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# The Anti- and Pro-oxidative Effects of Orally Administered Flavonoids in Normal Rats

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**The present study was designed to investigate the effects of genistein, daidzein, and quercetin on the antioxidative systems of normal rats. Male Sprague-Dawley rats were divided randomly into seven groups and treated with flavonoids at either 2 or 20 mg/day or through vehicle for four weeks. Lipid peroxidation in the liver was inhibited significantly following administration of quercetin. Genistein and daidzein did not have significant effects except in rats treated with 20 mg daidzein/day. Genistein and daidzein treatment did not affect the content of $\alpha$-tocopherol in the serum and liver, while quercetin caused a slight increase. In hepatic glutathione and its related enzymes, genistein and daidzein treatment tended to cause a decrease in $\alpha$-tocopherol content, although no significant difference was found. However, quercetin treatment significantly decreased the content of glutathione together with the activity of glutathione reductase in all doses in the liver but there was no significant difference in the brain. Interestingly, daidzein treatment in the brain at 2 mg/day significantly increased glutathione (27.1 %, $p<0.05$) compared with the control group, while at 20 mg/day glutathione decreased significantly (26.6 %, $p<0.05$). In conclusion, genistein has not antioxidant effects. Daidzein and quercetin may have the capacity to produce not only antioxidants but also have adverse effects including the production of pro-oxidants. Therefore, people should consider consumption at a high dosage.**

**Key words :** Flavonoids, Antioxidant, Prooxidant, Lipid peroxidation, Glutathione-related mechanism

## INTRODUCTION

Recently, a great deal of attention has focused on so-called "functional food" that is said to have specific medical and physiological benefits for the body in terms of health and reducing the risk of disease. Flavonoids have lately attracted considerable attention as they offer a number of functional food ingredients. Flavonoids are a subclass of polyphenols, which generally consist of two aromatic rings, each containing at least one hydroxyl that are connected through a three-carbon "bridge" and become part of a six-member heterocyclic ring.[1)]

Generally, high consumption of fruits and vegetables is beneficial to human health, which may be an important factor in building resistance to chronic disease. Also, fruits and vegetables play a preventive role through the biological action of a variety of constituents, including minerals, fiber, antioxidants such as vitamins, and numerous flavonoids. Thus, it is possible that flavonoids also contribute to the protective effect of fruits and vegetables, and this seems to be associated with their antioxidative ability. Many isolated polyphenolic compounds have been shown to have strong antioxidative properties *in vitro* cell cultures and cell free-systems, which suggests there may be potential antioxidative actions involving these compounds in humans.

The total intake of flavonoids (16.7 mg/day of flavonol and 47.2 mg/day of isoflavones) in Japanese women showed an inverse correlation with plasma LDL cholesterol concentrations.[2)] LDL isolated from human subjects who were on diets enriched in isoflavones daily for 2 weeks showed a greater resistance to copper-dependent oxidation.[3,4)] Furthermore, consumption of flavonoids is associated with decreases in lipid peroxidation markers such as isoprostanes and malondialdehyde.[5)]

Unfortunately, evidence of this possibility *in vivo* is still limited and somewhat controversial at present.[6,7)] Therefore, the present study was performed to investigate the effects of flavonoids such as genistein, daidzein and quercetin on lipid peroxidation, and glutathione and enzymes involved in its metabolism of normal rats.

## MATERIALS AND METHODS

### 1. Classification of Animals

Male Sprague-Dawley rats (250-260 g; Daehan Biolink, Chungbuk, Korea) were housed individually in cages

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(22±2 ℃, 40-50% relative humidity) under controlled lighting (12 h light:dark cycle). The rats were fed a standard diet [AIN 93 M diet; Dyets, Bethlehem, PA, USA] and allowed free access to water. After a one-week adaptation period, the rats were divided randomly into seven groups and treated with each compound at either 2 or 20 mg/day or through vehicle for four weeks. Genistein, daidzien (95% purity; TCI Tokyo Kasei Kogyo Co., Tokyo, Japan) and quercetin (Sigma, St. Louis, MO, USA) was dissolved in corn oil and administered orally to two of the three groups at 2 and 20 mg/day, respectively, for 4 weeks. The rats in the control group were given the corn oil alone. Animal care in this study conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

After 4 weeks, the rats were anesthetized by intravenous injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). Their livers and brains were isolated, blotted, weighed, frozen in liquid nitrogen and stored at -70 ℃ until assayed. Tissue was homogenized in 0.1 M phosphate-EDTA buffer (pH 7.4) with 25% $HPO_3$ at 4 ℃.

