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Improving the Lipid Profile in Hypercholesterolemia-Induced Rabbit by Supplementation of Germinated Brown Rice

Norhaizan Mohd. Esa,*,† Khairul-Kamilah Abdul Kadir,‡ Zulkhairi Amom,‡ and Azrina Azlan†

†Department of Nutrition and Dietetics and ‡Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT: It is imperative that there be a diet designed specifically to improve lipid profile in order to impede the progress of atherosclerosis. Because rice is a staple food in Asia, it will be chosen as the diet of interest. This study sets out to discover whether consumption of different processed rice diets may result in a change of the lipid profile. The experiment was done on male New Zealand white rabbits after 10 weeks of treatment with diet containing 0.5% cholesterol. The experimental diets include white rice (WR), brown rice (BR), and germinated brown rice (GBR). Among them, rabbits fed a GBR diet demonstrated significantly lower levels of total cholesterol (TC), low-density lipoprotein (LDL), LDL/HDL, and atherogenic index (AI) and a higher level of high-density lipoprotein (HDL). Results from atherosclerotic plaque assessment further support the findings. The level of malondialdehyde (MDA), which acts as an indicator for oxidative stress, was also reduced by GBR diet. The positive change in lipid profile in the rabbits fed GBR appeared to correspond with the higher amounts of γ-oryzanol, tocopherol, and monounsaturated fatty acid (MUFA) content.

KEYWORDS: germinated brown rice, hypercholesterolemia, lipid profile, MDA, atherogenic index

INTRODUCTION

Cardiovascular disease (CVD) is becoming a major health problem in developing countries. In Malaysia, it has been the leading cause of death over the past decade,[1] and the occurrence of CVD increased by 14% from 1995 to 2000. The link between elevated levels of total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol and decreased high-density lipoprotein (HDL) with the development of atherosclerosis has been well established. Statin drugs are widely prescribed to lower TC. However, they can cause adverse effects such as liver dysfunctions or myalgic.[2,3] Lowering TC through nondrug strategies such as consuming foods containing bioactive compounds with hypocholesterolemic effects would therefore be a desirable alternative. The treatment of CVD with rice diets was suggested several decades ago.

Rice (*Oryza sativa* L.) is classified according to the degree of milling, which makes a brown rice (BR) (unmilled rice) different from white rice (WR). Besides BR and WR, germinated brown rice (GBR) is a recent rice product that has gained popularity worldwide. Germinated brown rice has been developed by soaking BR in water to induce slight germination. This technique was used to soften the texture, enhance the flavor and nutrients such as γ-aminobutyric acid (GABA),[4] and improve the bioavailability of minerals[5] in brown rice. GBR, similar to BR, possesses high amounts of total ferulic acid and total, soluble, and insoluble dietary fibers as well as oryzanol.[4]

Previous studies have reported the beneficial effect of GBR in reducing the incidence of aberrant crypt foci (ACF) in azoxymethane-induced colon cancer rats,[6] protecting against diabetic deterioration, improving the physiological parameter of diabetic neuropathy,[7] and also mildly ameliorating hyperglycemia and imbalance of adipocytokine level in type 2 diabetes mellitus rats.[8] The effect of GBR in improving the lipid profile also has been studied by a few researchers using rats as an animal model.[9−11]

In the current study we investigate the effect of GBR supplementation on the lipid profile of hypercholesterolemia-induced rabbit. As an experimental model for the study of lipid metabolism and atherosclerosis, rabbits have several advantages over mice. For example, rabbits (LDL-mammals) have higher levels of apoB-containing lipoproteins than mice (HDL-mammals), a lipoprotein profile more like that of humans, and patterns of hepatic apoB100 and intestinal apoB48 synthesis resembling those of humans. Like humans, and unlike mice, rabbits have cholesteryl ester transfer protein, an important enzyme in lipoprotein metabolism and atherosclerosis.[12] Moreover, cholesterol-fed rabbits develop remnant-rich hypercholesterolemia and atherosclerosis.

