DOI:10.1021/jf900194s

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Antioxidant Activity of Tartary Buckwheat Bran Extract and Its Effect on the Lipid Profile of Hyperlipidemic Rats

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The effects of Tartary buckwheat bran extract (TBBE) on antioxidation status and on lipid profile were determined in hyperlipemic rats. Seven-week-old male Wistar rats were fed a high-fat diet to induce hyperlipemia with doses of TBBE at 0.2 (low), 0.5 (medium), and 1.0 (high) g/kg of body weight. The positive control group was fed the high-fat diet or supplemented with *Gynostemma pantaphyllum* total glucoside tablet at 0.032 g/kg of body weight. The negative control group was fed the basal diet. The blood lipids, liver lipids, and antioxidant-related parameters of the rats were measured. The rats fed TBBE indicated that TBBE could effectively reduce serum total triglycerides (TG) and total cholesterol (TC) when compared to the control groups ($P < 0.05$). TBBE also reduced liver TC and TG by 36.4 and 73.9% in the low-dose group when compared to the high-fat group ($P < 0.05$), respectively, presenting remarkable effects in serum triglyceride reduction, antiatherosclerosis, and serum-lipid oxidation resistance. TBBE also raised serum glutathione peroxidase (GSH-Px) activity and antiatheromatous plaque formation index (AAI) and lowered the atherogenic index of plasma (AIP), the artherogenic index (AI), and serum malondialdehyde (MDA) in comparison with the control groups ($P < 0.05$ or $P < 0.01$). In this study, TBBE was shown to significantly reduce the TG and TC in the serum and liver of rats, raise serum antioxidant activity, and inhibit serum lipid peroxide formation.

KEYWORDS: Tartary buckwheat bran extract; serum lipids; ORAC; hepatic lipids; antioxidant enzyme; malondialdehyde; hyperlipidemic rats

INTRODUCTION

Flavonoids are a large group of polyphenolic compounds found ubiquitously in plants including fruits, vegetables, and whole grains, which are regularly consumed in the human diet and are also taken as herbal supplements (*1*−*3*). They have been reported to exhibit a wide variety of beneficial biological effects such as anti-inflammatory (*4*), antioxidant (*5*, *6*), free radical scavenging (*7*), antiviral, antibacterial (*8*), cardiovascular health promotion (*9*, *10*), and chemopreventive activities (*11*, *12*). It was well established that rutin decreases the capillary fragility associated with hemorrhagic diseases, reduces high blood pressure (*13*−*15*), decreases the permeability of vessels, has an antiedema effect, reduces the risk of arteriosclerosis (*16*, *17*), and has strong antioxidant activity (*18*, *19*).

Common buckwheat (*Fagopyrum esculentum* Moench, Polygonaceae) originated in southwestern China and eventually spread to all continents, whereas Tartary buckwheat (*Fagopyrum tataricum*) is grown and used in the mountainous regions of southwestern China (Sichuan), northern India, Bhutan, and Nepal. As an important functional food, Tartary buckwheat grain contains proteins with high nutritional value including a balanced amino acid composition (*20*, *21*) and relatively high crude fiber and vitamin B_1, B_2, and B_6 contents (*20*). Furthermore, Tartary buckwheat is a rich source of flavonoids, including quercetin and its glycoside, rutin. They are found in its flowers, leaves, stems, and seeds. Its average flavonoid content is 9−300 times higher than that found in common buckwheat (*22*, *23*).

Epidemiological studies have revealed that buckwheat has the effect of reducing blood sugar and, to some extent, fat and blood pressure (*24*). For this reason, its effect on cardiovascular health has received increased attention (*24*). Tartary buckwheat bran is a byproduct of Tartary buckwheat processing. Previous research has shown that Tartary buckwheat is approximately 11.5% bran, and the bran contains about 7.7% total flavonoids, 1.4% shell powder, and 1.0% flour, meaning it can be an important raw

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 Published on Web 05/06/2009 © 2009 American Chemical Society

material for health foods. It has been reported that the flavonoids extracted from buckwheat leaves have the effect of reducing blood lipids and raising the activity of serum SOD in tested hyperlipemic animals (*25−28*). To our knowledge there has been no report on the effects of the extract from Tartary buckwheat bran on blood and liver lipid reduction and on antioxidant activity to date. The objectives of the present study were to determine Tartary buckwheat bran extract for its effect on blood and liver lipids and antioxidant activity in hyperlipemic rats.

MATERIALS AND METHODS

Chemicals and Reagents. Rutin (purity > 99.83%) and quercetin (purity > 99.92%) were bought from the Second Chemical productions factory (Shanghai, China). Trolox, ascorbic acid, and disodium fluorescein were obtained from Sigma (St. Louis, MO). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). Cholesterol was purchased from HuiSheng Biochemical Reagent Co. Ltd. (Shanghai, China); sodium chlorate was purchased from Microorganism Medium Factory (Beijing, China); *Gynostemma pantaphyllum* total glucoside tablets [Medical Approval no. (90)Z-04)] were purchased from Shaanxi An-Kang Factory of Chinese Medicines; triglyceride (TG) and cholesterol (TC) and high-density lipoprotein-cholesterol (HDL-C) test kits were produced by Biosino Biotechnology Co. Ltd. (Beijing, China); apolipoprotein A_1 ($apoA_1$) and apolipoprotein B (apoB) test kits were produced by Yilikang Biotech Co. Ltd. (Beijing, China); superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) test kits were produced by Jian-Cheng Bioengineering Institute (Nanjing, China).

