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ARTICLE

Chiral Separation of Catechins in Buckwheat Groats and the Effects of Phenolic Compounds in Mice Subjected to Restraint Stress

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This paper investigates the stereochemistry of catechin and epicatechin compounds in buckwheat (*Fagopyrum esculentum* Moench) groats and then examines the effects of an orally administered mixture of the phenolic compounds isolated from the groats in restrained mice. Phenolic compounds separated by Sephadex LH-20 chromatography contained catechin and epicatechin compounds with a sugar moiety or esterified with phenolic acid derivatives along with rutin. Other unidentified major compounds in the HPLC chromatogram were also deduced on the basis of the spectrometric data with LC-MS/MS analysis to be catechin or epicatechin compounds. A chiral HPLC separation technique under normal-phase conditions showed that catechin and its derivative compounds in buckwheat groats were (+)-isomers, whereas epicatechin and its derivative compounds were (−)-isomers. Propylene glycol (PPG) or buckwheat groats phenolic compounds (BGP) in PPG (10 or 100 mg/kg of body weight) were administered intragastrically once per day for 3 days to mice, which were then restrained for 24 h. Unrestrained mice were given PPG solution with or without free access to feed and water. Restraint stress induced an elevation in plasma arteriosclerotic index, hepatic total cholesterol, and the amount of thiobarbituric acid-reactive substances (TBARS) in plasma and liver tissues. In contrast, these variables were suppressed in the restrained mice that were given BGP. These results suggest that BGP has in vivo antistress effects against the reactions induced by immobilization in mice.

KEYWORDS: Buckwheat; catechin; epicatechin; enantiomer; restraint stress

INTRODUCTION

The importance of the daily intake of whole grains is receiving increasing attention because of the health benefits attributed to their dietary fibers, vitamins, and minerals, which are more abundant than in refined grains. In Japan, noodles from buckwheat grains are a popular traditional food, so much attention has been focused on the functionality of buckwheat groats. The seeds of buckwheat are utilized by removing the hulls. The groats are expected to contain various components, including the phenolic compounds found in whole groats. Buckwheat seeds have been reported to contain phenolic compounds, such as rutin, catechins (*1*), and procyanidins (*2*), and their total polyphenolic contents are higher than those of wheat, barley, rice, or millets (*3*). Therefore, the antioxidant activities of extracts from buckwheat groats are deduced to be higher than those of other cereals. Among the phenolic compounds in buckwheat groats, the antioxidant activities of catechins have been reported to be higher than that of rutin (*1*).

Recently, a chiral separation technique for enantiomers has been used to investigate differences in characteristics between enantiomers. The chiral separation of catechin and epicatechin enantiomers by HPLC using a chiral column (*4*) or capillary electrophoresis (*5*) has been reported. Although we isolated and identified catechin and epicatechin compounds from buckwheat groats (*1*), the stereochemistry of the compounds has not been fully investigated. We attempted to separate enantiomers of authentic catechin and epicatechin by normal-phase HPLC equipped with a chiral separation column and then used the technique to investigate the stereochemistry of catechin and epicatechin compounds isolated from buckwheat groats.

Seyle researched the effects of stressors on rats and other animals and proposed a general adaptation system, the first comprehensive biological theory of stress (*6*). The concept of the stress introduced by Seyle was broadened for the interpretation of the physiological phenomena in humans along with animals. Today, much attention has been focused on food components with antistress properties to promote health. Food materials with high antioxidant activity have been reported to be effective for the reactions induced by stress stimuli in restrained rodents (*7*−*9*). In this paper, we investigated the in vivo effects of phenolic compounds isolated from buckwheat groats in mice under restraint stress.