MATERIALS AND METHODS

Chemicals and Reagents. Cholesterol, Sudan IV, and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich (St. Louis, MO), and Simvastatin was obtained from Ranbaxy, Pharmaniaga Logistics Sdn Bhd. (Malaysia), whereas TC, LDL, HDL, and triglyceride (TG) estimation kits were supplied by Roche Diagnostic GmbH (Germany). Formalin, ethyl alcohol, and acetone were obtained from BDH Laboratory (Poole, U.K.), and ethanol was from Merck (Darmstadt, Germany). Petroleum ether, sodium hypochlorite, and hexane were purchased from Merck (Darmstadt, Germany), and standard of fatty acid methyl ester (FAMEs) was from AccuStandard (New Haven, CT). All other chemicals used were of analytical grade.

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Preparation of GBR. Malaysian local brown rice (*O. sativa* L. variety MR220) was obtained from a major rice miller (Bernas) in Malaysia. Brown rice was sterilized in 0.1% sodium hypochlorite for 30 min, followed by washing in sufficient water. Germination of brown rice was carried out according to the method of Suzuki and Maekawa[13] with a slight modification. Four kilograms of brown rice was soaked in 120 L of water at a controlled temperature of 30 °C. The soaking water was changed every 24 h. Samples were prepared and analyzed in triplicate.

Analysis of Rice Chemical Composition. Proximate analyses of WR, BR, and GBR including moisture, ash, crude fat, and protein were performed in accordance with the *Official Methods of Analysis of the AOAC* (1996).[14] Total dietary fiber was analyzed using an enzymatic and gravimetric method (AOAC, 1996).[14] Minerals contents of WR, BR, and GBR were determined from ashed samples using the flame system of an atomic absorption spectrophotometer (GBC, model 908AA) following the method described by Tee et al.[14]

Total lipids were extracted using the Soxhlet method (AOAC, 1996). Petroleum spirit 40−60 °C (AOAC, 1996) was the solvent used for the determination of fat content. Ten grams of rice was weighed into a predried extraction thimble, and the oil was extracted using the solvents mentioned above. The oil was recovered after the solvent had been stripped off in a rotary evaporator (Rotavapor R200, Buchi, Switzerland). A modified version of that by Ainie et al.[15] was used for fatty acid analysis. After the addition of approximately 10 mL of hexane into 100 mg of extracted lipids, the mixture was then methylated with 100 μL of 2 N KOH in dry methanol and vortexed for 30 s. The mixture was then centrifuged to separate the layers. The upper layer containing fatty acid methyl ester (FAME) was transferred into a small tube and stored at −20 °C until further analysis by gas chromatography.

γ-Oryzanol and vitamin E were measured from the lipid extract of the samples. Total lipid was extracted following a method by Suzuki et al.[16] using chloroform and methanol. γ-Oryzanol and vitamin E were analyzed using reverse-phase high-performance liquid chromatography (HPLC) using a method described by Rogers et al.[17] and further described by Azrina.[18]

Experimental Design. Male New Zealand white rabbits aged 9 weeks and weighing 1.5−2.0 kg were obtained from the Animal Source Unit, Faculty of Veterinary, Universiti Putra Malaysia (UPM). They were housed individually in standard stainless steel cages at 24 °C with a 12 h light/dark cycle and placed in the Animal House of Faculty of Medicine and Health Sciences, UPM, throughout the experimental period. The experiment was conducted in accordance with the guidelines established by the Animal Care and Use Committee (ACUC) of Universiti Putra Malaysia, Malaysia. Animals were acclimated for 1 week and given access to food and water ad libitum. The animals were then randomly divided into six diet groups (n = 7) consisting of normal diet (NC), normal diet with high cholesterol (0.5 g/100 g) (PC), high-cholesterol diet with 19.8% white rice powder (WR), high-cholesterol diet with 19.0% brown rice powder (BR), high-cholesterol diet with 19.5% germinated brown rice powder (GBR), and, finally, high-cholesterol diet with Simvastatin (10 mg/kg) (SG). The percentages of WR, BR, and GBR added into the diet were based on the composition of fiber determined in these three types of rice. The compositions of rabbits' diets are shown in Table 1. Treatments were given for a total of 10 weeks.

