Effects of a *Rubus coreanus* Miquel supplement on plasma antioxidant capacity in healthy Korean men

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**Abstract**

Korean raspberry, *Rubus coreanus* Miquel (RCM), contains high concentrations of phenolic compounds, which prevent oxidative stress. To determine the effect of RCM on antioxidant capacity in humans, we assessed *in vivo* lipid oxidation and antioxidant enzyme activities from plasma in 15 healthy men. The subjects ingested 30 g of freeze-dried RCM daily for 4 weeks. Blood was taken at baseline and at the end of the study to determine blood lipid profiles, fasting plasma glucose, liver function, lipid peroxidation, and antioxidant enzyme activities. RCM supplementation had no effect on blood lipid or fasting plasma glucose concentrations but decreased alkaline phosphatase activity. RCM supplementation increased glutathione peroxidase activities (*P* < 0.05) but had no effect on lipid peroxidation. These results suggest that short-term RCM supplementation may offer health benefits by enhancing antioxidant capacity in a healthy population.

**Key Words:** *Rubus coreanus* Miquel, antioxidant, lipid peroxidation, glutathione peroxidase

**Introduction**

Diets rich in fruits and vegetables protect against various chronic diseases such as cardiovascular diseases, stroke, and cancers [1-3]. These beneficial effects have been attributed to high concentrations of antioxidants such as vitamin C, vitamin E, carotenoids, and flavonoids [3-5]. Several epidemiological studies have reported that vitamin supplements or a high intake of fruits such as strawberries or blueberries increases plasma antioxidant capacity and lowers the risk for cardiovascular diseases [2,6,7]. However, other studies have shown that antioxidant supplements or a high intake of vegetables or fruits did not improve antioxidant capacity in healthy subjects [8,9]. These results suggest that different types of supplements or antioxidants could differentially affect the antioxidant system in humans.

The Korean raspberry, *Rubus coreanus* Miquel (RCM), has been used as a traditional remedy for liver and kidney diseases, spermatorrhea, prostate and urinary disease, and allergic diseases [10-12]. Scientists have elucidated the active components in RCM and found that it contains various antioxidants such as polyphenols, tannins, phenolic acids, organic acids, triterpenoids, flavonoids, gallotannin, ellagitannin, and anthocyanins [13-15]. Several studies have shown that an RCM extract has higher electron donation ability and prevents low-density lipoprotein (LDL) oxidation in *in vitro* studies [14-16]. Other studies have shown that a ripened RCM extract decreases gastric tumor growth and stimulates osteoblastic cell proliferation and differentiation [17,18].

RCM has traditionally been used as a Oriental medicine, and recent studies have demonstrated its antioxidant, anti-tumorigenic, and anti-inflammatory effects in *in vitro* studies [14-17]. These effects might be related to the antioxidants. Thus, we determined the antioxidant effect of RCM on healthy human subjects.

**Subjects and Methods**

**Subjects**

Fifteen healthy participants were recruited with an age range of 20-30 years, and all participants completed the study. None of the participants had a history of cardiovascular disease, diabetes, or renal or liver disease but had not taken vitamin/mineral supplements or any other functional food during this

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The procedures used were in accordance with the institutional guidelines and approved by the Chung-Ang University Institutional Review Board (Seoul, South Korea). Informed consent was obtained from all study participants before starting the study.

**Study protocol and analysis**

RCM was obtained from the Rural Development Administration and RCM supplements were prepared by Herbcure (Pocheon, Korea) (Table 1). Subjects consumed supplements containing 30 g of freeze-dried RCM per day for 4 weeks. After overnight fasting, blood samples were collected by venipuncture into evacuated tubes at 0 and 4 weeks. Blood samples were centrifuged at 3,000 × g for 15 min at 4℃, and plasma samples were collected. Samples were stored at -80℃ for further analysis.

Levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT), total cholesterol (TC), high-density lipoprotein (HDL), LDL cholesterol, triglyceride (TG), fasting plasma glucose, C-reactive protein (CRP), and testosterone were measured in plasma. ALP, AST, ALT, TC, TG, and CRP were measured enzymatically with the AVIDA 1650 instrument (Bayer), whereas HDL cholesterol levels were measured after precipitation of the other lipoproteins. LDL cholesterol levels were calculated by the Friedewald equation [19], and testosterone was measured by radioimmunoassay (Siemens).

Lipid peroxidation was measured by thiobarbituric acid-reactive substances (TBARS) using the malondialdehyde (MDA)-thiobarbituric acid assay adopted from Jentzsch et al. [20]. To measure antioxidant enzymes activity in plasma, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities were measured using assay kits, according to the manufacturer’s instructions (Caymen Chemical, Ann Arbor, MI, USA).