MATERIALS AND METHODS

Preparation of the Phenolic Compounds from Buckwheat Groats. Buckwheat (*Fagopyrum esculentum* Moench) seeds were hulled and then powdered using a vibrating mill. Phenolic compounds were extracted with

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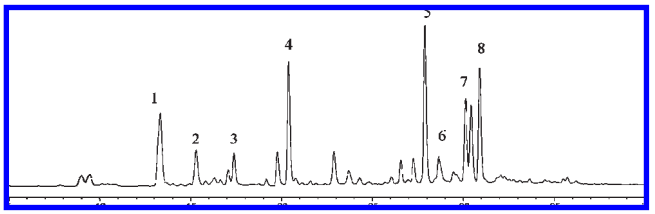

Figure 1. HPLC chromatogram (280 nm) of phenolic fraction separated by Sephadex LH-20. Compounds: **1**, catechin-7-*O*-glucoside; **2**, catechin; **3**, unknown; **4**, epicatechin; **5**, unknown; **6**, epicatechin-3-*O*-*p*-hydroxybenzoate; **7**, rutin; **8**, epicatechin-3-*O*-(3,4-di-*O*-methyl)gallate.

methanol (5 L) from the powdered groats (500 g) under reflux in a water bath at 75 °C, and the concentrated extract under reduced pressure was subjected to Sephadex LH-20 chromatography (46 × 900 mm). The fraction of buckwheat groats phenolic compounds (BGP), obtained by elution with ethanol by monitoring absorbance at 280 nm, was dried under reduced pressure and then dissolved in methanol. Total polyphenol content in the solution was analyzed according to the Folin−Denis method (*10*) to prepare the propylene glycol (PPG) solution (1 or 10 mg/mL of polyphenol) for the evaluation of the antistress effects in mice.

Analysis of the Phenolic Compounds by the Liquid Chromatography−Tandem Mass Spectrometry (LC-MS/MS) Technique. The composition of the phenolic compounds in BGP was determined by analysis with a QSTAR Pulsar-i LC-MS/MS system (Applied Biosystems, Foster City, CA), which was operated with a capillary voltage of 4500 V in the positive ion mode of electrospray ionization. The conditions of HPLC analysis were as follows: column, Symmetry C18 (2.1 × 150 mm, Waters, Milford, MA); column temperature, 35 °C; eluate, linear gradient of acetonitrile/water containing 0.005% trifluoroacetic acid (TFA), 5−58% acetonitrile over 50 min; flow rate, 0.2 mL/min. Four major catechin compounds [compound **1**, catechin 7-*O*-glucoside; compound **2**, catechin; compound **4**, epicatechin; compound **8**, epicatechin 3-*O*-(3,4-di-*O*-methyl) gallate in the HPLC chromatogram of extracts from buckwheat groats (**Figure 1**)], reported in previous literature (*1*), were isolated by preparative HPLC equipped with a Cosmosil 5C18 column (20 × 250 mm, Nacalaitesque, Kyoto, Japan) with a linear gradient of acetonitrile to determine the stereochemistry.

Chiral Separation Technique for Determination of Catechin and Epicatechin Compounds. Separation of (+)- and (−)-catechins in standard solution was achieved using a CHIRALPAK 1A column (Daicel Chemical, Tokyo, Japan) under normal-phase HPLC conditions. The chiral separation conditions using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) were as follows: eluate, hexane/ethanol (8:2) containing 0.5% TFA; flow rate, 1 mL/min; column temperature, 40 °C; detection, 280 nm. Separation of (+) and (−)-epicatechin in standard solution was achieved under the same conditions with a different eluate: hexane/ethanol (5:5) containing 0.5% TFA. Compound **1** (catechin 7-*O*-glucoside) and compound **8** [epicatechin 3-*O*-(3,4-di-*O*-methyl)gallate] were acid hydrolyzed with acidic methanol (2 mol/L HCl/methanol = 1:1) at 100 °C, and then the aglycone was recovered by extraction with ethyl acetate in the same manner as reported previously (*1*). Compound **2** (catechin), compound **4** (epicatechin), the aglycone of compound **1** (catechin), and that of compound **8** (epicatechin) were analyzed using a CHIRALPAK 1A column under the same conditions as the standard solution.