The rabbits were weighed every week. Blood samples from a marginal ear vein of rabbits were taken at weeks 0, 5, and 10 after treatment. A volume of 16 mL of blood was drawn into lithium heparin tubes for analysis of the lipid profile. At the end of the experimental period, the animals were sacrificed via exsanguinations.

Lipid Profile Assay. The plasma TC, TG, LDL, and HDL were determined using a diagnostic kit (Roche, Germany) on a Hitachi Automatic Analyzer 902 (Tokyo, Japan). The atherogenic index (AI) was calculated by using the equation ([total cholesterol] − [HDL-cholesterol])/(HDL-cholesterol).[10]

Table 1. Percentage of Ingredients in Rabbits' Diet[a]

	diet[b]					
ingredient	NC	PC	WR	BR	GBR	SG
soybean meal	15	15	15	15	15	15
corn	30	30	10.2	11	10.5	30
palm kernel meal	36	36	36	36	36	36
starch	10	10	10	10	10	10
molasses	2	2	2	2	2	2
corn oil	2	2	2	2	2	2
vitamin mixture	0.3	0.3	0.3	0.3	0.3	0.3
mineral mixture	3.5	3.5	3.5	3.5	3.5	3.5
DL-methionine	0.2	0.2	0.2	0.2	0.2	0.2
$CaCO_3$	0.5	0.5	0.5	0.5	0.5	0.5
$CaHPO_4$	0.5	0.5	0.5	0.5	0.5	0.5
cholesterol		0.5	0.5	0.5	0.5	0.5
Simvastatin (mg/kg), SG						10
WR			19.8			
BR				19.0		
GBR					19.5	
total	100	100.5	100.5	100.5	100.5	100.5

[a] Cholesterol was added without replacement of any nutrient. [b] Abbreviations: NC (negative control), rabbits were fed normal rabbit diet; PC (positive control), rabbits were fed a high-cholesterol diet; HC (normal diet coated with 0.5 g/100 g cholesterol); WR, HC with white rice; BR, HC with brown rice; GBR, HC with germinated brown rice; SG, high-cholesterol diet with Simvastatin.

Atherosclerotic Plaque Assessment. The large part of the aorta between its origin and bifurcation into the iliac arteries was opened longitudinally and prepared for plaque assay. Atherosclerotic plaque areas were assessed according to a previously described method.[19,20] Briefly, the aortic strips were immersed in 10% buffered formalin solution for 24 h and then rinsed in 70% alcohol. The tissue was then immersed in Herxheimer's solution containing Sudan IV, ethyl alcohol 70%, and acetone at room temperature for 15 min and washed in running water for 1 h. Photographs of the intimal surface of the aorta were taken using a digital camera (EOS Canon, Japan), and the initimal lipid lesions were determined quantitatively by estimation of the percentage of sudanophilic stained areas in the total aortic intimal area in photographs using image analysis software (Leica, Germany).

Lipid Peroxidation Measurement. Lipid peroxidation was measured according to the concentration of thiobarbituric acid reactive species (TBARs).[21] One milliliter of plasma was added to 2.0 mL of thiobarbituric acid reagent (15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid, and 0.25 N HCl). The solution was heated at 100 °C for 15 min. After cooling, the precipitate was removed by centrifugation at 1000g for 10 min, and the absorbance of the supernatant was determined at 535 nm against a blank containing distilled water instead of biological sample. The concentration of TBARs in the samples was calculated using the molar extinction coefficient of malondialdehyde (MDA) (1.56×10^5 M^{-1} cm^{-1}).

Statistical Analysis. The data are presented as the mean ± standard deviation (SD). The analysis of variance (ANOVA) and Tukey's test were used to detect whether significant differences existed in the proximate analysis and mineral composition of WR, BR, and GBR at the 5% level of significance.

