Effectiveness of Crowberry on Plasma Total Antioxidant Status, Lipid Profile and Homocysteine

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Abstract The objective of this study was to assess whether the consumption of crowberry could affect serum lipid profile, homocysteine (Hcy) level, and antioxidant status of healthy subjects. Out of 55 healthy volunteered subjects, 51 completed this investigation to final analysis. Each subject consumed 2 grams of powdered crowberries everyday for four weeks. Crowberry consumption led to significant increase in total antioxidant status (TAS) and superoxide dismutase (SOD), while it resulted in significant decrease in total cholesterol and low-density lipoprotein (LDL) levels. The differences in the levels of antioxidant markers and lipid profiles taken before and after crowberry intake were most significant. The levels of Hcy, catalase, T-cho, triglyceride, and LDL in the higher T-cho group decreased significantly after crowberry intake. Furthermore, this study revealed a significant positive correlation between Hcy level and body weight index (BMI). Crowberry intake improved lipid profile as demonstrated by having decreased T-cho, TG and LDL; increased antioxidative reactions (such as TAS or SOD enzymatic activities) and decreased Hcy levels in healthy subjects.

Keywords: crowberry, antioxidative activity, homocysteine, lipid profile


1. Introduction

Many in vitro studies have shown that flavonoids in foods have strong antioxidant and metal-chelating properties, which protect cells and tissues from free oxygen radicals.

A large number of studies support the hypothesis that oxidative damage to DNA, lipids, and proteins may contribute to the development of cardiovascular disease (CVD), cancer, and neurodegenerative diseases. Accordingly, dietary antioxidants may be particularly essential for the protection of individuals against chronic diseases [1,2,3]. The consideration is that high dietary intake of antioxidant compounds may prove to be a preventative measure against diseases associated with reactive oxygen species (ROS). Consumption of flavonoid -rich foods, particularly fruits and vegetables, is associated with low incidences of heart disease, ischemic stroke, cancer, and other chronic diseases. The aspect that 1) these chronic diseases are associated with increased oxidative stress, and 2) flavonoids are strong antioxidants concurs with the suggestion made by several studies that dietary flavonoids exert health benefits through antioxidant mechanisms [4,5,6].

Homocysteine (Hcy) is an amino acid arising by methylation of methionine, which does not participate in protein synthesis. Recently, owing to association with the pathogenesis of several neurological, cerebral and cardiovascular disorders, Hcy has received particular attention. Hyperhomocysteinemia is a common and independent risk factor for atherosclerosis and other CVD. The inhibitory effect of Hcy levels on endothelial cells may be mediated by oxidative stress [7]. In fact, a strong correlation between high plasma Hcy levels and ROS production, detected by plasma malondialdehyde, was identified in patients with CVD. Consequently, ROS suppression might be a potential therapeutic strategy for the prevention or impediment of atherosclerosis in patients with hyperhomocysteinemia. It was recently demonstrated that plasma Hcy could activate pathogenic mechanisms leading to oxidative stress [7,8]. Indeed, it showed that hyperhomocysteinemia decreased vascular reactivity and was associated with increased cardio-vascular morbidity and mortality.

Crowberry is one of the most common wild berries found in northern Norway, while Empetrum nigrum var. japonicum, a species of crowberry, grows naturally in Korea. The Korean crowberry (named Shiromi) is a narrow endemic species found at altitudes above 1,300 meters at Mt. Halla in the Island of Jeju.

These authors recently reported results from our previous study showing antioxidant activities of Korean crowberries. This study indicated that strong antioxidant activities of the native crowberry species were associated with reduced LDL peroxidation and protection of LDL from copper ion (Cu”) induced oxidation [9]. Therefore,
the hypothesis was derived that crowberries would exert a beneficial antioxidant effect on human health. In an attempt to inquire into the hypothesis, this study assessed the effect of crowberries on antioxidative activities, homocysteine level, and lipid profile of subjects.

Quercetin was shown to have blood pressure lowering properties in animal models due to vasorelaxation secondary to decreased oxidative stress on the arterial walls [10].

Ogawa et al. [11] investigated the anthocyanin composition and antioxidant activities of nine berry species. Among them, crowberries had the highest total concentration of anthocyanin and showed the strongest antioxidant activities.

Many studies on strawberries have linked their antioxidant activities with suppression of chronic diseases. Nevertheless, it lacks clinical trials with crowberry to date.

The aim of this study was to investigate whether moderate intake of black crowberry could exert beneficial effects on human health. Volunteered subjects were asked to consume dried encapsulated crowberries, "Shiromi," daily for 4 weeks; then, analysis was made on several parameters including antioxidant status, serum homocysteine level, and serum lipid profile.