**Dietary assessment**

Dietary intake was assessed with a 2-day food record method. Study subjects were provided with instructions to complete their food record and were asked to document their food consumption on any one weekday and any one weekend day. Subjects were asked to include food labels and recipes for mixed dishes and were encouraged to avoid any alterations to their normal diet. To ensure an adequate level of detail when describing foods and food preparation methods, the food records were carefully reviewed by a trained nutritionist immediately after completion. Dietary data were analyzed using the Can-Pro nutrient analysis software package version 3.0 (The Korean Nutrition Society, Seoul, Korea), which contains the Korean food composition table. When the food recorded could not be matched exactly with the database, the best possible match was found and used as a substitute.

**Statistical analysis**

All analyses were performed with a significance level of α = 0.05 using the SAS system (version 9.1 SAS Institute Inc, Cary, NC, USA). Continuous variables are presented as mean ± SD, and categorical variables are presented as frequencies (percentages). The paired t-test was performed to identify differences in the means before and after RCM supplementation.

**Results**

The nutrient content of freeze-dried RCM is shown in Table 2. At an energy level of 378 kcal per 100 g, the participants received 117 kcal from RCM per day. RCM contained 9.6 mg vitamin E and 1.05 g phenols, which included flavonol, flavonoids, and anthocyanins.

The general characteristics of the participants are shown in Table 3. Their average age, weight, height, and body mass index were 24.3 years, 67.5 kg, 174.9 cm, and 22.1, respectively. None

### Table 1. Components of the *Rubus coreanus* Miquel (RCM) supplement

| Components               | Amounts (g) |
|--------------------------|-------------|
| Freeze-dried *Rubus* C. Miquel | 30.0 g      |
| Carboxymethyl cellulose  | 0.3 g       |
| Sucrose                  | 7.0 g       |
| Methyl-β-Hydroxybenzoate | 0.1 g       |
| Water                    | 150 ml      |

### Table 2. Composition of freeze-dried *Rubus coreanus* Miquel powder

| Nutrients               | Amounts (g) |
|-------------------------|-------------|
| Energy (kcal)           | 387.0       |
| Water (g)               | 6.6         |
| Lipids (g)              | 5.4         |
| Proteins (g)            | 7.5         |
| Carbohydrates (g)       | 77.1        |
| Dietary fibers (g)      | 27.7        |
| Vitamin A (mg)          | 1.3         |
| Vitamin C (mg)          | 0.0         |
| Vitamin E (mg)          | 9.6         |
| Total phenol (g)        | 1.05        |

### Table 3. General subject characteristics

| Subjects                  | Subjects |
|---------------------------|----------|
| Age (yrs)                 | 24.3 ± 1.9 |
| Weight (kg)               | 67.5 ± 6.1 |
| Height (cm)               | 174.9 ± 5.6 |
| BMI                       | 22.1 ± 1.7 |

| Previous functional food intake | Subjects |
|--------------------------------|----------|
| Yes                            | 13 (86.7) |
| No                             | 2 (13.3)  |

1) Mean ± SD  
2) N (%)
The nutrient intakes of the subjects are presented in Table 4. The participants consumed 2,077 kcal per day for energy, which was lower than the recommended intake for Koreans. Calcium, zinc, vitamin B<sub>2</sub>, vitamin C, and folate intakes were lower than the recommended levels, whereas phosphorus, iron, vitamin A, vitamin B<sub>6</sub>, and vitamin E intakes were above the recommended intake.

We examined whether RCM supplementation could modulate liver function, blood lipid profiles, fasting plasma glucose, inflammation, and testosterone level (Table 5). Every parameter was in the normal range and 4 weeks of RCM supplementation had no effect on blood lipid profiles, fasting plasma glucose, CRP, or testosterone levels, but it significantly lowered ALP levels ($P < 0.05$).

To investigate the effect of RCM supplementation on lipid peroxidation and antioxidant enzyme activities, we determined the lipid peroxidation and antioxidant enzyme activities in plasma (Table 6). RCM supplementation did not significantly lower lipid peroxidation in plasma. However, GPx activity in plasma increased significantly after the 4-week supplementation ($P < 0.001$).

**Discussion**

We investigated if Korean raspberry, RCM, could reduce oxidative stress by affecting antioxidant enzyme activity in healthy men. Our results showed that while RCM did not reduce lipid peroxidation, it increased plasma GPx activity.

A high fruit and vegetable intake is recognized to reduce the incidence and development of chronic diseases such as cardiovascular disease and cancer [1,2,21-23]. Several studies have shown that antioxidants in fruits and vegetables prevent chronic diseases by reducing oxidative stress, LDL oxidation, and DNA damage [24-27]. Among the antioxidants in fruits and vegetables, the health benefits of phenolic compounds have been studied, because of their high quantities and antioxidant capacity. High phenolic compound-containing foods such as fruits, teas, and wines increase plasma antioxidant capacity and reduce inflammation, cardiovascular disease, and tumorigenesis [23-28]. Recent studies have also shown that berries such as strawberries, cranberries and blueberries are good sources of phenolic compounds and dietary fiber, and that a high intake of berries increases antioxidant capacity and prevents atherosclerosis and hepatic fibrosis in human and animal studies [29-33].