Table 2. Proximate Analysis of White Rice (WR), Brown Rice (BR), and Germinated Brown Rice (GBR)[a]

	g/100 g sample		
analysis	WR	BR	GBR
ash	0.60 ± 0.04 a	1.63 ± 0.04 b	1.44 ± 0.03 c
moisture	7.73 ± 1.08 a	18.28 ± 0.02 b	18.62 ± 0.07 b
protein	8.15 ± 0.06 a	8.78 ± 0.02 b	8.47 ± 0.06 c
fat	0.60 ± 0.01 a	2.67 ± 0.06 b	2.57 ± 0.01 b
total dietary fiber	3.90 ± 0.30 a	16.27 ± 0.50 b	10.74 ± 0.30 c
insoluble dietary fiber	2.75 ± 0.09 a	13.01 ± 0.06 b	10.13 ± 0.80 c
soluble dietary fiber	1.15 ± 0.08 a	3.26 ± 0.34 b	0.61 ± 0.06 a
carbohydrate	78.7 ± 1.00 a	52.5 ± 0.60 b	57.8 ± 0.80 c

[a] All values are expressed as the mean ± SD. Values in a row without the same letter are significantly different ($P < 0.05$).

Table 3. Mineral Composition of White Rice (WR), Brown Rice (BR), and Germinated Brown Rice (GBR)[a]

	mg/kg sample		
mineral	WR	BR	GBR
sodium (Na)	123.29 ± 2.87 a	147.72 ± 2.01 b	246.75 ± 5.74 c
magnesium (Mg)	431.40 ± 2.40 a	914.45 ± 10.30 b	904.05 ± 20.10 b
potassium (K)	902.9 ± 4.28 a	2609.65 ± 50.30 b	1552.97 ± 37.20 c
calcium (Ca)	94.26 ± 1.63 a	130.56 ± 1.28 b	162.92 ± 5.63 c
zinc (Zn)	22.27 ± 1.21 a	28.12 ± 0.55 b	26.05 ± 0.70 b

[a] All values are expressed as the mean ± SD. Values in a row without the same letter are significantly different ($P < 0.05$).

RESULTS

Chemical Composition of WR, BR, and GBR. Table 2 shows the nutritional composition of the WR, BR, and GBR. The ash contents of BR and GBR are significantly higher ($P < 0.05$) than that of WR. The moisture content of GBR was quite similar to that of BR but significantly higher than that of WR. The protein contents for all three types of rice (WR, BR, and GBR) were significantly different ($P < 0.05$), which were 8.15, 8.78, and 8.47 g/100 g sample, respectively. Both BR and GBR also contained significantly higher amounts of fat compared to WR. The total dietary fiber of GBR was significantly higher than that of WR but significantly lower than that of BR. Similar to the total dietary fiber, the insoluble dietary fiber of GBR also was significantly higher than that of WR but lower than that of BR. The soluble dietary fiber of GBR, however, was not significantly different from that of WR (1.15 g/100 g) but was significantly lower (0.61 g/100 g) than that of BR (3.26 g/100 g). Mg and K are the most abundant mineral elements in BR and GBR, and these samples also are good sources of Ca and Zn (Table 3). The concentrations of tocopherol, tocotrienol, and γ-oryzanol of WR, BR, and GBR are summarized in Table 4. The data showed that tocopherol was detected only in the GBR sample. Data from the same table show that tocotrienol was found to be highest in BR, followed by GBR and WR. Table 5 represents the level of fatty acids in WR, BR, and GBR. The level of saturated fatty acid in GBR was not significantly different from those in WR and BR. The level of monounsaturated fatty acid (MUFA), however, was significantly higher in GBR than in BR and WR. Some of the results from this study were different from those of other studies.[9,22] This might be due to the different techniques used for the germination process.

Table 4. Oryzanol and Vitamin E Concentrations of White Rice (WR), Brown Rice (BR), and Germinated Brown Rice (GBR)[a]

	type of rice		
analysis	WR	BR	GBR
oryzanol (ppm)	1912.33 ± 152.90 a	3807.57 ± 266.52 b	7225.69 ± 361.28 c
vitamin E (ppm)			
tocopherol	ND	ND	593.33 ± 36.00
tocotrienol	8637.97 ± 518.28 a	28361.68 ± 2552.55 b	24764.12 ± 1733.49 b

[a] All values are expressed as the mean ± SD. Values in a row without the same letter are significantly different ($P < 0.05$) according to Tukey's HSD post hoc test.