2. Materials and Methods

2.1. Participants

This study enrolled 55 healthy volunteers from Jeju Island, who satisfied the inclusion criteria. The inclusion criteria comprised: 1) men or women over the age of 20 years, 2) having no known chronic diseases, 3) who are not currently using any medication, and 4) have no known allergy or hypersensitivity to any food or material. The characteristics of 55 voluntary subjects enrolled in the study are listed in Table 1.

This investigation conformed to the Ethical Principles of the Declaration of Helsinki. All subjects read and gave informed consent prior to participation in the study.

2.2. Procedure

Natural crowberries (shiromi) were harvested and dried in the island of Jeju before they were powdered and encapsulated for easy consumption. Each capsule contained 2g of powdered crowberry. Every participant was instructed to take one capsule a day. During the study period of four weeks, all participants maintained their usual dietary habits and no additional dietary supplements were allowed throughout the study.

2.3. Analysis of Total Antioxidant Status

Serum total antioxidant status (TAS) was quantitatively measured for the assessment of in vivo antioxidant status using a commercially available kit (RX Daytona, Randox, Antrim, United Kingdom). This kit was based on the method of trolox equivalent antioxidant capacity (TEAC), introduced by Aprikan et al. 2003. The TAS of heparinized plasma was determined according to the method of Caio and Prior [12].

The assay was carried out on a Selectra Analyzer (Selectra E, Vital Scientific, Dieren, Netherland) using commercially available kits (Randox). Blood samples were collected in heparin-treated tubes in the beginning (T=0 days) and at the end (T=28 days) of the experiment.

After separating the plasma by centrifugation (1500 × g at 4 °C for 10 min), erythrocyte packets were prepared by washing them with a saline solution three times. Then, erythrocyte haemolysates for antioxidant enzymes were prepared and stored at -80°C deep freezer until the time of analysis. Oxidative stress was documented by utilizing changes in antioxidant enzymatic activities in the erythrocytes.

With respect to enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were examined. For the analysis of SOD, CAT and GPx, heparinized erythrocytes were washed with a saline solution and butylated hydroxytolune was added to prevent oxidation. These samples were stored at -80°C until utilization. Ransod (SD 125) and Ransel (RS 504) from Randox laboratories (Antrim, United Kingdom), commercially available kits, were used for determination of SOD and GPx activities, respectively. The catalase-520 kit was used for the CAT spectrophotometric assay, and the activities were determined according to the product instructions (Oxis Research, Portland, Oregon, USA).

For the analysis of lipid profile, Selectra E, a clinical chemistry analyzer, was used to measure the serum concentrations of total cholesterol (T-cho), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TG).

Selectra E, a commercial kit (Diazyme, Hannover, Germany), was also utilized for the analysis of the level of total serum homocysteine (Hcy).

The enzymatic Hcy assay kit is based on a novel assay principle that assesses the co-substrate conversion product instead of co-substrate or Hcy conversion product. The co-substrate conversion product is a molecule that is not a substrate of the Hcy conversion enzyme, and does not contain any element from sample Hcy. Body mass index (BMI) was calculated as the individual's body weight (kg) divided by the square of his or her height (m²).

2.4. Statistical Analysis

Excel 2010 (Microsoft, Redmond, Washington, USA) was used for data entry, validation, and restructuring, as well as for calculating changes in variables over time, reorganizing/reformatting results, and graphical preparation. Statistical analysis was performed using the SPSS Base System ver. 19 (IBM SPSS, Chicago, Illinois, USA).

3. Results

<table>
<thead>
<tr>
<th>Table 1. Demographics of participants stratified by gender (n=51)</th>
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<td>Age(years)</td>
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<td>BMI(kg/m²)</td>
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Values expressed as mean ± standard deviation

Of 55 subjects voluntarily participating in this experiment early on, 51 completed this study. Of 4 individuals excluded, 2 acquired an acute febrile illness during the study and the remaining 2 had errors in blood
sampling. Table 1 shows the demographics of 51 participants.

The serum levels of TAS, Hcy and lipid profile were measured before (T=0) and after (T=28) crowberry intake. Similarly, GPx, SOD and CAT activities in the erythrocytes were measured before and after crowberry intake. The results were analyzed and compared with various biological parameters. Changes in antioxidant markers and Hcy levels of test subjects are shown in Table 2.

After crowberry consumption, the TAS levels increased about 16% from 1.44±0.09 to 1.71±0.07 mmol·l⁻¹ (p<0.001). SOD activities increased 9% while CAT activities decreased 17%. The levels of SOD and CAT activities changed from 199±35 to 223±25 U·ml⁻¹ (p<0.01) and 272±51 to 225±42 U·l⁻¹ (p<0.01), respectively. The serum Hcy concentrations and GPx enzymatic activities showed statistically insignificant (p>0.05) changes before and after crowberry consumption. The serum lipid profiles showed significant changes after crowberry consumption (Table 3). The T-cho and LDL cholesterol levels decreased 14% and 13%, respectively, with statistical significance (each component: p<0.001). Changes in HDL showed statistically insignificant (p>0.05) changes before and after crowberry consumption.