Extraction from Tartary Buckwheat Bran (TBBE). Tartary buckwheat bran used in this study was the byproduct of flour processing using Kuqiao 1, a variety of Tartary buckwheat that was planted in Liang-Shan Yi Autonomous Prefecture of Sichuan Province in 2003. Tartary buckwheat oil was removed with petroleum ether at 60−90 °C in a 100 L vacuum extractor. Tartary buckwheat bran was extracted three times with 95% ethanol at 55−65 °C. The extraction rate was 9.83%, and the extract (TBBE) was evaluated for the contents of rutin, quercetin, and total phenolics and for antioxidant activity and was used for the animal study.

Determination the Contents of Rutin and Quercetin. *Nonhydrolyzed Sample.* TBBE was dried at 100−105 °C for 4 h. One hundred milligrams was then dissolved in 0.4% phosphoric acid and methanol (40:60, v/v) in a 50 mL volumetric flask. Next, the solution was diluted 4 times with 0.4% phosphoric acid and methanol solvent for analysis by HPLC. The final concentration of the sample was 0.5 mg/mL.

Hydrolyzed Sample. One hundred micrograms of dried TBBE was dissolved in 35 mL of methanol and then mixed with 5.0 mL of 25% hydrochloric acid. The solution was refluxed at 80 °C for 1 h and cooled. The residue was then dissolved in 0.4% phosphoric acid/methanol (40:60, v/v) for analysis by HPLC. The final concentration was 0.2 mg/mL.

Analysis of Contents of Rutin and Quercetin. Twenty microliters of nonhydrolyzed or hydrolyzed sample, rutin standards, or quercetin standards was loaded in a Perkin-Elmer series 200 HPLC (200E pump, UV−vis detector, and Tata Chrom workstation 6.2.0), and the column was a Kromasil C_{18} (4.6 mm × 300 mm, 5 μm). The mobile phase was 0.4% of phosphoric acid/methanol (40:60, v/v), and the flow rate was 1.0 mL/min. The column temperature was set at 20 °C, and compounds were measured at 358 nm. All solvents and samples were filtered through a 0.45 μm filter before loading. Individual compounds were identified by retention time and quantified by comparison to standard curves for rutin and quercetin. The retetion times of rutin and quercetin were 6.0 and 3.3 min, respectively. Samples were measured in triplicate.

Determination of Total Phenolic Content. The total phenolics content was analyzed according to the Folin−Ciocalteu colorimetric method described previously (*29*). Briefly, 125 μL of the standard gallic acid solution or appropriate dilutions of TBBE was mixed with 0.5 mL of distilled water in a test tube followed by the addition of 125 μL of Folin−Ciocalteu reagent. The samples were mixed well and then allowed to stand for 6 min; then 1.25 mL of 7% aqueous sodium carbonate was added. Water was used to adjust the final volume to 3 mL. Samples were allowed to stand for 90 min at room temperature before the absorbance was measured at 760 nm versus the blank using a 1420 Multilabel Counter (Perkin-Elmer, Waltham, MA). The results were expressed as mean (μmol of gallic acid equiv/g of sample) ± SD. Triplicate measurements were taken.

Quantification of the Total Antioxidant Activity. A modified method of the oxygen radical absorbance capacity (ORAC) assay was used for determining total antioxidant capacity of the extract. The ORAC−fluorescein assay was performed as described previously (*30*) with minor modifications. Briefly, 20 μL of antioxidant (TBBE or Trolox standards), and 200 μL of 0.117 μM fluorescein in 75 mM phosphate buffer (pH 7.4) were pipetted into a 96-well microplate. The mixture was preincubated for 20 min at 37 °C, and then 20 μL of 40 mM AAPH was added rapidly using a multichannel pipet. The fluorescence (λ_{ex} = 485 nm; λ_{em} = 520 nm) was recorded every min for 90 cycles using a 1420 Multilabel Counter (Perkin-Elmer). Calibration solutions of Trolox (6.25−50 μmol/L) were also carried out in each assay. Data were exported to Excel (Microsoft) for further calculations. The area under the fluorescence decay curve (AUC) was calculated as $AUC = 1 + f_1/f_0 + f_2/f_0 + ... + f_{35}/f_0$, where f_0 is the initial fluorescence at $t = 0$ and f_i the fluorescence at $t = i$. ORAC-FL values were expressed as Trolox equivalents by using a standard curve, and regression analysis was performed using Sigmaplot 11.02 software (Systat Software Inc., San Jose, CA).