Table 5. Fatty Acid Composition (Percent) of White Rice (WR), Brown Rice (BR), and Germinated Brown Rice (GBR)[a]

	type of rice		
fatty acid profile	WR	BR	GBR
C14:0	0.69	1.27	0.65
C16:0	17.85	14.76	18.21
C16:1	0.28	0.30	0.28
C18:0	2.38	2.19	1.81
C18:1	41.43	41.70	41.2
C18:2	33.97	33.93	35.82
C18:3	1.05	3.29	0.75
C20:0	1.09	1.24	0.62
C20:1	0.49	0.53	0.36
C20:3	ND	ND	ND
C20:4	ND	ND	ND
C22:0	0.19	0.41	0.11
C22:2	0.46	0.08	0.04
C23:0	0.12	0.30	0.14
C24:0	ND	ND	0.12
C24:1	ND	ND	6.24
SFA	22.32	20.17	21.66
UFA	42.20	42.53	48.08
MUFA	35.48	37.29	30.26
PUFA			

[a] All values are expressed as the mean ± SD. Abbreviations: SFA, saturated fatty acid; UFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; ND, not detected.

Lipid Profile. The changes of plasma TC, TAG, LDL, HDL, LDL/HDL ratio, and AI of the six groups are summarized in Table 6. It could be observed that giving high-cholesterol diet caused the plasma levels of TC to increase. However, the level of TC gradually reduced significantly ($P < 0.05$) by week 10 by

Table 6. Plasma Lipoprotein Concentration and Malondialdehyde (MDA) in Rabbits Fed a Normal (NC) or High-Cholesterol Diet (PC) or the High-Cholesterol Diet Containing White Rice (WR), Brown Rice (BR), or Germinated Brown Rice (GBR) before, during, and after 10 Weeks of Treatment[a]

		weeks of treatment		
parameter		0	5	10
TC (mmol/L)	NC	1.89 ± 0.06 a1	4.304 ± 0.58 b1	3.54 ± 0.51 b1
	PC	1.96 ± 0.18 a1	29.74 ± 1.39 b2	46.87 ± 1.35 c2
	WR	1.24 ± 0.24 a1	31.48 ± 1.01 b2	41.48 ± 0.82 c3
	BR	1.34 ± 0.02 a1	23.96 ± 1.32 b3	19.37 ± 0.75 c4
	GBR	1.43 ± 0.18 a1	40.85 ± 0.86 b4	28.26 ± 0.88 c5
	SG	1.48 ± 0.29 a1	8.07 ± 0.36 b5	3.01 ± 0.66 c1
TG (mmol/L)	NC	1.56 ± 0.29 a1	1.99 ± 0.21 a1	1.38 ± 0.38 a1
	PC	0.70 ± 0.18 a1	1.42 ± 0.33 b2	4.81 ± 0.15 c2
	WR	0.88 ± 0.05 a2	1.58 ± 0.33 b2	4.30 ± 0.61 c2
	BR	0.80 ± 0.17 a2	1.11 ± 0.54 b2	5.30 ± 0.35 b3
	GBR	0.77 ± 0.07 a2	1.24 ± 0.28 b2	5.11 ± 0.55 c3
	SG	0.83 ± 0.07 a2	1.14 ± 0.50 a2	3.68 ± 0.48 b2
HDL (mmol/L)	NC	0.76 ± 0.06 a1	1.43 ± 0.24 b1	1.23 ± 0.05 b1
	PC	1.18 ± 0.13 a2	3.59 ± 0.41 b2	3.31 ± 0.21 b2
	WR	0.75 ± 0.17 a1	3.48 ± 0.58 b2	5.59 ± 0.39 c3
	BR	1.00 ± 0.05 a2	2.78 ± 0.68 b2	4.83 ± 0.73 c3
	GBR	0.71 ± 0.22 a1	4.67 ± 0.12 b3	7.92 ± 0.66 c4
	SG	0.59 ± 0.25 a1	1.16 ± 0.22 b1	0.49 ± 0.22 a5
LDL (mmol/L)	NC	1.18 ± 0.25 a1	3.74 ± 0.82 b1	2.66 ± 0.30 b1
	PC	0.51 ± 0.05 a2	38.94 ± 1.50 b2	39.34 ± 1.17 b2
	WR	0.20 ± 0.09 a2	27.45 ± 1.12 b3	34.05 ± 0.50 c3
	BR	0.22 ± 0.15 a2	21.70 ± 1.54 b4	14.17 ± 1.04 c4
	GBR	0.30 ± 0.14 a2	34.88 ± 0.61 b5	18.46 ± 0.41 c5
	SG	0.42 ± 0.18 a2	3.17 ± 0.15 b1	0.69 ± 0.24 a6
LDL/HDL	NC	1.55 ± 0.34 a1	2.61 ± 0.72 a1	2.16 ± 0.26 a1
	PC	0.43 ± 0.06 a2	10.85 ± 1.30 b2	11.88 ± 0.83 b2
	WR	0.26 ± 0.13 a2	7.88 ± 1.33 b2	6.09 ± 0.43 c3
	BR	0.22 ± 0.15 a2	7.80 ± 1.98 b2	2.93 ± 0.49 c1
	GBR	0.42 ± 0.23 a2	7.46 ± 0.23 b2	2.33 ± 0.20 c1
	SG	0.71 ± 0.42 a2	2.73 ± 0.53 b1	1.41 ± 0.80 b4
AI	NC	1.48 ± 0.13 a1	2.00 ± 0.43 b1	1.87 ± 0.28 b1
	PC	0.66 ± 0.09 a2	7.28 ± 0.90 b2	13.16 ± 0.91 c2
	WR	0.65 ± 0.19 a2	8.04 ± 1.36 b2	6.42 ± 0.46 c3
	BR	0.34 ± 0.02 a2	7.61 ± 1.82 b2	3.01 ± 0.47 c4
	GBR	1.01 ± 0.33 a1	7.74 ± 0.25 b2	2.56 ± 0.22 c4
	SG	1.5 ± 0.45 a1	5.95 ± 1.16 b4	5.14 ± 2.56 b4
MDA	NC	0.95 ± 0.24 a1	1.39 ± 0.36 a1	1.09 ± 0.21 a1
	PC	1.40 ± 0.33 a1	4.95 ± 0.29 b2	6.76 ± 0.21 c2
	WR	1.50 ± 0.36 a1	4.83 ± 0.27 b2	4.69 ± 0.38 b3
	BR	1.53 ± 0.34 a1	4.38 ± 0.25 b2	3.94 ± 0.35 b3
	GBR	1.77 ± 0.30 a1	5.09 ± 0.09 b3	3.95 ± 0.38 c3

