulation of lipid metabolism. There are many reports suggesting that ginseng reduces serum cholesterol and triglycerides.[35-37] Although it is difficult to expect with only these data, only certain single component does not seem to contribute to the reduction of free radical damage because major components of water extract are mostly saponins and polysaccharides and the amount of antioxidant components such as maltol are relatively rare. As mentioned above, a certain metabolite(s) of ginseng might have antioxidant action regulating free radical metabolism. Many byproducts were observed in the incubation mixture of saponins with liver microsomes and in the digests by gastic iuice.[38,39]

We observed another interesting action of ginseng in rats exposed to the stress. Its antioxidant activity was more effective in the animal of abnormal status than normal. This is valuable evidence for the understanding of mysterious efficacy of ginseng.

From the data of all of these, we concluded as follows. First, ginseng preserves the activity of major antioxidant enzymes *in vivo*. Secondly, such an efficacy seems to be attributed to the normalizing effect by the harmony of various biological active commponents rather than by a single component alone. And thirdly, such an comprehensive action of ginseng reveals more obviously when it is supplemented on long-term basis, for example, for 8 to 9 months to rats. It shows more effective antioxidant activity in the abnormal status. Based on these evidences we propose here that such affirmative action of ginseng is the real mechanism on its antioxidant activity.

## 요 약

인삼의 항산화작용을 생체내에서 확인하기 위하여 인삼의 물추출물을 흰쥐에 장기간 투여하고 항산화 활성과 관련이 있는 여러가지 지표성분의 활성도 변화를 조사하였다. 그 결과 인삼을 투여한 쥐는 항상 높은 활성도를 유지하였으며, 노화와 더불어 나타나는 활성도의 감소도 지연되었다. 간 마이크로솜에서의 활성산소생성은 인삼의 투여로 인해 변화되지 않았으나 free radical generator의 첨가에 의한 지질과산화반응의 유도는 오히려 낮아졌다. 또한 혈장의 항산화활성은 인삼투여군이 높은반면 산화생성물인 TBARS의 함량은 낮았다. 인삼추출물의 지질과산화반응에 대한 인삼의억제작용은 정상쥐에서 보다 움직이지 못하도록 스트레스를 가한 쥐에서 더 효과적이었다.

이와같은 인삼의 포괄적인 항산화작용은 어떤 단일성분의 직접적인 작용에 의한것 보다는 장기적으로 급여했을때 여러성분들의 조화를 통해 나타나는 정상화효과에 의한 것으로 사료된다.

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