Hypocholesterolemic metabolism of dietary red pericarp glutinous rice rich in phenolic compounds in mice fed a high cholesterol diet

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BACKGROUND/OBJECTIVES: The purpose of the current study was to investigate the effect of red pericarp glutinous rice rich in polyphenols (Jakwangchalbyeo, red rice) on serum and hepatic levels of cholesterol and hepatic protein expression linked to synthesis and degradation of cholesterol in a hypercholesterolemic mice diet as compared with brown rice.

MATERIALS/METHODS: C57BL/6 male mice were randomly divided into four groups (n = 5 each), which were fed different diets for a period of 12 weeks: American Institute of Nutrition (AIN)-93G diet, AIN-93G diet with 2% cholesterol, brown rice with 2% cholesterol, or red rice with 2% cholesterol.

RESULT: Consumption of red rice resulted in a significant decrease in serum level of low-density lipoprotein cholesterol and hepatic levels of triglyceride and total-cholesterol. Expression of acyl-coenzyme A cholesterol acyltransferase-2 (ACAT-2), sterol regulatory element binding protein-2 (SREBP-2), and 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase was decreased, while expression of phosphorylated adenosine monophosphate activated protein kinase (p-AMPK)/AMPK ratio, cholesterol 7α-hydroxylase (CYP7a1), and sterol 12α-hydroxylase (CYP8b1) was increased in mice fed red rice. Brown rice had similar effects on cholesterol metabolism, but the effect of red rice was significantly greater than that of brown rice.

CONCLUSIONS: The current study suggested that red rice had a hypocholesterolemic effect by lowering hepatic cholesterol synthesis through ACAT-2, HMG-CoA reductase, and SREBP-2, and by enhancing hepatic cholesterol degradation through CYP7a1 and CYP8b1 in mice fed a hypercholesterolemic diet.

INTRODUCTION

Rice (Oryza sativa L.) is widely cultivated in the world, and half of the world’s population depends on rice as their main source of food [1]. The outer bran and germ portion of intact rice grains (brown rice) has gained popularity because of its high contents of multiple nutrients, including fiber, vitamins, minerals, and phytochemicals [2]. Consumption of brown rice has been shown to decrease serum cholesterol concentration in human [3,4] and hypercholesterolemia-induced animals [5-7], suggesting that polyphenols in brown rice may have a hypocholesterolemic effect [2].

Pigmented rice bran, such as black, red, and purple rice has also been reported to have higher health promoting potential depending on the type, contents, and structural properties of phenolic compounds [8-10]. Most studies focused on the anti-oxidative effect of phenolic compounds [11-14]; however, those have also been known to reduce serum concentration of cholesterol in vivo [15-17], possibly through inhibition of 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase and acyl-coenzyme A cholesterol acyltransferase-2 (ACAT-2).

Consumption of black rice [11,18] and black rice extract [12,19] has been shown to reduce serum cholesterol concentration in dyslipidemic animals. Red rice intake also reduced the ratio of LDL/HDL-cholesterol in rabbits fed high cholesterol diets [11], however, little is known about regulation of cholesterol metabolism by bioactive compounds in pigmented rice.

We previously reported that the developed red pericarp glutinous rice (red rice), Jakwangchalbyeo, contained seven times more total phenolic compounds, mainly ferulic acid, hesperidin, and catechin, than brown rice [20]. Therefore, the current study investigated the hypothesis that the newly developed red rice rich in polyphenols has cholesterol lowering effects through regulation of protein expression linked to synthesis and degradation of cholesterol in hypercholesterolemic mice as compared with brown rice.

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MATERIALS AND METHODS

Animals and diets
The protocol was approved by the Institutional Animal Care and Use Committee of Hanyang University (HY-IACUC-12-026). Five-week old C57BL/6 male mice (Orient Bio, Gyeonggi-do, Korea) were housed in individual ventilated cages in an air-conditioned room maintained at 22 ± 2°C with a 12 h light-dark cycle. After acclimatization for one week, 20 mice were randomly divided into four isoenergetic diet groups (Table 1; n = 5 each); American Institute of Nutrition (AIN)-93G diet (normal control, NC), AIN-93G diet with 2% cholesterol (high cholesterol, HC), brown rice with 2% cholesterol (BR-HC), or red rice with 2% cholesterol (RR-HC). Amount of cholesterol in the diet was previously confirmed to cause development of hypercholesterolemia in a rodent model [21-24].