Table 6. Continued

		weeks of treatment		
parameter		0	5	10
	SG	1.05 ± 0.23 a1	0.63 ± 0.29 a1	0.46 ± 0.18 b4

[a] Values are expressed as the mean ± SD (n = 3). Means with different letters within a row are significantly different (P < 0.05). Means with different numbers within a column are significantly different (P < 0.05). SG, simvastatin group.

Figure 1. Photographs of the aortas of rabbits from six groups stained with Sudan IV after 10 weeks of treatment. Note marked brick-red lipid deposits in all groups except NC group. NC group, negative control; PC group, 0.5% cholesterol diet; WR, 0.5% cholesterol with white rice; BR, 0.5% cholesterol diet with brown rice; GBR group, 0.5% cholesterol with germinated brown rice; SG, 0.5% cholesterol diet with Simvastatin.

giving BR and GBR following the high-cholesterol diet as compared to the PC group.

The level of plasma LDL significantly increased at week 5 for all groups; however, regular diet with BR and GBR supplementation following a high-cholesterol diet gradually reduced the levels of LDL in these two groups, the decrease at 10 weeks being 34.7 and 21.8%, respectively.

With regard to HDL, a high-cholesterol diet with WR, BR, and GBR supplementation for 10 weeks significantly increased the levels of HDL as compared to the initial value. The highest value was found in GBR groups as compared to other groups. The level also was significantly higher than that of the SG group.

Even though the level of TAG increased in rabbits fed BR and GBR, at the same time these diets also caused a marked increase in the HDL level, resulting in a significant lowering of the AI, an atherosclerosis CVD risk factor, relative to those fed only a high-fat diet.