Animals. Female ddY mice were purchased from Japan SLC Inc. (Hamamatsu, Japan). They were housed in plastic cages in a temperature-controlled room (23 ± 1 °C) under light and dark (12 h each) conditions and had free access to water and feed (CE-2, Japan CLEA, Tokyo, Japan) until the experiment began. At 8 weeks old, the mice were randomly divided into five groups of 10−11 mice each. The mice in the restraint stress−high BGP group (RHP) were intragastrically administered 100 mg/kg of body weight (BW) of BGP solution (10 mg/mL in PPG) three times at 24 h intervals. The mice in the restraint stress−low BGP group (RLP) were administered 10 mg/kg of BW of BGP solution (1 mg/mL in PPG) in the same manner as the RHP group. The RHP and RLP groups were subsequently restrained for 24 h. The mice in the restraint stress−control (RC) group were intragastrically administered PPG solution and subsequently restrained for 24 h. The mice in the not restrained stress−fasting (NF) group were intragastrically administered PPG solution and subsequently fasted for 24 h. The mice in the not stressed−control (NC) group were administered PPG solution with free access to water and feed. The treatment of the mice complied with the Guide for the Care and Use of Experimental Animals (National Agricultural Research Center for Tohoku Region).

Stress Protocol. Thirty minutes after the last oral administration of BGP or PPG solution, the mice were subjected to restraint stress in the same method previously reported (*11*) according to other studies (*12*−*14*). In brief, the mice were restrained individually by placing them in 50 mL plastic tubes with holes (0.4 cm diameter) for respiration for 24 h. After being immobilized, the mice were anesthetized with sodium pentobarbital and killed by collecting whole blood from the heart in heparinized tubes, and the liver was excised. The plasma was prepared by centrifuging the collected blood at 11000*g* for 5 min at 4 °C. The plasma and liver were stored at −80 °C until used.

Measurement of Plasma Corticosterone. Plasma corticosterone, which is the main glucocorticoid in rodents, was extracted with ethyl acetate and measured by HPLC as previously reported (*11*) on the basis of Hayashi's method (*15*). Cortisol (0.13 μg in 2% metanol/saline) was added to 0.2 mL of plasma as an internal standard to correct the corticosterone content. Calibration of corticosterone was performed using a standard corticosterone solution.

Measurement of Plasma Glucose. Plasma glucose was measured with a Glucose C-II test kit (Wako Pure Chemical Ltd., Osaka, Japan) according to the instruction manual.

Extraction of Lipids from Liver Tissues. Total lipids in liver tissues were extracted according to the Folch method with a chloroform/methanol (2:1 v/v) solvent (*16*). After removal of the solvent from the extracts, the residue was dissolved in 2-propanol containing 10% Triton X-100. This crude lipid solution was used for the measurement of lipids.

Measurement of Plasma and Hepatic Lipids. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were measured with Cholesterol E, HDL cholesterol E, and triglyceride E test kits, respectively (all from Wako Pure Chemical Ltd.).

Assay for Plasma Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT). Plasma AST and ALT activities—enzyme markers for the injury of liver and other tissues—were assayed with a Transaminase C-II test kit (Wako Pure Chemical Ltd.) according to the instruction manual. The colored blue dye as end product of the enzymatic reactions was measured at 555 nm.

Assay for Thiobarbituric Acid-Reactive Substances (TBARS) in Liver Tissues and Plasma. The concentrations of TBARS in liver tissue homogenate were analyzed by using Kikugawa's method (*17*). Plasma TBARS were analyzed according to Yagi's method (*18*). TBARS in the reaction mixture, formed from malondialdehyde and thiobarbituric acid under acidic condition, were measured at 532 nm.

Measurement of Protein Content in the Liver Homogenate. The protein concentration in liver homogenate was measured by a Protein Quantification Kit—Rapid (Dojindo, Kumamoto, Japan) according to the instruction manual.

Statistics. Statistically significant differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's Kramer test using Prism4 software (GraphPad Software Inc., La Jolla, CA). Differences at $P < 0.05$ were considered to be significant.

RESULTS

Characterization of the Phenolic Compounds in BGP. The HPLC chromatogram (280 nm) of BGP by Sephadex LH-20 chromatography (**Figure** 1) showed that extracts from buckwheat groats contain many phenolic compounds. The percentages of phenolic compounds (w/w) were as follows: catechin (compound **2**), 4.2%; epicatechin (compound **4**), 11.6%; catechin-7-*O*-glucoside (compound **1**), 10.6%; epicatechin-3-*O*-*p*-hydroxybenzoate (compound **6**), 5.1%; epicatechin 3-*O*-(3,4-di-*O*-methyl)gallate (compound **8**), 11.7%; rutin (compound **7**), 7.3%; compound **3**, 3.1%; compound **5**, 15.3%; others (unknown), 31.1%. LC-MS/MS analysis revealed that unknown compounds **3** and **5** contained catechin or epicatechin as their aglycons. The ions detected in compound **3** (m/z 453 $(M + H)^+$, m/z 291 $(M - C_6H_{10}O_5)^+$) were also detected in