Jakywangoalbeo (red pericarp glutinous rice; red rice) and Dongjincheolbeo (white pericarp glutinous rice; brown rice) were cultivated at the Konkuk University Experimental Farm. Dongjincheolbeo is widely cultivated and consumed in Korea. Harvested rice seeds were dried, hulled using a milling machine, and ground by a pin-type mill (DK-201, Sejong Tech, Daegu, Korea). We previously reported that red rice contained 1245 μg/g of phenolic compound, such as 83.4 μg/g of catechin, and 28.0 μg/g of hesperidin, while brown rice contained 159 μg/g of phenolic compound [20].

Diet and water were allowed ad libitum for 12 weeks. Food intake was measured daily and body weight was measured weekly. At the end of the experiment, mice were fasted overnight and euthanized by exsanguination under anesthesia with an intraperitoneal injection of tiletamine (25 mg/kg), zolazepam (25 mg/kg), and xylazine (10 mg/kg). Blood was collected into serum separation tubes (BD Vacutainer, Franklin Lakes, NJ, USA) from the abdominal aorta and centrifuged at 3,000 g for 15 min (HA 10000-3, Hanil Sciences Industrial CO. Ltd., Incheon, Korea).

Table 1. Composition of the experimental diets

| Ingredients (g/100g diet) | NC   | HC   | BR-HC  | RR-HC  |
|---------------------------|------|------|--------|--------|
| Corn starch               | 39.75| 39.75| 39.75  | 39.75  |
| Dyetrose                  | 13.20| 13.20| 13.20  | 13.20  |
| Succrose                  | 10.00| 10.00| 10.00  | 10.00  |
| Brown rice                | 42.80|      |        |        |
| Red rice                  | 42.80|      |        |        |
| Casein                    | 20.00| 20.00| 16.17  | 16.17  |
| Soybean oil               | 7.00 | 7.00 | 5.53   | 5.53   |
| Cellulose                 | 5.00 | 5.00 | 5.00   | 5.00   |
| Mineral mixture           | 3.50 | 3.50 | 3.50   | 3.50   |
| Vitamin mixture           | 1.00 | 1.00 | 1.00   | 1.00   |
| L-Cystine                 | 0.30 | 0.30 | 0.30   | 0.30   |
| Choline bitartrate        | 0.25 | 0.25 | 0.25   | 0.25   |
| Cholesterol               | 2.00 | 2.00 | 2.00   | 2.00   |
| Cholic acid               | 0.25 | 0.25 | 0.25   | 0.25   |
| Carbohydrate : Protein : Fat (%) | 63 : 20 : 7 | 63 : 20 : 7 | 63 : 20 : 7 | 63 : 20 : 7 |
| Total calories (kcal/100g) | 400  | 400  | 400    | 400    |

* NC, normal control; HC, 2% cholesterol diet; BR-HC, brown rice with 2% cholesterol diet; RR-HC, red rice with 2% cholesterol diet

Organs were harvested, rinsed with saline, and then weighed. Serum and tissue samples were stored at -80°C until further analysis.

Biochemical assays
The serum levels of triglyceride, total-cholesterol, low-density lipoprotein (LDL)-cholesterol, alanine transaminase (ALT), and aspartate transaminase (AST) were determined using commercial kits (ID Labs Inc., London, Ontario, Canada). All experiments were performed according to the manufacturer’s instructions and the results were measured using a microplate reader (iMark Microplate Reader, BioRad, Richmond, CA, USA).