### 2. Measurement of Concentrations of $\alpha$-tocopherol

The concentration of $\alpha$-tocopherol was analyzed by HPLC analysis. The HPLC system consisted of a Waters 501 pump (Waters Ltd., Watford, UK), fitted with a UV spectrophotometer or electrochemical detector (Shimadzu, Kyoto, Japan). Samples were analyzed on a 4.6×250 mm, RP C-18 Mightysil column (5 μm) (Kanto Chemical Co., Japan) and HPLC-grade $\alpha$-tocopherol (Sigma Chemical Co., St. Louis, MO, USA) was used as a standard. The mobile phase was filtered through a 0.45-μm nylon filter and sonicated for 5 min. The injection volume was 50 μl.

Determination of $\alpha$-tocopherol concentration in serum, liver and brain homogenate was performed according to a modified method of Talwar et al (1998). Briefly, each sample was deproteinized with ethanol containing 0.1% ascorbic acid. The sample solution was extracted twice with 1 ml of n-hexane. The separation and injection procedure was the same as that described above. The mobile phase consisted of methanol and water (95:5, *v/v*) flowed at a rate of 1.5 ml/min at an absorbance of 290 nm.

### 3. Measurement of Lipid Peroxidation

Lipid peroxidation was determined by measuring the concentration of malondialdehyde in the liver homogenates according to the method of Ohkawa et al..[8] Lipid peroxidation was calculated from the standard curve using malondialdehyde tetrabutylammonium salt and expressed as a concentration of nmol malondialdehyde per mg of protein.

### 4. Measurement of Glutathione Concentration and Gluta- Thione Peroxidase and Reductase Activity

The concentration of glutathione was measured with a spectrophotometer (412 nm) using the 5,5'-dithiobis (2-nitrobenzoic acid), DTNB-glutathione disulfide reductase recycling assay for glutathione based on the method of Anderson.[9] Glutathione concentration was expressed as a concentration of glutathione per mg protein. Glutathione peroxidase and reductase activity were determined with a spectrophotometer by measuring the rate of NADPH oxidation at 340 nm, based on the methods of Flohe et al.[10] and Carlberg et al..[11] Activity was expressed as a concentration of nmol $NADP^+$/min/mg protein. Protein concentrations were measured using the method of Bradford.[12]

### 5. Statistical Analyses

All values are expressed as means ± S.D. Data were analyzed by unpaired student's t-test or one-way analysis of variance followed by Dunnett's multiple comparison test (Sigma Stat, Jandel, San Rafael, CA, USA). For all comparisons, differences were considered statistically significant at $P<0.05$.

## RESULTS

### 1. Lipid Peroxidation

The basal content of hepatic malondialdehyde was 2.16±0.18 nmol/mg protein. It decreased significantly following the administration of quercetin (27.8 and 34.3 % for 2 and 20 mg quercetin/day, respectively, compared with the respective vehicle-treated groups, $p<0.05$, Fig. 1). Genistein and daidzein did not result in a significant change, but did so in rats treated with 20 mg daidzein/day.

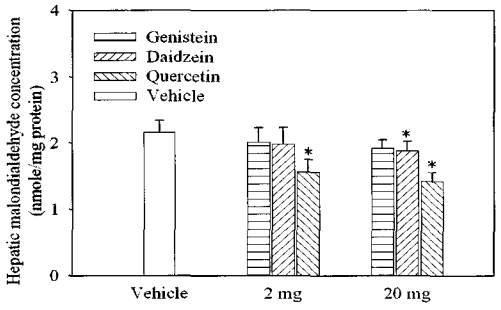


**Fig. 1.** Concentration of malondialdehyde in liver.
Each group was treated for four weeks with either 2 or 20 mg/day genistein, daidzein and quercetin alone or with vehicle. Values are means±S.D. (n=6-9). *$P<0.05$, significantly different from the group treated with vehicle.

## 2. Concentrations of α-tocopherol

In serum, the basal content of α-tocopherol in vehicle-treated rats was 1.82±0.32 μg/ml (Fig. 2A). Genistein and daidzein treatment did not have much affect while quercetin caused a slight increase.

As for the liver, the basal content of α-tocopherol in vehicle-treated rats was 3.74±0.27 μg/ml. In rats treated with quercetin, α-tocopherol content increased slightly even though differences of between 2 and 20 mg /day in the treated group could not be recognized (3.47±0.26 and 4.00±0.21 μg/ml in rats treated with 2 or 20 mg/day quercetin, respectively, Fig. 2B).