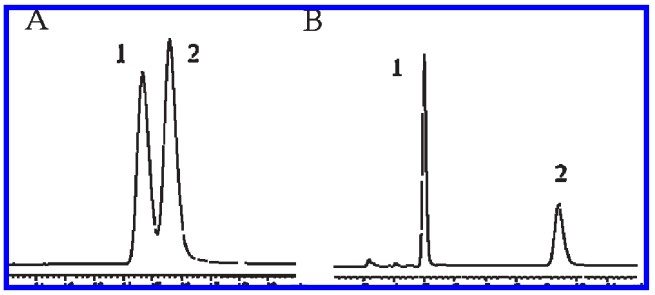

Figure 2. Separations of catechin and epicatechin enantiomers (authentic standards) by chiral HPLC column with normal-phase: (**A**) catechin enantiomers [peak 1, (+)-catechin; peak 2, (−)-catechin]; (**B**) epicatechin enantiomers [peak 1, (−)-epicatechin; peak 2, (+)-epicatechin].

compound **1** and had a retention time which suggested that compound **3** contained a glucose moiety with epicatechin as an aglycone. The ions detected in compound **5** (*m*/*z* 743 $(M + H)^+$, *m*/*z* 471, *m*/*z* 273) showed that this compound would be a conjugate consisting of epicatechin (or catechin) and its derivative.

Determination of Catechin and Epicatechin Compounds in Buckwheat Groats by Chiral Separation Technique. Figure 2 shows the HPLC chromatogram of the separation for standard solutions of catechin and epicatechin enantiomers. The standards of catechin and epicatechin were divided into (+)- and (−)-isomers by the same chiral separation column for each condition of the normal phase. Using these analysis conditions, the optical rotations of both isolated catechin (compound **2**) and the hydrolysate of catechin 7-*O*-glucoside (compound **1**) were determined to be the (+)-form, and that of epicatechin (compound **4**) and epicatechin 3-*O*-(3,4-di-*O*-methyl)gallate (compound **8**) was the (−)-form.

Effects of BGP on Plasma Corticosterone and Glucose Level. Figure 3 shows the effects of BGP on plasma corticosterone level in restrained mice. The plasma corticosterone level was higher in the NF and RC groups than in the NC group, and the concentration in the RC group tended to be higher than in the NF group. The elevation of plasma corticosterone in mice in the NF and RC groups indicated that stressor stimuli—food restriction and immobilization—activated the hypothalamic−pituitary−adrenal axis in these groups. Although the corticosterone levels in the RLP and RHP groups tended to be lower than in the RC group, there was no significant difference among these groups. The effect of BGP on plasma glucose concentrations in restrained mice is shown in **Figure** 4. The plasma glucose levels in the NF, RC, RLP, and RHP groups were significantly lower in than the NC group. Although the plasma glucose concentration was higher in the RC and RHP groups than in the NF group, there was no significant difference in the NF and RLP groups. In contrast, administration of BGP tended to suppress glucose concentrations in the RLP and RHP groups more than in the RC group.

Effects of BGP on Cholesterol and Triglyceride Levels in Plasma and Liver Tissues. Concentrations of total cholesterol, HDL cholesterol, triglycerides, and the arteriosclerotic index (total cholesterol − HDL cholesterol/HDL cholesterol) in the plasma are shown in **Table** 1. Although the concentration of plasma total cholesterol was significantly higher in the RC, RLP, and RHP groups than in the NC group, it was lower in the RLP and RHP groups than in the RC group. In contrast, the concentration of plasma HDL cholesterol tended to be higher in the RLP and RHP groups than in the RC group, so that the arteriosclerotic index in the RHP group was substantially lower than in the RC group, and that of the RLP group tended to be lower than in the RC group. However, the concentration of plasma triglyceride was significantly lower in the NF, RC, RLP, and RHP groups