Western blotting
Liver tissues were homogenized in a 0.8 M ice-cold lysis buffer (0.25 M sucrose, 20 mM HEPES, 2 mM dithiothreitol, 0.5 mM EDTA, 10 μg/mL leupeptin, 1 mM PMSF, 10 μg/mL aprotonin, and 1 mM Na3VO4, pH 7.5). The homogenous liver tissues were centrifuged at 10,000 g for 15 min at 4°C (Eppendorf, Hamburg, Germany), and the supernatant was centrifuged at 20,000 g for 1 h at 4°C to obtain the cytosolic fraction. The protein concentrations were determined using a Bradford assay with bovine serum albumin (Bio-Rad, Hercules, CA, USA) as the standard. Equal amounts of protein (30 μg) were separated on 8% SDS-PAGE and transferred to a polyvinylidene fluoride membrane (0.45 μm, Immobilon-P transfer membrane, Millipore, Billerica, MA, USA). Membranes were blocked for 1 h at room temperature with 5% skim milk in Tris-buffered saline with Tween 20 (TBST) and then incubated with a primary antibody for ACAT-2 (1:250, Cayman chemical, Ann Arbor, MI, USA), sterol regulatory element binding protein-2 (SREBP-2, 1:500, abCam, Cambridge, UK), HMG-CoA reductase (1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA), adenosine monophosphate activated protein kinase (AMPK, 1:1,000, Cell Signaling Technology, Beverly, MA, USA), phosphorylated AMPK (p-AMPK, 1:500, Cell Signaling Technology, Beverly, MA, USA), cholesterol 7-α-hydroxylase (CYP7a1, 1:1,000, abCam, Cambridge, UK) or sterol 12-α-hydroxylase (CYP8b1, 1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA) in TBST containing 5% skim milk at 4°C. After washing three times with TBST, the membranes were incubated with goat anti-rabbit (1:20,000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or mouse IgG secondary antibody (1:5,000, Cell Signaling Technology, Beverly, MA, USA) for 1 h at room temperature. The immunoreactive bands were developed using the enhanced chemiluminescence kit (GE Healthcare Life Sciences, Piscataway, NJ, USA) and exposed to X-ray film (Kodak Inc., Rochester, NY, USA). The relative amounts of protein were calculated by normalizing the value to β-actin (1:1,000, BD Transduction Laboratories, NJ, USA).

Liver lipid extraction
The lipids extract with Folch solution was centrifuged at 3,000 g for 10 min to obtain the supernatants. Hepatic triglyceride and total cholesterol were determined using a commercial kit (Asan Pharm., Hwaseong, Korea) with a spectrophotometer (DU 600, Beckman Coulter, Inc., Indianapolis, IN, USA).
**Immunofluorescence**

Liver (~4 mm) was fixed in 4% paraformaldehyde in phosphate-buffered saline at 4°C for 24 h, and paraffin sections were cut and deparaffinized using ethanol and xylene. After incubation in 10% normal goat serum with 0.3% Triton X-100 for 1 h for blocking, samples were incubated with primary antibody (1:200, AbCam, Cambridge, UK) in 10% normal goat serum overnight at 4°C. After rinsing, secondary antibody (1:500, Invitrogen, Carlsbad, CA) was applied with 10% NGS at room temperature for 2 h in the dark. The nuclei were stained with 4, 6-Diamidino-2-phenylindole (DAPI) and the sections were mounted. The slides were left in the dark overnight and then sealed using clear nail polish. Images were then taken using a Leica microscope (Leica Microsystems GmbH, Wetzlar, Germany) and Leica Application Suite Advanced Fluorescence software (Leica Microsystems GmbH, Wetzlar, Germany).

**Statistical analysis**

Values were expressed as mean ± standard error of mean (SEM). Statistical differences among the groups were calculated by analysis of variance (ANOVA) followed by Duncan’s multiple range test (SPSS 18.0 version., Chicago, IL, USA). Differences with \( P < 0.05 \) were considered significant.

**RESULTS**

**Food intake, bodyweight, and organ weight**

There were no significant differences in dietary intake, initial and final body weight, and weight of kidney, muscle, heart, visceral fat, and epididymal fat (Table 2). However, weight of liver was significantly higher in mice with HC, BR-HC, and RR-HC than in those with NC.