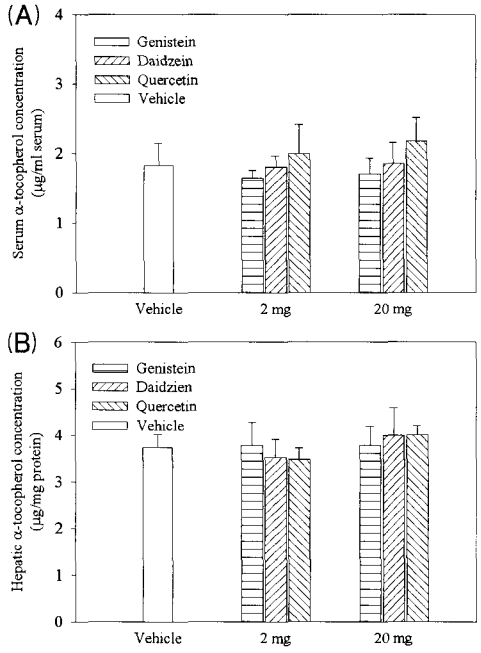


**Fig. 2.** Concentration of α-tocopherol in serum (A) and liver (B).
Each group was treated for four weeks with either 2 or 20 mg/day genistein, daidzein and quercetin alone or with vehicle. Values are means±S.D. (n=6-9).

## 3. Concentrations of Glutathione

In the liver, the basal content of glutathione was 16.93±1.56 nmol/mg of protein. Genistein and daidzein treatment tended to decrease glutathione content, although no significant difference was found. However, quercetin treatment significantly decreased glutathione content in all doses (8.27±2.26 and 9.44±2.26 nmol/mg protein for 2 and 20 mg/day, respectively, Fig. 3A).

In the brain, the basal content of glutathione was 58.60±4.82 nmol/mg protein. It increased significantly following administration of quercetin at a dose of 20 mg/day (Fig. 3B). The effects of genistein on glutathione in the brain were similar to those in the liver. Daidzein treatment at 2 mg/day significantly increased glutathione (27.1%, $p<0.05$) compared with the control group, while at 20 mg/day glutathione significantly decreased (26.6%, $p<0.05$).

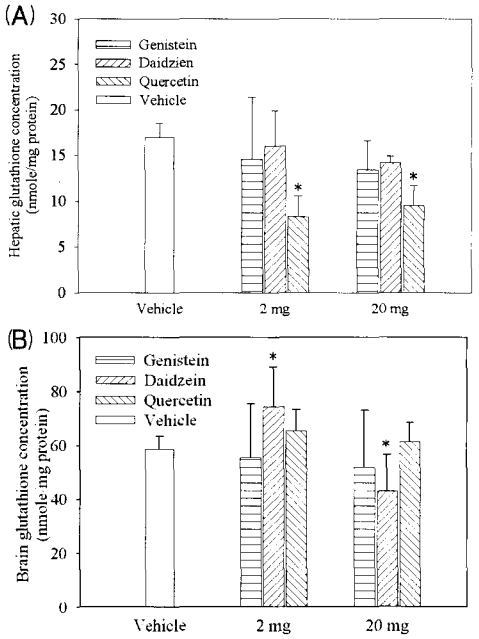


**Fig. 3.** Concentration of glutathione in liver (A) and brain (B).
Each group was treated for four weeks with either 2 or 20 mg/day genistein, daidzein and quercetin alone or with vehicle. Values are means±S.D. (n=6-9).
*$P<0.05$, significantly different from the group treated with vehicle.

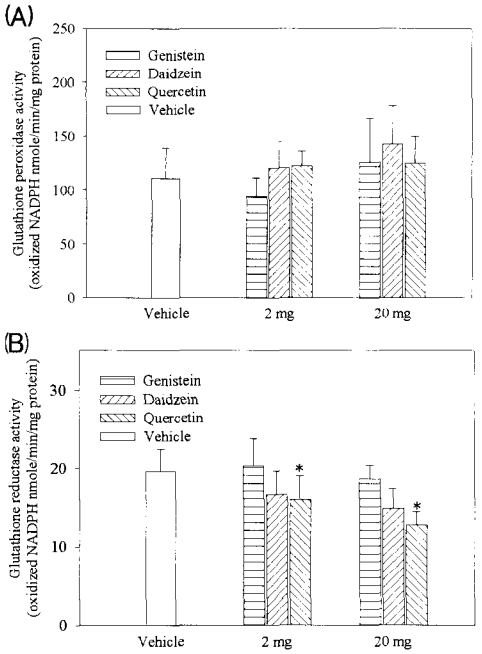


**Fig. 4.** Activities of glutathione peroxidase (A) and reductase (B) in the liver.
Each group was treated for four weeks with either 2 or 20 mg/day genistein, daidzein and quercetin alone or with vehicle. Values are means±S.D. (n=6-9).
*$P<0.05$, significantly different from the group treated with vehicle.