There were differences in the parameters between men and women. Men showed higher levels of Hcy, T-cho, TG, and LDL compared to women. In particular, women showed a 30% lower Hcy level than men. After crowberry consumption, both genders showed significant reduction in Hcy, CAT, T-cho, TG, and LDL, but showed increase in TAS and SOD. The TAS levels increased about 25% in both groups after crowberry consumption.

TAS and SOD increased similarly in both groups, but Hcy, CAT, T-cho, TG, and LDL considerably decreased in the high T-cho group after crowberry consumption. There were significant differences in TAS, CAT, T-cho, and LDL levels of the over-weight subgroup after consumption of crowberry. The levels of CAT, T-cho, and LDL of the over-weight subgroup were almost normalized similar to the normal-weight subgroup (Table 4).

4. Discussion

With rising costs of health care management and pharmaceutical products, the role of natural dietary measures in prevention and treatment of diseases has gained special attention; in particular, with reference to diseases related to oxidative stress.

Free radicals or reactive oxygen/nitrogen species may play major roles in many diseases such as cancer, atherosclerosis, and the entire aging process [4,5]. Many chronic diseases are associated with increased oxidative stress, while flavonoids are strong antioxidants in vitro. Thus, it was suggested that dietary flavonoids could exert health benefits through antioxidant mechanisms. The health benefits of dietary sources of flavonoids were investigated in previous studies. It was reported that strawberries, as a fine source of flavonoids among these dietary products, improved dyslipidemia in type 2 diabetics or hyperlipidemia in mice. Likewise, the protective effect of blueberry consumption was also investigated. The benefits were realized by upregulation in expression of antioxidant enzymes, and by decreased insulin resistance with reduction in adipose cell death and inflammation [13,14].

The antioxidant activities of various berry species have been evaluated before. However, there has been no similar study conducted on the effects of crowberries to our knowledge. In this study, the antioxidant activities of the species of crowberry “Shiromi” were analyzed. Powdered...
and encapsulated crowberries were consumed and their effects on total antioxidant status (TAS), antioxidant enzymes, serum lipid profile, and homocysteine level were analyzed. Oxidative metabolites are continuously produced in the human body through complex biological processes. The combination of a high level of oxidative product and low level of antioxidants could accelerate cellular damage by oxidative stress. Many studies have shown antioxidant activities of strawberries, which are linked to suppression of chronic diseases. Nevertheless, it lacks clinical studies on crowberry.

In this experiment, total antioxidant status increased considerably after crowberry intake (p<0.001). Increased antioxidant status from crowberry intake was due to the high concentration of flavonoids, which had a strong countervailing effect on the total oxidant contents in the human body. Ogawa et al. [11] investigated the anthocyanin composition and antioxidant activities of ten berry species including crowberry. Of those ten berry species, crowberry had the highest total concentration of anthocyanins and also exhibited the strongest antioxidant activities.

Among thirteen varieties of anthocyanins identified in the HPLS analysis, cyanidin-3-galactoside and delphinidin-3-galactoside were major components. Delphine and cyanidin have more hydroxyl groups on the benzene ring, which provides strong scavenging activities of hydrogen peroxide-induced lipid peroxidation [15]. As Ogawa et al. [11] pointed out, owing to higher hydrogen peroxide -induced lipid peroxidation [15]. As Ogawa et al. [11] pointed out, owing to higher antioxidative activities; and, according to the results, SOD, GPx and antioxidative enzymes such as SOD, CAT, and GPx, oxidative products such as superoxide are detoxified in water. These enzymatic cellular antioxidants have been well studied.

In this study, the enzymatic level of SOD increased and that of CAT decreased after crowberry intake (p<0.001); while the GPx levels did not change significantly (Table 2) [10]. Wu et al. [17] studied the effect of blueberry on atherosclerosis in mice and analyzed the enzymatic activities; and, according to the results, SOD, GPx and thioredoxin reductase increased in blueberry fed mice due to upregulation of gene expressions in the aorta.

T-cho, LDL, HDL, and TG were analyzed to determine the effect of crowberry on lipid profile. Among them, T-cho and LDL cholesterolers dropped 7% and 11%, respectively, with their figures showing statistical significance (p<0.001) (Table 3). The TG levels also decreased. However, owing to high variability in daily consumption of foods containing starch and carbohydrates, statistically significant change was not found. The results of this report showed that crowberry intake improved the lipid profile. LDL is one of the major causes of atherosclerosis. Thus, substances that control LDL have positive roles to play in cardiovascular health. For instance, anthocyanins in berries inhibit LDL oxidation and suppress atherogenic stimuli of macrophages. It was demonstrated that anthocyanin components in mulberry extracts could prevent atherosclerosis. Chen et al. [18] reported similar results from their study that dietary sesame intake decreased cholesterol levels of patients with hypercholesterolemia.