**Serum and hepatic levels of lipids**

Serum triglyceride concentrations did not differ significantly between groups, but serum concentrations of total cholesterol, alanine transaminase, and aspartate transaminase were significantly higher in HC, BR-HC, and RR-HC than in NC (Table 3). However, serum concentration of LDL-cholesterol, and hepatic concentration of triglyceride and total cholesterol were significantly lower in RR-HC and BR-HC than in HC, and similar to that in NC.

**Expression of protein associated with cholesterol metabolism**

Expression of ACAT-2 and SREBP-2 was significantly decreased, but the expression of p-AMPK/AMPK ratio was significantly increased in RR-HC, BR-HC, and NC compared with HC (Fig. 1). In particular, the effect of red rice on expression of SREBP-2 was greater than that of brown rice. Expression of HMG-CoA reductase was significantly increased in HC, but decreased in RR-HC as compared with NC and BR-HC.

Immunofluorescence staining of liver also showed increased expression of SREBP-2 (light green) in HC compared with NC, BR-HC, and RR-HC (Fig. 2). In addition, more foam cells (black circle) were detected in HC than in NC, BR-HC, and RR-HC. DAPI (nucleus) showed no difference in all mice. As shown in Fig. 2, SREBP-2 expression in mice fed red rice was consistently more similar to that of normal control than that of mice fed brown rice.

Expression of CYP7a1 and CYP8b1 was significantly increased in RR-HC, BR-HC, and NC compared with HC (Fig. 3). In particular, CYP8b1 expression showed a greater increase in mice fed red rice than in mice fed brown rice, suggesting that the effect on cholesterol degradation was greater by red rice than brown rice.

**Table 2. Dietary intake, body weight, and organ weights**

|                | NC     | HC     | BR-HC  | RR-HC  |
|----------------|--------|--------|--------|--------|
| Dietary intake (g/day) | 2.64 ± 0.24 | 2.67 ± 0.01 | 2.69 ± 0.05 | 2.65 ± 0.01 |
| Initial body weight (g) | 16.83 ± 0.20 | 17.0 ± 0.14 | 17.1 ± 0.14 | 17.1 ± 0.14 |
| Final body weight (g) | 30.25 ± 0.75 | 31.3 ± 0.49 | 31.2 ± 0.86 | 30.9 ± 0.86 |
| Liver (g) | 0.97 ± 0.02abc | 1.6 ± 0.03b | 1.53 ± 0.05bc | 1.64 ± 0.06b |
| Kidney (g) | 0.13 ± 0.01 | 0.13 ± 0.01 | 0.13 ± 0.01 | 0.13 ± 0.01 |
| Muscle (g) | 0.33 ± 0.01 | 0.31 ± 0.01 | 0.32 ± 0.01 | 0.31 ± 0.01 |
| Heart (g) | 0.13 ± 0.01 | 0.12 ± 0.01 | 0.13 ± 0.01 | 0.13 ± 0.01 |
| Visceral fat (g) | 0.33 ± 0.01 | 0.37 ± 0.02 | 0.35 ± 0.04 | 0.32 ± 0.02 |
| Epididymal fat (g) | 0.58 ± 0.03 | 0.58 ± 0.02 | 0.57 ± 0.02 | 0.56 ± 0.03 |

* Values are expressed as the mean ± SEM (n = 5); NC, normal control; HC, 2% cholesterol diet; BR-HC, brown rice with 2% cholesterol diet; RR-HC, red rice with 2% cholesterol diet.

† Values in the rows with different letters are significantly different at \( P < 0.05 \) using ANOVA with Duncan’s multiple range test.