Animals and Treatment. Sixty pathogen-free male Wistar rats (7 weeks old), 180−220 g, were purchased from the Center of Experimental Animals of the Fourth Military Medical University (Xi-An, China). The rats were fed basal feed and were housed with 12 h light/12 h dark cycle. They were acclimated to the surroundings for 1 week prior to experimentation. Care and treatment followed the recommended guidelines of the National Research Council (1985). The rats were randomly assigned to six groups (n = 10): (1) control group fed basal feed (NC), which was based on the AIN-93 recommendations; (2) high-fat feed group (HFC), which was 10% fat and was adjusted by changing the fat and carbohydrate contents based on the normal diet; (3) positive control group fed high-fat feed and *G. pantaphyllum* total glucoside tablet at a dose of 0.032 g/kg of body weight (GPC) delivered by gavage; (4−6) groups fed the high-fat feed and the TBBE at low dose (LD), 0.2 g/kg of body weight, medium dose (MD), 0.5 g/kg of body weight, and high dose (HD), 1.0 g/kg of body weight, respectively, delivered by gavage. The doses were chosen by the toxicity study of TBBE in rats determined from our preliminary experiment. NC was fed basal feed, and the other five groups were fed the high-fat feed. The formula of basal feed was composed of 30% corn flour, 20% bean cake powder, 25% wheat bran, 16% wheat flour, 5% fish meal, 2% bone meal, 1% yeast powder, and 1% common salt. The formula of the high-fat feed was 87.7% basal feed, 2% cholesterol, 0.3% sodium cholate, and 10% lard. The NC rats were fed the basal feed, and the other groups were fed the high-fat feed for 10 days. The NC rats and three randomly selected from the high-fat fed groups were measured for their TG and TC. The results showed that the TG and TC of all the high-fat feed groups were >3 times higher than the NC group, meaning the study could continue. Next, GPC and TBBE (LD, MD, and HD) were delivered by gavage. Distilled water was gavaged into the stomachs of the rats in NC and HFC at 10 mL/kg of body weight per day for 6 weeks. During this period the rats were allowed to drink and feed freely, and their weights were measured weekly after an overnight fast. The blood of the rats was taken from the eye socket in the second and fourth weeks after TBBE was administered and was used to determine TG and TC. At the end of week 6 after a 12 h fast, blood was obtained from the carotid artery and used to determine TG, TC, HDL-C, $apoA_1$, apoB, SOD, GSH-Px, and MDA; at the same time, the livers of the rats were removed and weighed, and then some liver tissue was collected and homogenized for determining the following liver parameters: TG, TC, SOD, GSH-Px, and MDA.

Serum and Liver Lipid Profile Antioxidant Enzymes and Oxidative Measurements. Serum and liver homogenates were obtained to determine TG, TC, HDL-C, $apoA_1$, apoB, SOD, GSH-Px, and MDA using the test kits from companies mentioned above. Total cholesterol was measured using an enzymatic photometric method (CHOD-PAP), and TG was measured using an enzymatic colorimetric method (GPO-PAP). HDL-C was determined by the phosphotungstic acid (PTA)/$MgCl_2$ precipitation procedure. Serum $apoA_1$ and apoB were measured by ELISA. Total SOD activity in the plasma was assayed