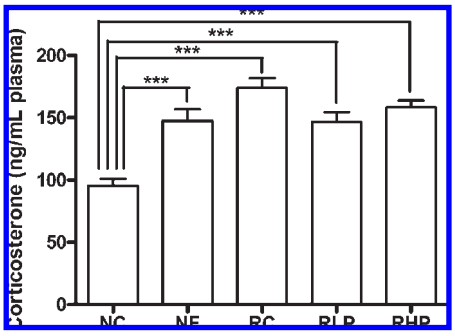

Figure 3. Effect of BGP on plasma corticosterone concentrations in restrained mice. NC, not-stressed group; NF, not restrained stress−fasting group; RC, restraint stress−control group; RLP, restraint stress−low BGP group; RHP, restraint stress−high BGP group. Data are mean ± SE (n = 10−11). ***, $P < 0.001$.

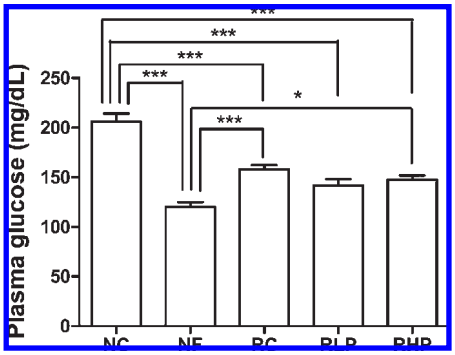

Figure 4. Effect of BGP on plasma glucose concentrations in restrained mice. NC, not-stressed group; NF, not restrained stress−fasting group; RC, restraint stress−control group; RLP, restraint stress−low BGP group; RHP, restraint stress−high BGP group. Data are mean ± SE (n = 10−11). *, $P < 0.05$; ***, $P < 0.001$.

than in the NC group, but it was not significantly different among the NF, RC, RLP, and RHP groups. As shown in **Table** 2, the concentrations of hepatic triglycerides were higher in the NF, RC, RLP, and RHP groups than in the NC group, but the concentrations were not significantly different in the NF, RC, RLP, and RHP groups. Although the concentration of hepatic total cholesterol in the RC group was higher than in the NC, NF, RLP, RHP groups, there was no difference in the NC, NF, RLP, and RHP groups.

Effects of BGP on Plasma AST and ALT Activities. Plasma AST and ALT activities—enzyme markers of injury to tissues such as the liver—are shown in **Table** 3. Although plasma ALT activity was significantly higher in the NF and RC groups than in the NC group, pretreatment with BGP suppressed the elevation in the activity of the enzyme compared with the RC group. In addition, the pattern of AST activity among the groups was similar to that of ALT activity; that is, ALT activity tended to be lower in the RLP and RHP groups than in the RC group. These results indicate that the damage to tissues induced by stress stimuli, including the liver, decreased with the administration of BGP in restrained mice.

Effects of BGP on TBARS Concentrations in Plasma and Liver Tissues. TBARS concentrations in plasma and liver tissues are shown in **Figure** 5. Although TBARS concentrations were not elevated in the plasma by food restriction, restraint stress induced an elevation in both plasma and liver tissues. Pretreatment with BGP at high level (RHP) significantly suppressed TBARS concentrations in the plasma and liver tissues, and pretreatment

Table 1. Effects of BGP on Plasma Triglycerides Concentrations, Cholesterol Concentrations, and Arteriosclerotic Index in Restrained Mice

group[a]	triglycerides (mg/dL)	total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	arteriosclerotic index[b]
NC (n = 10)	117.2 ± 11.6 b	92.6 ± 5.7 a	67.2 ± 5.8	0.41 ± 0.06 ab
NF (n = 10)	58.9 ± 4.0 a	88.0 ± 4.2 a	67.3 ± 3.8	0.31 ± 0.01 a
RC (n = 11)	53.0 ± 3.6 a	144.3 ± 9.3 b	72.9 ± 4.8	0.79 ± 0.08 c
RLP (n = 10)	53.4 ± 3.3 a	125.4 ± 9.0 b	82.7 ± 8.1	0.56 ± 0.07 bc
RHP (n = 10)	50.5 ± 2.7 a	126.2 ± 8.0 b	85.7 ± 7.1	0.50 ± 0.05 ab

[a] NC, not-stressed group; NF, not restrained stress−fasting group; RC, restraint stress−control group; RLP, restraint stress−low BGP group; RHP, restraint stress−high phenolic compounds group. Data are mean ± SE. Different letters within a column are significantly different (P < 0.05). [b] Arteriosclerotic index = (total cholesterol − HDL cholesterol)/HDL cholesterol.