**Table 3. Lipid profiles and liver function in serum, and hepatic levels of triglyceride and total cholesterol**

|                | NC         | HC         | BR-HC       | RR-HC       |
|----------------|------------|------------|-------------|-------------|
| Serum          |            |            |             |             |
| Triglyceride (mmol/L) | 0.87 ± 0.03 | 0.85 ± 0.04 | 0.81 ± 0.03 | 0.80 ± 0.02 |
| Total cholesterol (mmol/L) | 2.56 ± 0.02abc | 3.06 ± 0.07b | 3.00 ± 0.08b | 3.02 ± 0.08b |
| LDL-cholesterol (mmol/L) | 2.11 ± 0.15a | 3.01 ± 0.12b | 2.26 ± 0.13a | 2.20 ± 0.23a |
| Alanine transaminase (IU/ L) | 21.01 ± 1.35a | 45.26 ± 3.15b | 45.56 ± 3.73b | 52.06 ± 5.76b |
| Aspartate transaminase (IU/ L) | 132.84 ± 12.93a | 197.30 ± 10.00b | 180.84 ± 5.83b | 190.64 ± 13.71b |
| Liver          |            |            |             |             |
| Triglyceride (mg/g liver) | 40.30 ± 3.32a | 60.26 ± 2.74b | 47.57 ± 2.15a | 45.66 ± 1.04a |
| Total cholesterol (mg/g liver) | 12.38 ± 1.45a | 27.83 ± 2.02b | 23.33 ± 3.61abc | 23.21 ± 2.36abc |

* Values are expressed as the mean ± SEM (n = 5); NC, normal control; HC, 2% cholesterol diet; BR-HC, brown rice with 2% cholesterol diet; RR-HC, red rice with 2% cholesterol diet.

† Values in the rows with different letters are significantly different at \( P < 0.05 \) using ANOVA with Duncan’s multiple range test.
Fig. 1. Expression of acyl-coenzyme A cholesterol acyltransferase-2 (ACAT-2), sterol regulatory element binding protein-2 (SREBP-2), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) and phosphorylated adenosine monophosphate activated protein kinase (p-AMPK)/adenosine monophosphate activated protein kinase (AMPK) ratio. NC, normal control; HC, 2% cholesterol diet; BR-HC, brown rice with 2% cholesterol diet; RR-HC, red rice with 2% cholesterol diet. Values are expressed as the mean ± SEM (n = 5) and the different letters in the rows are significantly different at P < 0.05 using ANOVA with Duncan’s multiple range test.

Fig. 2. Immunofluorescence microscopic imaging of sterol regulatory element binding protein-2 (SREBP-2) in liver. NC, normal control; HC, 2% cholesterol diet; BR-HC, 2% cholesterol with brown rice diet; RR-HC, 2% cholesterol with red rice diet. Images of SREBP-2 antibody (light green), nuclei (DAPI) and merged image were captured at identical times of exposure (4s and 500ms). Bar represents, 100 μm.

Fig. 3. Expression of cholesterol 7α-hydroxylase (CYP7a1) and sterol 12α-hydroxylase (CYP8b1). NC, normal control; HC, 2% cholesterol diet; BR-HC, brown rice with 2% cholesterol diet; RR-HC, red rice with 2% cholesterol diet. Values in the rows with different letters are significantly different at P < 0.05 using ANOVA with Duncan’s multiple range test.

DISCUSSION

The current study demonstrated that consumption of red rice rich in polyphenols caused a decrease in serum and hepatic level of LDL-cholesterol in mice fed a high cholesterol diet, which was due to inhibition of hepatic cholesterol synthesis by the decreased expression of HMG-CoA reductase, ACAT-2 and SREBP-2 expression, and the increased degradation of cholesterol by the increased expression of CYP7a1 and CYP8b1. Brown rice had a similar effect on cholesterol metabolism, but red rice showed greater effects, as we hypothesized.

Previous studies consistently showed that pigmented rice contained higher levels of phenolic compounds [8], and in particular, red rice contained more phenolic compounds than black rice [9]. Only a few studies of red rice have been reported;
effect of red rice on radical-scavenging activity and antioxidation capacity [13]. Only one previous study examining the effect of red rice on cholesterol showed an increase in plasma HDL-cholesterol concentration and antioxidant status in rabbits fed a high cholesterol diet, but not in the concentration of LDL-cholesterol or total cholesterol [11]. Other pigmented rice, black rice and black rice extracts decreased serum cholesterol concentration in vivo [11-12, 18-19], suggesting that cholesterol degradation and/or synthesis were modulated.