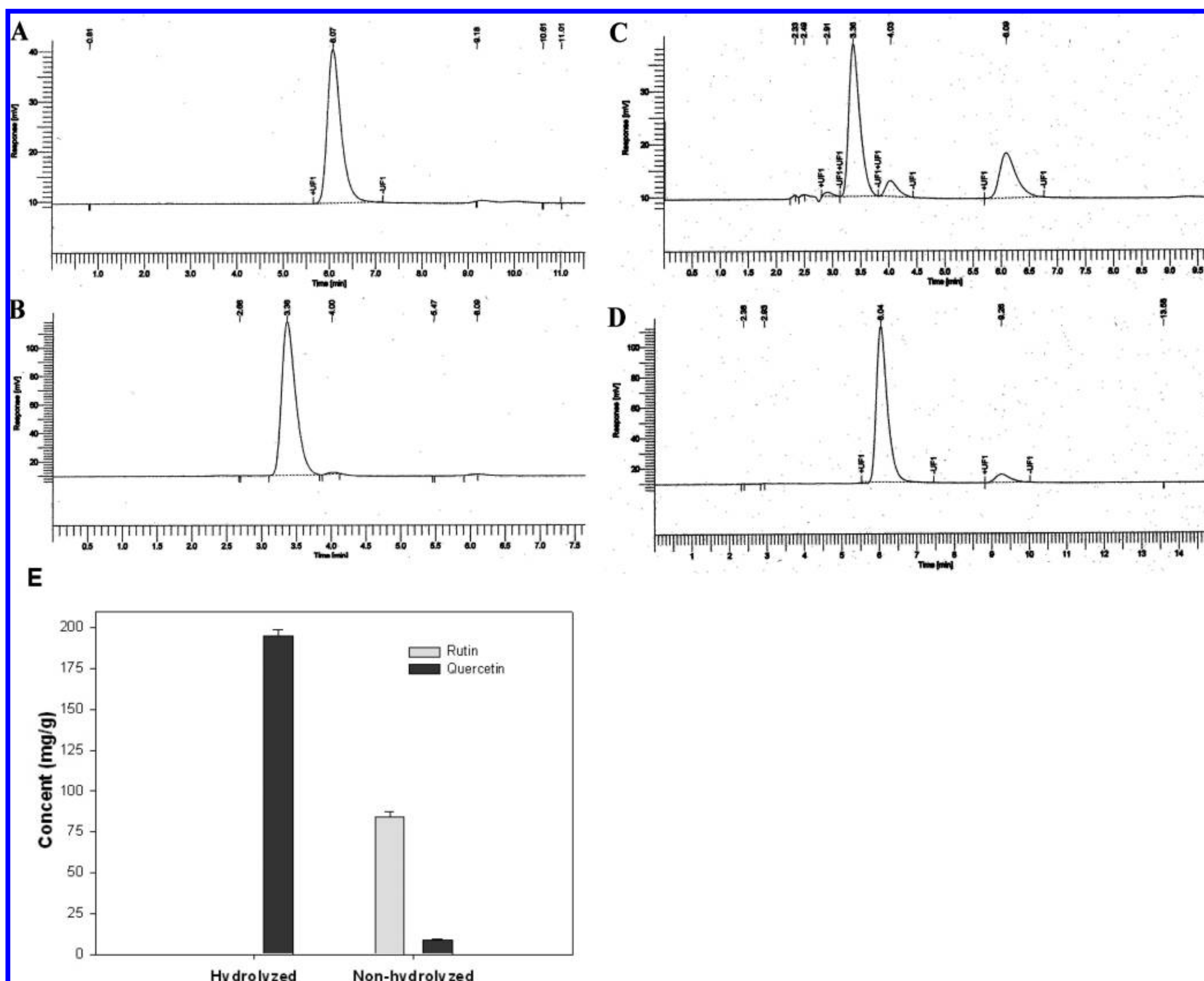

Figure 1. Determination of rutin and quercetin in Tartary buckwheat by reversed-phase high-performance liquid chromatography: (**A**) quercetin; (**B**) rutin; (**C**) nonhydrolyzed sample; (**D**) hydrolyzed sample. (**E**) Contents of rutin and quercetin in the samples of hydrolysis and nonhydrolysis.

by the nitroblue tetrazolium (NBT) reduction method (*31*). One unit of enzyme activity was defined as the amount of enzyme necessary to inhibit the reaction by 50%. SOD activity was expressed as units per minute per milliliter of plasma. Plasma GSH-Px activity was measured according to the method of Paglia and Valentine (*32*). GSH-Px catalyzes the reaction between reduced glutathione (GSH) and H_2O_2. The product of this reaction—oxidized glutathione—is reduced to GSH using nicotinamide adenine dinucleotide phosphate ($NADPH + H^+$) catalyzed by glutathione reductase (GR). The decrease in absorbance was measured at 340 nm. Activity of GP-x was determined as micromoles of $NADPH + H^+$ used to reduce GSH per minute in plasma (units/L). Lipid peroxidation was estimated according to the methods of Hunter et al. (*33*) and Ohkawa et al. (*34*) and was expressed as *n* moles of malondialdehyde (MDA) formed per liter of plasma. Samples were measured at 532 nm with an extinction coefficient of 0.152 $mmol^{-1}$ cm^{-1}. Low-density lipoprotein-cholesterol (LDL-C) was calculated by using the Friedewald formula (*35*): LDL-C (mmol/L) = TC (mmol/L) − HDL-C (mmol/L) − TG (mmol/L) × 0.456. The artherogenic index (AI) was calculated by using the formula AI = (TC − HDL-C)/HDL-C, and the antiatheromatous plaque formation index (AAI) was calculated by using the formula AAI = HDL-C/TC. Liver index (%) was equal to liver weight (wet) (g)/body weight (g) × 100. The atherogenic index of plasma (AIP) (*36*) was also calculated using the following equation: AIP = log(TG/HDL-C).

Statistical Analyses. Data were expressed as mean ± SD. A one-way analysis of variance was used to analyze body weight. Serum and liver levels of TC, TG, MDA, GSH-Px, SOD, and serum of HDL-C, LDL-C, $apoA_1$, and apoB were compared by using one-way ANOVA or the Mann−Whitney *U* test. Data analyses were generated and plots were constructed using SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL) and SigmaPlot version 11.02 for Windows (Systat Software Inc.). Statistical significance was set at $P < 0.05$ and $P < 0.01$, and all *P* values were unadjusted for multiple comparisons.

RESULTS

Contents of Rutin and Quercetin in TBBE. The contents of rutin and quercetin in TBBE were analyzed by HPLC. The recovery rates of rutin and quercetin were 99.9 and 98.5%. The relative standard deviations (RSD) were 1.16 and 1.50% for rutin and quercetin, respectively. The retention times of rutin and quercetin were 3.36 and 6.09 min, respectively (**Figure 1**). Both rutin and quercetin were determined in the nonhydrolyzed sample. The results showed concentrations of 8.96 ± 0.26 and 83.84 ± 3.29 mg/g of dry weight of extract for quercertin and rutin, respectively. Only quercetin was determined in the hydrolyzed sample (194.68 ± 4.22 mg/g) (**Figure 1**).

Effect of TBBE on Antioxidation. Phenolic compounds have been shown to have strong antioxidant activity (*37*). In this study, the total phenolic content and ORAC assay were evaluated. The total phenolic content of the TBBE was 158.44 ± 7.30 μmol

of gallic acid equiv/gram, and the ORAC value was determined to be 55514 ± 5850 μmol of Trolox equiv/g of dry weight.

Effect of TBBE on the Growth and Development of the Rats. Rats in the NC group had the highest weight gain, whereas the MD group had the lowest (**Table 1**). However, the differences were not significant, indicating that the administration of TBBE to the rats by gavage had no toxic effect at the tested doses.

Effects of TBBE on TG and TC Contents in Serum of the Rats. The serum TC and TG in the HFC group was higher than the NC group from weeks 2 to 6 ($P < 0.01$) (**Table 2**), indicating that the high-fat feed had caused the hyperlipemia. Throughout the study the TG and TC in the GPC group were lower than in the HFC group, but the TG content was higher than that of the NC group ($P < 0.01$). The serum TG of the GPC group was lower than that of the HFC group but was higher than that of the NC group. By the second week and throughout the study, the three TBBE groups showed a decrease in TG and TC. After 6 weeks, the serum TG in the TBBE groups decreased by 73.9% (LD), 49.8% (MD), and 42.0% (HD), and their TC contents decreased by 36.4% (LD), 35.6% (MD), and 36.8% (HD) compared to the HFC group. The TG content in the serum of the LD group was much lower than that of the GPC group ($P < 0.01$) and was also slightly lower than that of the NC group.