RCM is prepared from a Korean raspberry that contains high concentrations of phenolic compounds. The freeze-dried RCM used as the supplement in this study contained 1.05 g total phenols per 100 g; thus, affording a daily RCM supplementation level of about 315 mg of total phenols. This was a relevant amount of phenolic compound in comparison with other high phenolic compound-containing fruits. Pears and grapes provide approximately 300 mg of total phenols per serving, and cranberries and blueberries provide about 200-400 mg [34,35]. Therefore, RCM is a good source of phenolic compounds.

RCM has traditionally been used as a Oriental medicine, and recent studies have demonstrated its antioxidant, anti-tumorigenic,
and anti-inflammatory effects in *in vitro* studies [14-17]. These effects might be related to the antioxidants present in RCM. However, as no such study has been performed with human subjects, we determined the antioxidant effect of RCM on healthy human subjects.

Our subjects ingested 65.2 g of lipids, which provided about 28% of total energy and 363.6 mg of cholesterol. These amounts were within the recommended intake range, so their blood lipid profiles were in the normal range before and after RCM supplementation. Although vitamin C intake was lower than the recommended level, vitamins A and E intake was higher than the recommended level. Other studies have reported that a high intake or supplementation with vitamins A, C, and/or E reduces lipid peroxidation and increases antioxidant enzyme activity [36,37]. Our subjects ingested high levels of antioxidants such as vitamin A and E, which means that their antioxidant capacity was already high, so the RCM supplementation may not have significantly reduce lipid peroxidation and/or increased SOD and CAT activities.

We showed that 4 weeks of RCM supplementation slightly, but not significantly, reduced lipid peroxidation from 4.84 to 4.26 μM. We tested the antioxidant effects of RCM in healthy males who did not smoke or drink alcohol, suggesting that oxidative stress levels were relatively low in our subjects. Other studies with healthy subjects also showed similar results. Six weeks of a diet with a high intake of vegetables, berries, and apples combined with linoleic acid does not reduce lipid peroxidation in healthy subjects, and moderate antioxidant supplementation does not reduce lipid and protein oxidation in healthy men [8,9]. However, strawberry supplementation significantly reduces oxidative damage and lowers TC and LDL-cholesterol levels in women with metabolic syndrome or hyperlipidemia [29-31]. These results suggest that the endogenous antioxidant defense system and antioxidant intake from the diet were adequate to prevent oxidative stress in healthy subjects, so supplementation with extra antioxidants did not reduce oxidative stress. Furthermore, the reduced level of oxidative damage may have been insufficient to produce measurable improvement.

Even though RCM supplementation did not reduce lipid peroxidation, plasma GPx activity increased. GPx is an endogenous catalytic H2O2 scavenger that protects against oxidative stress in cultured cells and animals [38,39]. Other studies have shown that antioxidant enzyme activities can be modulated by antioxidant or fruit supplementation [40-42]. Two months of blueberry supplementation in children with type 1 diabetes improves SOD activity in erythrocytes, and mulberry fruit supplementation increases GPx and SOD activities of red blood cells and liver in hyperlipidemic rats [41,43]. Additionally, several studies have reported that enhanced GPx activity or expression reduces the incidence of chronic diseases such as cancer, cardiovascular disease, neurodegeneration, autoimmune disease, and diabetes [44-47]. Guo et al. and Hoehn et al. showed that GPx overexpression inhibits the development of atherosclerosis and experimental stroke in animals [44,46]. However, in this study, we showed that SOD and CAT activities decreased slightly, but the difference was not significant. This result may have been caused by either the major type or concentration of phenolic compounds in RCM. Another study also showed that types or concentrations of phenolic compounds such as quercetin and rutin differentially modulate GPx, CAT, and SOD expression in HepG2 cells [48]. They showed that low-dose quercetin significantly decreases SOD but increases GPx expression levels, whereas high dose treatment significantly increases SOD but does not change CAT and GPx expression levels.

In this study, we showed that RCM supplementation may offer health benefits by enhancing antioxidant capacity in a healthy population. However, it had some limitations. First, TBARS is not a good indicator of oxidative damage because the assay relies on the reaction of thiobarbituric acid with MDA as a marker of oxidative damage to lipids. During the reaction, thiobarbituric acid may react with other oxidized products, which could modulate TBARS values. Second, self-administered dietary assessment methods tend to either under- or over-estimate diet. Even though dietary data was collected using a 2-day food record method, we could not completely prevent food intake measurement errors. Finally, we tested the antioxidant effects of RCM for 4 weeks in healthy males. Healthy males, who do not smoke and drink alcohol, have low levels of oxidative stress and high antioxidant capacity, so 4 weeks of supplementation might not have been long enough to change the antioxidant capacity and lower lipid peroxidation.

In summary, a 4-week supplementation with RCM, a Korean berry with a high phenolic content, slightly reduced lipid peroxidation and significantly increased GPx activity. Therefore, RCM is useful as a good source of fruit, which can reduce oxidative stress and provide health advantages.

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