Salgado et al. [18] reported that the hypocholesterolemic effect of black rice was mediated by increasing cholesterol excretion in hypercholesterolemic rats. Similarly, brown rice intake has been shown to result in up-regulation of cholesterol catabolism by enhancing fecal bile acid excretion and improving the activity of CYP7a1 in hepatoma-bearing rats [6]. Consumption of rice protein also resulted in increased biliary secretion and fecal excretion of bile acid, and expression CYP7a1 in hypercholesterolemic rats [25]. Cholesterol 7-α-hydroxylase is the rate-limiting enzyme for cholesterol degradation to bile acid, and regulates CYP8b1 [26]. In the current study, expression of CYP7a1 and CYP8b1 was significantly increased in mice fed red and brown rice. In particular, CYP8b1 expression showed a greater increase in mice fed red rice than in mice fed brown rice, suggesting that the effect on cholesterol degradation was greater by red rice than brown rice.

Although the identities of the bioactive compounds in pigmented rice and the mechanisms involved are not well known, phenolic compounds such as ferulic acid [15,27], hesperidin [28], and catechin [16,17] have consistently been reported to decrease serum levels of total cholesterol and LDL-cholesterol in vivo. Catechin has also been shown to increase cholesterol degradation by activation of CYP7a1 in mice fed high fat diet [17], and fecal cholesterol excretion in hamsters fed a high fat diet [29].

Phenolic compounds have also been shown to reduce hepatic cholesterol synthesis by regulating the expression of HMG-CoA reductase, the rate-limiting enzyme in the de novo synthesis of cholesterol [30-32]. In addition, HMG-CoA reductase is associated with SREBP-2, AMPK, and ACAT-2 in hepatic cholesterol synthesis [33,34].

Administration of ferulic acid and hesperidin resulted in decreased hepatic activity of HMG-CoA reductase and ACAT in apolipoprotein E-deficient mice fed a western diet [15] and rats fed a high cholesterol diet [16], respectively. Catechin also decreased cholesterol synthesis by inhibition of HMG-CoA reductase and SREBP-2 in mice fed a high fat diet [17], and by inhibition of HMG-CoA reductase and p-AMPK in hepatoma cells [30]. In addition, Kim et al. [33] suggested that individual phenolic compounds had a cholesterol lowering effect, and combination of polyphenol had a synergistic effect. Pigmented rice, particularly red rice contained several types of phenolic compounds [35], however, their effect on cholesterol metabolism has not been studied. Therefore, this was the first study to examine how consumption of pigmented rice can regulate cholesterol metabolism in vivo. In the current study, red rice consumption resulted in significantly decreased synthesis of hepatic cholesterol by modulating the expression of ACAT-2, SREBP-2, HMG-CoA reductase, and p-AMPK/AMPK ratio. In addition, immuno-fluorescence staining of liver consistently showed increased expression of SREBP-2 in mice fed red rice.

The current study had a limitation. Red rice and brown rice contain not only phenolic compounds but also dietary fiber, protein, fatty acids, vitamins, and minerals, which may also affect hepatic cholesterol metabolism. Thus, we were unable to determine which components of red rice were responsible for the hypocholesterolemic effect. However, red rice was more potent than brown rice, which could be due to the higher phenolic compounds in red rice.

In conclusion, red rice had a hypocholesterolemic effect by lowering hepatic cholesterol synthesis through HMG-CoA reductase, ACAT-2, p-AMPK/AMPK, and SREBP-2, and by enhancing hepatic cholesterol degradation through CYP7a1 and CYP8b1 in mice fed a hypercholesterolemic diet. Red rice may therefore have potential use in treatment and prevention of pathological states associated with cholesterol metabolism. Care may be required, but polyphenol rich red rice could be used in hypercholesterolemic patients, particularly those who consume a high carbohydrate diet.