Effects of TBBE on HDL-C, LDL-C, ApoA$_1$, ApoB, AI, AAI, and AIP in Serum. After 6 weeks, the rats in the LD group increased their AAI ($P < 0.01$) and decreased their apoA$_1$ contents ($P < 0.05$) and AI ($P < 0.01$). The rats in the MD group increased their AAI ($P < 0.05$) and decreased their AI ($P < 0.01$) and apoB contents ($P < 0.05$) (**Table 3**). The AI in the TBBE groups showed a dose-dependent increase from HD to LD, and all of the AI values in TBBE groups were lower than those in the HFC group. The HDL-C and LDL-C in the LD group were not significantly different compared to HFC group, and the AI and AAI of the rats in the HD group were not significantly different compared to the HFC group. The AIP in the TBBE groups were significantly different from the NC and HFC groups ($P < 0.01$ or $P < 0.05$). An increasing trend was observed within the TBBE treatment (**Table 3**). The apoB to apoA$_1$ ratio in the TBBE groups was slightly lower compared to the NC and HFC control groups (**Table 3**).

Effects of TBBE on SOD and GSH-Px Activities and MDA Contents of the Serum of the Rats. The rats in the HFC group had a lower SOD activity ($P < 0.01$) and a higher MDA content ($P < 0.01$) compared to the NC group. This indicated that high-fat feed lowered the SOD activity and accelerated lipid oxidation, thereby resulting in an increased quantity of lipid peroxides. Compared to the HFC group, the rats in the LD, MD, and HD groups had significantly higher SOD activity with increasing doses of TBBE ($P < 0.01$) (**Table 4**). The rats in the MD and LD groups had significantly higher GSH-Px activity when compared to the NC group ($P < 0.01$), and the LD group had a significantly lower MDA concentration compared to the HFC group ($P < 0.01$) (**Table 4**). It was shown that TBBE raised the antioxidant enzymes in rat serum, but these were varied among the dose levels.

Table 1. Initial Body Weights and Gains in Body Weight for Different Groups of Rats (Mean ± SD)[a]

group	*n*	dose (g/kg of bw)	initial body wt (g)	final body wt (g)	wt gain (g)
NC	10	0	320.2 ± 89.7	431.5 ± 167.4	111.8 ± 89.0
HFC	10	0	352.9 ± 69.9	461.7 ± 125.7	100.8 ± 55.9
GPC	10	0.032	341.6 ± 78.8	426.0 ± 137.3	84.4 ± 60.7
LD	10	0.2	344.0 ± 89.9	437.5 ± 149.6	93.5 ± 59.4
MD	10	0.5	349.0 ± 80.7	419.7 ± 129.0	70.7 ± 55.8
HD	10	1.0	341.7 ± 80.0	427.8 ± 139.6	86.1 ± 61.7

[a] NC, basal diet control group; HFC, high-fat diet control group; GPC, high-fat + *Gynostemma pantaphyllum* total glucoside tablet group; LD, high-fat diet + low dose of Tartary buckwheat extract group; MD, high-fat diet + medium dose of Tartary buckwheat extract group; HD, high-fat diet + high dose of Tartary buckwheat extract group.

Table 2. Contents of TG and TC in Rat Sera of Different Groups (Mean ± SD)[a]

		TG (mmol/L)				TC (mmol/L)			
group	*n*	0 weeks	2 weeks	4 weeks	6 weeks	0 weeks	2 weeks	4 weeks	6 weeks
NC	10	0.67 ± 0.28	0.82 ± 0.21	0.83 ± 0.25	0.92 ± 0.24	1.86 ± 0.21	1.94 ± 0.41	1.72 ± 0.19	1.78 ± 0.47
HFC	10	2.65 ± 0.81 b	2.56 ± 0.78 b	2.95 ± 0.58 b	2.83 ± 0.72 b	3.70 ± 0.85 b	4.23 ± 1.92 b	4.40 ± 1.83 b	4.07 ± 1.17 b
GPC	10	2.65 ± 0.81 b	2.10 ± 0.51 b	1.58 ± 0.57 bc	1.50 ± 0.54 bd	3.70 ± 0.85 b	3.57 ± 1.16 b	2.56 ± 1.09 ac	2.12 ± 0.63 d
LD	10	2.65 ± 0.81 b	1.99 ± 0.63 b	2.15 ± 1.0 b	0.74 ± 0.33 df	3.70 ± 0.85 b	4.02 ± 1.24 b	2.88 ± 0.87 bc	2.59 ± 0.23 bde
MD	10	2.65 ± 0.81 b	1.62 ± 0.59 bd	1.66 ± 0.84 bd	1.42 ± 0.40 bd	3.70 ± 0.85 b	3.52 ± 0.85 b	2.45 ± 0.50 bd	2.34 ± 0.62 ad
HD	10	2.65 ± 0.81 b	2.19 ± 0.70 b	1.52 ± 0.82 bd	1.46 ± 0.57 ad	3.70 ± 0.85 b	3.27 ± 0.86 b	2.63 ± 0.55 bd	2.62 ± 0.89 ad

[a] NC, basal diet control group; HFC, high-fat diet control group; GPC, high-fat + *Gynostemma pantaphyllum* total glucoside tablet group; LD, high-fat diet + low dose of Tartary buckwheat extract group; MD, high-fat diet + medium dose of Tartary buckwheat extract group; HD, high-fat diet + high dose of Tartary buckwheat extract group; TG, triglyeride; TC, total cholesterol. Letters after entries indicate the following: a, b, $P < 0.05$ and $P < 0.01$, compared to normal the NC group; c, d, $P < 0.05$ and $P < 0.01$, compared to the HFC group; e, f, $P < 0.05$ and $P < 0.01$, compared to the GPC group.