Table 2. Effects of BGP on Hepatic Lipid Concentrations in Restrained Mice

group[a]	triglycerides (mg/g of tissue)	total cholesterol (mg/g of tissue)
NC (n = 10)	6.3 ± 0.4 a	2.7 ± 0.1 a
NF (n = 10)	25.6 ± 3.9 b	3.0 ± 0.1 a
RC (n = 11)	26.0 ± 2.1 b	3.9 ± 0.3 b
RLP (n = 10)	21.2 ± 3.6 b	3.2 ± 0.2 a
RHP (n = 10)	26.9 ± 3.7 b	2.8 ± 0.1 a

[a] NC, not-stressed group; NF, not restrained stress−fasting group; RC, restraint stress−control group; RLP, restraint stress−low BGP group; RHP, restraint stress−high BGP group. Data are mean ± SE. Different letters within a column are significantly different (P < 0.05).

Table 3. Effects of BGP on Plasma AST and ALT Activities in Restrained Mice

group[a]	AST activity (IU/L)	ALT activity (IU/L)
NC (n = 10)	16.2 ± 1.3 a	4.0 ± 0.2 a
NF (n = 10)	24.4 ± 2.1 ab	7.2 ± 0.6 b
RC (n = 11)	46.4 ± 6.9 c	10.0 ± 0.8 c
RLP (n = 10)	37.1 ± 11.9 bc	7.0 ± 1.4 b
RHP (n = 10)	37.8 ± 2.2 bc	6.4 ± 0.5 ab

[a] NC, not-stressed group; NF, not restrained stress−fasting group; RC, restraint stress−control group; RLP, restraint stress−low BGP group; RHP, restraint stress−high BGP group. Data are mean ± SE. Different letters within a column are significantly different (P < 0.05).

at low level (RLP) significantly suppressed the value in the plasma.

DISCUSSION

Differences in characteristics between catechin enantiomers have been demonstrated as follows: the bioavailability of (+)-catechin was reported to be higher than that of (−)-catechin (*4*), and catechin enantiomers exhibited the opposite effects on glycogen metabolism in isolated rat hepatocytes (*19*). These results indicated that investigation of the stereochemistry of catechin and epicatechin enantiomers in food materials is important to clarify the characteristics of each enantiomer and the materials. HPLC chiral separations under normal-phase conditions gave a rapid (within 20 min) and good separation of (+)- and (−)-isomers of catechin and epicatechin. In addition, this research demonstrated that major catechins and epicatechins in buckwheat groats were (+)- and (−)-isomers, respectively. The most widely distributed members of naturally occurring phenolic flavan-3-ols are the diastereoisomeric pair (+)-catechin and (−)-epicatechin (*20*). We could not use standards that were evident in their optical rotation to compare with the chromatogram for chiral HPLC separation of catechin and epicatechin derivatives, such as the glucoside of catechin and esterified epicatechin. Therefore, their acid-hydrolyzed products were used for analysis in the investigation of the stereochemistry of the aglycons. Further studies for chiral separation of catechin and epicatechin derivatives in the normal-phase HPLC analysis are required. On the other hand, roasted buckwheat groats are utilized for tea in Japan. It is speculated that roasting of buckwheat groats might