REFERENCES

1. Khush GS. What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol Biol 2005;59:1-6.
2. Slavin JL, Martini MC, Jacobs DR Jr, Marquart L. Plausible mechanisms for the protective effects of whole grains. Am J Clin Nutr 1999;70:4595-4635.
3. Shimabukuro M, Higa M, Kinjo R, Yamakawa K, Tanaka H, Kozuka C, Yabiku K, Taira S, Sata M, Masuzaki H. Effects of the brown rice diet on visceral obesity and endothelial function: the BRAVO study. Br J Nutr 2014;111:310-20.
4. Sun Q, Spiegelman D, van Dam RM, Holmes MD, Malik VS, Willett WC, Hu FB. White rice, brown rice, and risk of type 2 diabetes in US men and women. Arch Intern Med 2010;170:961-9.
5. Mohd Esa N, Abdul Kadir KK, Amom Z, Azlan A. Improving the lipid profile in hypercholesterolemia-induced rabbit by supplementation of germinated brown rice. J Agric Food Chem 2011;59:7985-91.
6. Miura D, Ito Y, Mizukuchi A, Kise M, Aoto H, Yagasaki K. Hypcholesterolemic action of pre-germinated brown rice in hepatoma-bearing rats. Life Sci 2006;79:259-64.
7. Rooheinijad S, Omidizadeh A, Mirhosseini H, Saari N, Mustafa S, Yusof RM, Hussin AS, Hamid A, Abd Manap MY. Effect of pre-germination time of brown rice on serum cholesterol levels of hypercholesterolaemic rats. J Sci Food Agric 2010;90:245-51.
8. Deng GF, Xu XR, Zhang Y, Li D, Gan RY, Li HB. Phenolic compounds and bioactivities of pigmented rice. Crit Rev Food Sci Nutr 2013;53:296-306.
9. Jun HI, Song GS, Yang EI, Youn Y, Kim YS. Antioxidant activities and phenolic compounds of pigmented rice bran extracts. J Food Sci 2012;77:C759-64.
10. Min B, McClung AM, Chen MH. Phytochemicals and antioxidant capacities in rice brans of different color. J Food Sci 2011;76: C117-26.
11. Ling WH, Cheng QX, Ma J, Wang T. Red and black rice decrease atherosclerotic plaque formation and increase antioxidant status in rabbits. J Nutr 2001;131:1421-6.
12. Jang HH, Park MY, Kim HW, Lee YM, Hwang KA, Park JH, Park DS, Kwon O. Black rice (Oryza sativa L.) extract attenuates hepatic steatosis in C57BL/6 J mice fed a high-fat diet via fatty acid oxidation. Nutr Metab (Lond) 2012;9:27.

13. Finocchiaro F, Ferrari B, Gianinetti A, Dall’asta C, Galaverna G, Scazzina F, Pellegrini N. Characterization of antioxidant compounds of red and white rice and changes in total antioxidant capacity during processing. Mol Nutr Food Res 2007;51:1006-19.

14. Wang L, Sun J, Yi Q, Wang X, Ju X. Protective effect of polyphenols extract of adlay (Coix lachryma-jobi L. var. ma-yuen Stapf) on hypercholesterolemia-induced oxidative stress in rats. Molecules 2012;17:8886-97.

15. Kwon EY, Do GM, Cho YY, Park YB, Jeon SM, Choi MS. Anti-atherogenic property of ferulic acid in apolipoprotein E-deficient mice fed Western diet: comparison with clofibrate. Food Chem Toxicol 2010;48:2298-303.

16. Kim YJ, Houng SJ, Kim JH, Kim YR, Ji HG, Lee SJ. Nanoemulsified green tea extract shows improved hypocholesterolemic effects in C57BL/6 mice. J Nutr Biochem 2012;23:186-91.

17. Raederstorff DG, Schlachter MF, Elste V, Weber P. Effect of EGCG on lipid absorption and plasma lipid levels in rats. J Nutr Biochem 2003;14:326-32.

18. Salgado JM, Oliveira AG, Mansi DN, Donado-Pestana GM, Bastos CR, Marcondes FK. The role of black rice (Oryza sativa L) in the control of atherogenic cholesterol in rats. J Med Food 2010;13:1355-62.