Table 3. Contents of HDL-C and LDL-C, ApoA$_1$ and ApoB Levels, and AI and AAI Values in Rat Sera of Different Groups (Mean ± SD)[a]

group	*n*	HDL-C (mmol/L)	AIP	LDL-C (mmol/L)	apoA$_1$ (g/L)	apoB (g/L)	ratio of apoB/apoA$_1$	artherogenic index (AI)	antiatheromatous plaque formation index (AAI)
NC	9	1.00 ± 0.16	−0.04 ± 0.13 df	0.51 ± 0.49	0.55 ± 0.19	0.60 ± 0.33	0.99 ± 0.56	0.86 ± 0.62	0.60 ± 0.19
HFC	10	1.02 ± 0.41	0.46 ± 0.20 bf	1.76 ± 1.41 a	0.58 ± 0.20	0.73 ± 0.16	1.06 ± 0.13	3.71 ± 2.59 b	0.28 ± 0.15 b
GPC	8	0.90 ± 0.25	0.21 ± 0.19 bd	0.74 ± 0.41	0.44 ± 0.19	0.44 ± 0.28	1.07 ± 1.47	1.47 ± 0.86 c	0.49 ± 0.16 c
LD	10	1.22 ± 0.21 ab	−0.26 ± 0.24 df	0.93 ± 0.33 a	0.37 ± 0.10 ac	0.46 ± 0.30	0.55 ± 0.89	1.17 ± 0.38 d	0.47 ± 0.08 d
MD	8	1.12 ± 0.21 d	0.89 ± 0.45 ad	0.10 ± 0.15	0.41 ± 0.14	0.22 ± 0.17 ac	0.50 ± 0.58	1.23 ± 0.63 d	0.54 ± 0.29 c
HD	10	0.70 ± 0.05 be	1.30 ± 1.02 bd	0.23 ± 0.26	0.35 ± 0.08 ac	0.57 ± 0.38	0.74 ± 0.91	2.72 ± 1.48 b	0.29 ± 0.10 b

[a] NC, basal diet control group; HFC, high-fat diet control group; GPC, high-fat + *Gynostemma pantaphyllum* total glucoside tablet group; LD, high-fat diet + low dose of Tartary buckwheat extract group; MD, high-fat diet + medium dose of Tartary buckwheat extract group; HD, high-fat diet + high dose of Tartary buckwheat extract group; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; AIP, atherogenic index of plasma (AIP); apoA$_1$, apolipoprotein A$_1$; apo B, apolipoprotein B. Letters after entries indicate the following: a, b, $P < 0.05$ and $P < 0.01$, compared to the NC group; c, b, $P < 0.05$ and $P < 0.01$ compared to HFC group; e, f, $P < 0.05$ and $P < 0.01$, compared to the GPC group.

Table 4. Effects of Tartary Buckwheat Bran Extract (TBBE) on Levels of SOD, GSH-Px, and MDA in Serum of Rats (Mean ± SD)[a]

group	*n*	SOD (units/mL)	increase[b] (%)	GSH-Px (units/mL)	increase[b] (%)	MDA (μmol/L)	decrease[b] (%)
NC	10	116.55 ± 22.95		1943.18 ± 143.56		4.35 ± 1.26	
HFC	10	38.17 ± 37.30 b		2029.55 ± 88.08		7.87 ± 2.92 b	
GPC	10	93.24 ± 25.91 ad	144.3	2124.55 ± 194.71 a	4.7	6.00 ± 3.85	23.8
LD	10	53.70 ± 15.06 b	40.7	2254.92 ± 34.88 bd	11.1	4.18 ± 2.07 d	46.9
MD	10	94.37 ± 17.88 ad	147.2	2213.61 ± 117.82 bd	9.1	6.67 ± 4.09	15.3
HD	10	103.87 ± 38.64 d	172.1	1904.86 ± 311.12	−6.1	5.69 ± 2.22	27.7

[a] NC, basal diet control group; HFC, high-fat diet control group; GPC, high-fat + *Gynostemma pantaphyllum* total glucoside tablet group; LD, high-fat diet + low dose of Tartary buckwheat extract group; MD, high-fat diet + medium dose of Tartary buckwheat extract group; HD, high-fat diet + high dose of Tartary buckwheat extract group; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde. Letters after entries indicate the following: a, b, $P < 0.05$ and $P < 0.01$, compared to the NC group; c, d, $P < 0.05$ and $P < 0.01$, compared to the HFC group. [b] Compared to the HFC group.