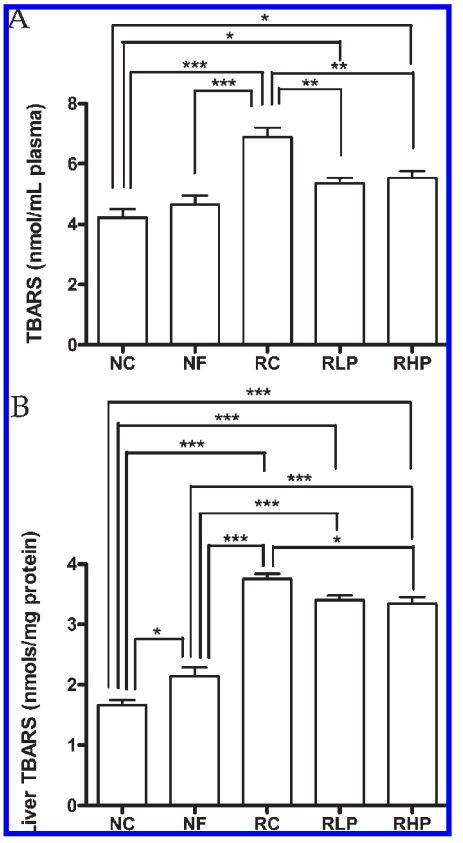

Figure 5. Effects of BGP on lipid peroxidation in plasma (**A**) and liver tissues (**B**) in restrained mice. NC, not-stressed group; NF, not restrained stress−fasting group; RC, restraint stress−control group; RLP, restraint stress−low BGP group; RHP, restraint stress−high BGP group. Data are mean ± SE (n = 10−11). ∗, P < 0.05; ∗∗, P < 0.01; ∗∗∗, P < 0.001.

affect the epimerization of catechin, epicatechin, and their derivatives in groats as well as cocoa beans (*5*). Investigation of the stereochemistry of catechin and epicatechin enantiomers in roasted groats may be important for investigation of their functions. Moreover, identification of unknown catechin or epicatechin compounds (compounds **3** and **5**) in BGP is in progress.

With respect to the effects of the phenolic compounds in buckwheat groats in restrained mice, oral administration of BGP in restrained mice significantly suppressed the elevation of the plasma arteriosclerotic index in the RHP group and the content of hepatic total cholesterol in the RLP and RHP groups compared with the control restrained stress mice (RC group). Ricart-Jane et al. showed that chronic stress by immobilization induced elevations in total and HDL cholesterol levels in the plasma (*21*). Although the result of this research was in agreement with their paper, the arteriosclerotic index in the RC group was increased compared with the NC and NF groups. Improvement in the plasma arteriosclerotic index in

the administered BGP groups (RLP and RHP) compared to the RC group was attributable to the suppression of total cholesterol level in the plasma. Furthermore, Igarashi et al. reported that the arteriosclerotic index in catechin-fed Goto−Kakizaki rats was lower than in the control rats (*22*). Yokozawa et al. demonstrated that green tea polyphenols increased the levels of HDL cholesterol, leading to dose-dependent improvement of the arteriosclerotic index in cholesterol-fed rats (*23*). These results suggested that BGP, which contains catechins as major components, would be effective in improving lipid metabolism in restrained mice.

Yun et al. reported that epigallocatechin-3-gallate (EGCG), the most abundant catechin polyphenol in green tea, reduced AST and ALT levels in ethanol-fed rats. This result suggested that EGCG prevents ethanol-induced hepatotoxicity (*24*). The suppressive effect of BGP on ALT activity indicated that BGP has a protective effect on liver damage induced by restraint stress in mice. Decrements in TBARS in liver tissues were reported along with liver transaminase levels, due to the hepatoprotective effect of green tea extract in tamoxifen-intoxicated rats (*25*). The elevation of TBARS concentrations in plasma and liver tissues in mice subjected to restraint stress indicated that restraint stress induces in vivo oxidative stress in mice. In contrast, oral administration of BGP effectively suppressed the elevation of TBARS in both plasma and liver tissues, suggesting that BGP would be effective against reactive oxygen species induced by stress stimuli. The activity would be attributable to the antioxidant activities of phenolic compounds, including epicatechin and catechin compounds along with rutin.

We previously reported that flavonoids with antioxidant activities from buckwheat sprouts affected reactions induced by restraint stress in mice (*11*). In this experiment, we investigated the stereochemistry of catechins and epicatechins in BGP and their beneficial effects on restrained mice. Consequently, it was demonstrated that buckwheat seeds and sprouts are useful food materials with antistress effects.

ABBREVIATIONS USED

BGP, buckwheat groats phenolic compounds; LC-MS/MS, liquid chromatography−tandem mass spectrometry; PPG, propylene glycol; TFA, trifluoroacetic acid; TBARS, thiobarbituric acid-reactive substances; HDL cholesterol, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminnotransferase; SE, standard error.

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