19. Um MY, Ahn J, Ha TY. Hypolipidaemic effects of cyanidin 3-glucoside rich extract from black rice through regulating hepatic lipogenic enzyme activities. J Sci Food Agric 2013;93:3126-8.

20. Chi HY, Lee CH, Kim KH, Kim SL, Chung IM. Analysis of phenolic compounds and antioxidant activity with H4IIE cells of three different rice grain varieties. Eur Food Res Technol 2007;225:887-93.

21. Ando H, Tsuruoka S, Yamamoto H, Takamura T, Kaneko S, Fujimura A. Regulation of cholesterol 7α-hydroxylase mRNA expression in C57BL/6 mice fed an atherogenic diet. Atherosclerosis 2005;178: 265-9.

22. Hu X, Wang T, Li W, Jin F, Wang L. Effects of NS Lactobacillus strains on lipid metabolism of rats fed a high-cholesterol diet. Lipids Health Dis 2013;12:67.

23. Green CO, Wheatley AO, McGrowder DA, Dilworth LL, Asemota HN. Citrus peel polymethoxylated flavones extract modulates liver and heart function parameters in diet induced hypercholesterolemic rats. Food Chem Toxicol 2013;51:306-9.

24. Azuma K, Ippoushi K, Terao J. Evaluation of tolerable levels of dietary quercetin for exerting its antioxidative effect in high-cholesterol-fed rats. Food Chem Toxicol 2010;48:1117-22.

25. Yang L, Chen JH, Xu T, Nie MH, Yang HK. Hypcholesterolemic effect of rice protein is due to regulating hepatic cholesterol metabolism in adult rats. Gene 2013;512:470-6.

26. Chiang JY. Regulation of bile acid synthesis. Front Biosci 1998;3:d176-93.

27. Jung EH, Kim SR, Hwang IK, Ha TY. Hypoglycemic effects of a phenolic acid fraction of rice bran and ferulic acid in C57BL/KsJ-db/db mice. J Agric Food Chem 2007;55:9800-4.

28. Wang X, Hasegawa J, Kitamura Y, Wang Z, Matsuda A, Shinoda W, Miura N, Kimura K. Effects of hesperidin on the progression of hypercholesterolemia and fatty liver induced by high-cholesterol diet in rats. J Pharmacol Sci 2011;117:129-38.

29. Chan PT, Fong WP, Cheung YL, Huang Y, Ho WK, Chen ZY. Jasmine green tea epicatechins are hypolipidemic in hamsters (Mesocrictetus auratus) fed a high fat diet. J Nutr 1999;129:1094-101.

30. Singh DK, Banerjee S, Porter TD. Green and black tea extracts inhibit HMG-CoA reductase and activate AMP kinase to decrease cholesterol synthesis in hepatoma cells. J Nutr Biochem 2009;20:867-22.

31. Yang Y, Andrews MC, Hu Y, Wang D, Qin Y, Zhu Y, Ni H, Ling W. Anthocyanin extract from black rice significantly ameliorates platelet hyperactivity and hypertriglyceridemia in dyslipidemic rats induced by high fat diets. J Agric Food Chem 2011;59:6759-64.

32. Suckling KE, Stange EF. Role of acyl-CoA: cholesterol acyltransferase in cellular cholesterol metabolism. J Lipid Res 1985;26:647-71.

33. Kim HJ, Jeon SM, Lee MK, Cho YY, Kwon EY, Lee JH, Choi MS. Comparison of hesperetin and its metabolites for cholesterol-lowering and antioxidative efficacy in hypercholesterolemic hamsters. J Med Food 2010;13:808-14.

34. Chen MH, Choi SH, Kozukue N, Kim HJ, Friedman M. Growth-inhibitory effects of pigmented rice bran extracts and three red bran fractions against human cancer cells: relationships with composition and antioxidative activities. J Agric Food Chem 2012; 60:9151-61.

35. Muntana N, Prasong S. Study on total phenolic contents and their antioxidant activities of Thai white, red and black rice bran extracts. Pak J Biol Sci 2010;13:170-4.