Table 5. Effects of Tartary Buckwheat Bran Extract (TBBE) on Levels of TG, TC, SOD, GSH-Px, and MDA in the Liver of Rats (Mean ± SD)[a]

					SOD (units/g)		GSH-Px (units/g)		MDA (μmol/g)	
group	*n*	TG (mmol/100 g)	TC (mmol/100 g)	liver index	mean ± SD	increase[b] (%)	mean ± SD	increase[b] (%)	mean ± SD	decrease[b] (%)
NC	10	0.74 ± 0.33	0.67 ± 0.27	3.47 ± 0.24	283.34 ± 31.52		258.88 ± 23.50		1.52 ± 0.37	
HFC	10	1.55 ± 0.52 b	1.76 ± 0.72 b	3.88 ± 0.72	211.17 ± 95.74 a		304.52 ± 77.42		2.21 ± 1.17	
GPC	10	0.99 ± 0.40 c	1.05 ± 0.29 bc	3.58 ± 0.23	274.11 ± 18.53	29.8	322.73 ± 41.01 b	6.0	1.80 ± 0.45	18.6
LD	10	0.62 ± 0.42 d	0.71 ± 0.22 d	3.51 ± 0.18	247.27 ± 24.29 c	17.1	301.41 ± 22.22 b	−1.0	1.57 ± 0.38	29.0
MD	10	0.87 ± 0.34 d	0.89 ± 0.28 d	3.74 ± 0.26 a	221.57 ± 60.83 a	4.9	370.29 ± 156.51 a	21.6	1.47 ± 0.72	33.5
HD	10	0.98 ± 0.49 c	1.02 ± 0.65 c	3.84 ± 0.34 c	231.44 ± 28.78 b	9.6	284.26 ± 72.67	−6.7	1.42 ± 0.68	35.7

[a] NC, basal diet control group; HFC, high-fat diet control group; GPC, high-fat + *Gynostemma pantaphyllum* total glucoside tablet group; LD, high-fat diet + low dose of Tartary buckwheat extract group; MD, high-fat diet + medium dose of Tartary buckwheat extract group; HD, high-fat diet + high dose of Tartary buckwheat extract group; TG, triglyeride; TC, total cholesterol; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde. Letters after entries indicate the following: a, b, $P < 0.05$ and $P < 0.01$, compared to the NC control; c, d, $P < 0.05$ and $P < 0.01$, compared to the HFC control. [b] Compared to the HFC group.

Effects of TBBE on TG and TC Contents in the Livers and Liver Indices of the Rats. Rats in the HFC group had significantly higher liver TC and TG compared to NC ($P < 0.01$) (**Table 5**). The rats in the TBBE treatments had increasing liver TG and TC from low to high dose, but were all lower than that in the HFC group. They were significantly lower in the LD and MD in comparison with the HFC group ($P < 0.01$) (**Table 5**) and were similar to the GPC group. In addition, the liver indices in the MD and HD groups significantly differed from those in the NC group ($P < 0.05$). With an increase in TBBE doses, the TC levels decreased by 60.0, 43.9, and 36.8% ($P < 0.05$ or $P < 0.01$), respectively, and the TG levels decreased by 59.7, 49.4, and 42.0% ($P < 0.05$ or $P < 0.01$) when compared to the HFC group, respectively.

Effects of TBBE on SOD and GSH-Px Sctivities and MDA Contents in Rat Livers. Table 5 presents SOD and GSH-Px activities and MDA levels in the livers of the rats at different TBBE doses. When compared to the NC group, the rats in the HFC group had significantly lower SOD activity ($P < 0.05$). Compared to the rats in the HFC group, the LD, MD, and HD groups had higher SOD activity by 17.1, 4.9, and 9.6%, respectively. The GSH-Px activity varied irregularly, increasing by 21.6% in the MD group. The MDA content was significantly lower with increasing doses of TBBE, and the MDA content at the high TBBE dose decreased by 35.7%.

DISCUSSION

Atherosclerosis leads to cardiovascular disease and stroke. Lipid metabolic disorders characterized by hyperlipemia are among the pathological factors inducing atheromatous plaque formation. An atherogenic lipoprotein profile is an important risk factor for coronary artery disease (CAD). It is characterized by a high ratio of LDL-C to HDL-C and an increased level of TGs. Some nutraceuticals and functional foods such as dietary fiber, phytosterols, grape polyphenols, and buckwheat have been shown to lower plasma TC in hypercholesterolemia in clinical and animal studies (*9*). The present study indicates that TBBE administrations at different doses could effectively control the increases in the TC and TG contents of the serum and liver of hyperlipemia rats. The TBBE administration at the low dose did not cause the liver indices to vary but raised the GSH-Px activity, inhibited the formation of lipid peroxides in the rat serum, and increased the antiatherosclerosis index, thus enhancing its anti-atheromatous plaque formation effect. Herein, the antiatheromatous plaque formation effect appeared to be strongest at the low TBBE dosage. To some extent this is similar to previous research (*27*, *28*). However, it is still not clear whether the reduction of TC in the liver and serum by TBBE was related to increased activity of the liver LDL receptors, increased activity of lipoprotein lipase, a combination, and/or other unknown factors. These results need to be further explored.

Free radicals are products of redox reactions in living organisms. Under normal conditions their formation and elimination are balanced, thus a concentration too high or too low will have negative effects on living organisms. Many data show that free radicals are major contributors to arteriosclerosis by their ability to readily oxidize biological lipids (*38*). In an organism, the oxidative defense system includes enzymatic and nonenzymatic antioxidant systems, and once the balance between the oxidative and antioxidant systems is disturbed, oxidative stress occurs, causing lipid peroxidation and leading to the formation of MDA. In addition, in the organism, lipid peroxidation occurs mainly in the blood serum and liver. Lipid peroxides have an inhibitory effect on cholesterol esterase activity and favor TC to form plaques, leading to the development of arteriosclerosis (*39*). The superoxide radical, hydroxyl radical, and various lipid peroxides can induce lipid oxidation in LDL-C membranes. Organisms can make use of antioxidant enzymes, such as SOD and GSH-Px, and nonenzymatic substances, such as flavonoids, vitamin E (VE), vitamin C (VC), and β-carotene, to reduce the damage resulting from the free radicals. In particular, flavonoids have strong reducing capacity because they can donate electrons on their own to reduce various free radicals and inhibit LDL-C oxidation. The AIP has been shown to be directly related to the