## 4. Glutathione Peroxidase and Reductase Activity

The basal activity of glutathione peroxidase and reductase in the livers of rats were 110.55±28.75 and

19.54±2.89 oxidized NADPH nmole/min/mg protein, respectively (Fig. 4A). Glutathione peroxidase activity was not affected by treatment with flavonoid and, in rats treated with genistein and daidzein, glutathione reductase activity tended to decrease slightly, although there was no significant difference. However, in rats treated with quercetin, glutathione reductase activity decreased significantly (18.5 and 34.7% for 2 and 20 mg quercetin/day, respectively, $p<0.05$ for 20 mg/day, Fig. 4B).

## DISCUSSION

Flavonoids are widespread in nature, occurring in all plant families, and are categorized into six major subclasses including the flavones, flavonols, flavanones, catechins (flavanols), anthocyanidins, and isoflavones. Quercetin, a flavonol, is the most predominant flavonoid in the human diet and estimates of human consumption are in the range of 4 to 68 mg per day based on epidemiological studies in the U.S., Europe, and Asia.[13-15] Isoflavones, genistein and daidzein are mainly found in soybeans and other soy-based products such as tofu, miso, soy milk and tempeh.[16] Assuming that the total isoflavone concentration of soy-based products is in the range of 1 to 3 mg/g, expected daily isoflavone intake would range from 20 to 240 mg for Asians and from 1 to 9 mg for those in other populations.[17]

Flavonoids included in quercetin and isoflavones, such as genistein and daidzein, are considered antioxidant compounds in the human diet. According to Halliwell and Gutteridge,[18] mechanisms of antioxidant action can include suppressing reactive oxygen species formation either by the inhibition of enzymes or chelating trace elements involved in free radical production, scavenging reactive oxygen species and upregulating or protecting antioxidant defenses.

The present study investigated the effects of genistein, daidzein and quercetin on lipid peroxidation, glutathione and the enzymes involved in its metabolism in normal rats. Lipid peroxidation was inhibited significantly following the administration of flavonoids. In rats treated with 20 mg/day of genistein or daidzein, malondialdehyde concentration decreased significantly (11.1% and 13.0% for genistein and daidzein, respectively, compared with the vehicle group). Specifically, quercetin treatment significantly decreased the content of malondialdehyde (an indicator of lipid peroxidation) in all doses. In addition, daidzein and quercetin treatment progressively increased the content of $\alpha$-tocopherol in the serum and liver, although there was no difference. This is consistent with the study by Zhu et al.[19] who reported that flavonoids act as an antioxidant via the mechanism of maintaining and regenerating $\alpha$-tocopherol in low-density lipoprotein. Although the mechanism of such interactions between flavonoids and vitamins is not clear, regeneration of $\alpha$-tocopherol from its one-electron-oxidized form ($\alpha$-tocopheryl radical) by some flavonoids suggested an analogy to the well-known synergism between $\alpha$-tocopherol and ascorbate.[20-22] We postulate that interactions between flavonoids and $\alpha$-tocopherol (that is, increases in $\alpha$-tocopherol content by means of flavonoid administration) prevent the progression of lipid peroxidation.

On the other hand, glutathione and the enzymes involved in its metabolism in the liver were not affected by genistein and daidzein. Interestingly, daidzein treatment may have the capacity to produce not only antioxidants but also pro-oxidants including a decrease of glutathione in the brain. Rohrdanz et al. (2002) reported that daidzein affected the AOC expression (glutathione peroxidase, CuZn- and Mn-superoxide dismutase) pattern in rat hepatoma H4IIE cells, and these changes did not add to the antioxidant, but rather to the oxidant properties of daidzein. Similarly, the results of several *in vitro* studies have suggested that daidzein has pro-oxidant effects in the liver, although it remains unproved whether daidzein acts as a prooxidant in the brain.

In the case of quercetin, it has been shown to have adverse effects on the glutathione and antioxidant enzyme systems in the livers of rats but not so in their brains. Even though glutathione peroxidase increased slightly, glutathione and glutathione reductase activity acutely decreased (44.2-51.2 and 18.5-34.7% decrease, respectively).

The results of the present study did not reflect results reported previously that quercetin, genistein and daidzein possess antioxidant activity *in vitro* cell cultures and cell-free systems. These results, therefore, suggest that flavonoids may have the capacity to produce not only beneficial factors but also adverse effects including the production of pro-oxidants. Recently, in consuming flavonoids-containing foods, people may ingest more than what might be considered an effective amount. However, before that, it is important to know the part flavonoids play in the oxidation and antioxidant defense system in the body and more research through animal studies is needed.

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