In Chen’s [18] study, after consumption of sesame supplement, T-cho level above 240 mg·dl⁻¹ was decreased 6.4% and 9.5% for total and LDL cholesterol, respectively [1,18]. Chen et al. [18] reported that the T-cho and LDL cholesterol levels decreased by 6.4% and 9.5%, respectively, after sesame supplementation. The extent of decreased level of T-cho or LDL cholesterol in this crowberry study was remarkably greater than that of the results from the Chen’s study [18].

The results of this study showed that individuals with a T-cho level of higher than 200 mg·dl⁻¹ showed 15% and 18% decreases in the total and LDL cholesterol levels after crowberry consumption. Ever since the relation between hyperhomocysteinemia and atherosclerosis was first hypothesized by [19], hyperhomocysteinemia became a well-known independent risk factor for neurologic and cardiovascular disease [7]. These authors analyzed the effect of crowberry consumption on serum Hcy levels.

The most significant change was observed in high T-cho subjects, before and after crowberry consumption (>200 mg·dl⁻¹, p<0.05). In the normal cholesterol group, there was no significant change in the serum Hcy levels. However, in the hyper-cholesterolemic group, the mean Hcy level decreased from 18.9 µmol·l⁻¹ to 16.3 µmol·l⁻¹ (p<0.05). The results of this study showed that Hcy levels were significantly higher among smokers and obese subjects. After consumption of crowberries, the Hcy levels of non-smokers became much lower than smokers. The higher serum Hcy levels in smokers are consistent with the effect of dose-dependent cigarette smoking on plasma Hcy levels. Targher et al. [20] asserted that cigarette smoking adversely affected plasma Hcy levels in young adults with type 1 diabetes. The reason why plasma Hcy levels are higher in smokers is not fully understood. Though no straightforward explanation is available, smoking may directly inactivate enzymes of homocysteine remethylation (such as methionine synthase [18]). Smoking was also accompanied by changes in plasma thiol redox status, possibly due to a higher formation of reactive oxygen species [22].

Findings of this study concur with the cardio-protective role of fruits and vegetables in human diet [23]. Some of the known cardio-protective agents in fruits include phytochemicals, vitamin C, folic acid, potassium, fiber, and phytosterol. These agents have the antioxidant, anti-inflammatory, and hypocholesterolemic benefits of fruits.

Studies on dietary blueberry consumption showed that the overall antioxidant capacity of serum as measured by the Oxygen Radical Absorption Capacity (ORAC) was not significantly altered, which suggested that there might not be a direct radical-scavenging effect of a blueberry diet.

However, the blueberry diet may elevate cellular or tissue antioxidant status [16]. Dark-colored berries, such as blueberries, were found to contain extremely high levels of polyphenols and exhibited strong antioxidant capacities [23,24]. The mean Hcy level changed from 18.99 µmol·l⁻¹ to 18.49 µmol·l⁻¹ for all subjects before and after crowberry intake. However, the mean Hcy level significantly decreased among hypercholesterolemic subjects, changing (T-cho>200 mg·dl⁻¹) from 18.99 µmol·l⁻¹ to 16.39 µmol·l⁻¹ (p<0.05).
The CAT, T-cho, TG and LDL levels also significantly decreased in high T-cho subjects compared to those of the low T-cho subjects. The low T-cho subjects in this study exhibited low lipid profiles. Hence, it was assumed that there would be no lowering effect of crowberry intake on their lipid profile and Hey level. Aprikian et al. [25] reported that supplementation with lyophilized apples in hyperlipidemic rats significantly decreased plasma cholesterol and liver cholesterol levels. In their study, they found that cholesterol excretion increased in the feces of apple-fed rats, suggesting reduced cholesterol absorption. The study also suggested that high phenolics in apples contributed to this effect.

In conclusion, “Shiromi” showed a stronger antioxidant effect than any other known berry species, and may prove to be a good supplement for health promotion and reduction of chronic diseases. Food is nature’s best medicine. The food like “Shiromi” people eat can help people naturally treat and cure life-threatening diseases and improve their health.

**Abbreviations:**

CAT: catalase;  
BMI: body mass index;  
GPx: glutathione peroxidase;  
Hey: homocysteine;  
HDL: high density lipoprotein;  
LDL: low density lipoprotein;  
SOD: superoxide dismutase;  
TAS: total antioxidant status;  
T-cho: total cholesterol; and TG triglyceride.

**References**