risk of atherosclerosis in epidemiological cohort and clinical studies (*40*). In our study, TBBE reduced AIP in this animal model. However, an increasing trend was also observed within the TBBE treatments (**Table 3**). To further investigate this study it will be necessary to increase the number of animals used in the experiment. $ApoA_1$ is the major apo-lipoprotein in HDL particles and initiates the "reverse cholesterol transport". $ApoA_1$ can "pick up" excess cholesterol from peripheral cells and transfer it back to the liver through HDL. $ApoA_1$ also has anti-inflammatory and antioxidant effects. ApoB is present in very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), large buoyant LDL, and small dense LDL (sd-LDL), with one molecule of apoB in each of these atherogenic particles. ApoB, produced in the liver, also stabilizes and allows the transport of cholesterol and TG in plasma VLDL, IDL, large buoyant LDL, and sd-LDL. $ApoA_1$, apoB, and the apoB/$apoA_1$ ratio is now considered to be a new risk factor for CVD (*41*). In the present study, TBBE did not affect the serum levels of $apoA_1$ and apoB in this animal model. The ratio of apoB/$apoA_1$ was not significantly lower in the TBBE-treated groups compared to the control groups. A long-term animal study (over 6 weeks) is needed to determine if TBBE could affect the levels of $apoA_1$ and apoB as well as ratio of apoB/$apoA_1$.

Hayek et al. (*42*) fed apoE-deficient mice red wine and two major antioxidant compounds in red wine, quercetin and catechin, for 2 weeks. Next, LDL-C was extracted from the serum and incubated in a macrophage and $CuSO_4$ solution. Results showed that the red wine and quercetin effectively inhibited LDL-C oxidation; however, catechin had no significant inhibitory effect. They also examined the effect of quercetin and catechin on reducing the neutral free radical, 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$), and the results showed that quercetin reduced the DPPH free radical more effectively than catechin and, accordingly, protects LDL-C. Quercetin also performed well in resisting LDL-C oxidation in many other ways. For example, quercetin was able to inhibit 15-lipoxygenase (15-LO) activity. 15-LO, an enzyme that is secreted by monocytes and macrophages, is activated by ferric ions. It is capable of increasing LDL-C oxidation and participates in early athermanous plaque formation. Silva et al. (*43*) showed that quercetin and its monosaccharide derivatives also inhibited LO activity in vitro to a greater extent than VC and VE. This activity was possibly due to the reduction of ferric ions to ferrous ions or the chelation of ferric ions, which rendered 15-LO inactive, or 15-LO was indirectly inhibited through VE protection. Flavonoids are able to reduce lipid oxidation in macrophages by inhibiting oxidative enzymes and protecting reducing compounds, such as glutathione, thus inhibiting macrophage-mediated LDL-C oxidation (*44*). Although some oxidizing fragments of ox-LDL-C can become cytotoxic, flavonoids such as quercetin and other substances are capable of inhibiting this toxicity, therefore playing a protective role (*45*). In our study, rutin and quercetin were determined in TBBE by HPLC. The concentrations of rutin and quercetin were 8.96 ± 0.26 and 83.84 ± 3.29 mg/g in the nonhydrolyzed sample, and only quercetin was measurable in the hydrolyzed sample (194.68 ± 4.22 mg/g). The total phenolic content of the TBBE was 158.44 ± 7.30 μmol of gallic acid equiv/gram, and the ORAC value was determined to be 55514 ± 5850 μmol of Trolox equiv/g of dry weight. However, ORAC values of quercetin and rutin were 8.04 ± 2.37 and 13.7 ± 1.7 μmol of Trolox equiv/μmol, respectively (*46*). The pure compounds of quercetin and rutin have very high antioxidant activity determined by ORAC. The ORAC values of quercetin and rutin were determined to be 26601 ± 7841 and 22440 ± 2785 μmol of Trolox equiv/g, respectively. Wójcicki et al. (*26*) reported that rutin, as a positive control, did not significantly reduce the serum fat profile in rabbits fed a high-fat diet. Further study is needed to determine whether quercetin and rutin can improve the serum fat profile of rats fed a high-fat diet. The antioxidant activity of TBBE may be related to the healthier fat profiles of liver and serum. It may possibly regulate serum antioxidant enzymes such as SOD and GSH-Px and inhibit the formation of peroxides and free radicals. Much of the antioxidative activity of TBBE comes from quercetin and rutin, but there are many other phytochemicals present that contribute to the overall activity.

In summary, the effects of TBBE on antioxidant activity and on blood lipid reduction were investigated in hyperlipemic rats. TBBE significantly reduced blood and liver lipids, raised serum antioxidant activity, and inhibited peroxide formation of serum lipids. This may be attributed to the antioxidant action of the phenolic compounds in Tartary buckwheat bran. Although the total phenolics may be the major compounds responsible for the effects, further studies are needed to determine whether certain isolates in TBBE could make a significant contribution to the effects on antioxidant activity and improvements in blood lipids in hyperlipidemic rats.

ACKNOWLEDGMENT

We sincerely thank the Pharmaceutical Research Institute of the Fourth Military Medical University, where the present study was carried out. We are also grateful to Professor Si-Wang Wang for his guidance and support in conducting the study.

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Received January 18, 2009. Revised manuscript received April 21, 2009. The project was partly supported by Program for New Century Excellent Talents in University (NCET), People's Republic of China.