The basal risk ratio (LDL/HDL) values in PC, WR, BR, and GBR groups were not significantly different (P < 0.05) in all groups except the NC group, which were highest compared to the other groups. The values increased to a similar extent in all other groups at week 5 as compared to week 0. However, a high-cholesterol diet alone increased the risk ratio, but BR and GBR supplementation following a high-cholesterol diet reduced the

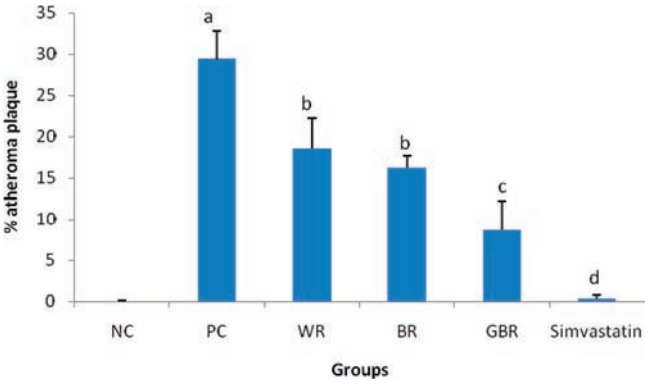

Figure 2. Atherosclerotic plaque of aorta in rabbits fed a normal (NC) or high-cholesterol diet (PC) or the high-cholesterol diet containing white rice (WR), brown rice (BR), germinated brown rice (GBR), or Simvastatin after 10 weeks. Means of percent atheroma plaque with different letters are significantly different ($P < 0.05$).

risk ratio significantly at the end of the treatment, the reduction from week 5 being 62.4 and 68.7%, respectively.

Atherosclerotic Plaque. Photographs of the atherosclerotic changes in the aortas from the six groups stained with Sudan IV are shown in Figure 1, and the extent of atherosclerosis in the groups is summarized in Figure 2. There were no visible atherosclerotic plaques in the aortas of rabbits fed the normal diet, but atherosclerotic plaques of various degrees were visible in the aortas of rabbits fed the high-cholesterol diet and rice diets. A significant area (30.00 ± 3.35%) of the aortic intimal surface from the PC group on 0.5% cholesterol for 10 weeks was covered with atherosclerotic plaques. The plaque in the WR group for 10 weeks following 0.5% cholesterol was 19.00 ± 3.67%. This shows that the atherosclerotic process was reduced in rice treatment. The plaque levels in the BR and GBR groups at 10 weeks were 16.00 ± 1.45 and 8.70 ± 3.54%, respectively. No significant difference in atherosclerotic plaque could be found for the GBR with SG group. These results suggest that a 0.5% cholesterol diet induced the progression of atherosclerosis and rice treatment prevented the progression of atherosclerosis. Rabbits consuming the GBR diet showed greater retardation of the progression of atherosclerosis than those consuming WR and BR diets.

Lipid Peroxidation Measurement. The changes in plasma MDA levels of the six groups for 10 weeks are summarized in Table 6. The basal value of plasma MDA was not significantly different in all groups NC, PC, WR, BR, GBR, and SG. For NC and SG, the levels of MDA remained unchanged throughout the 10 week period of study but increased significantly in other groups at week 5. The level of MDA in plasma, however, was significantly reduced ($P < 0.05$) in the group supplemented with GBR at week 10 compared to week 5.

DISCUSSION

Consumption of foods containing bioactive compounds as an alternative or a supplement to prescription drugs is gaining in popularity. Therefore, the present study aims to evaluate the effect of rice (WR, BR, and GBR) on the lipid profile, MDA level, and atheromatous plaque formation in rabbits with induced hypercholesterolemia. Supplementation of BR and GBR diets has been shown to ameliorate hypercholesterolemia in rabbits. Even though all rabbits fed rice diets that included high cholesterol showed markedly increased TC, TG, LDL, and AI after 5 weeks of supplementation, the level reduced significantly after 10 weeks for the BR and GBR groups. The level of MDA also decreased significantly at week 10 compared to week 5 for the GBR group. The result for AI is also parallel with the percentage of atheroma plaque, which showed that BR and GBR diets could reduce the formation of atheroma plaque.

There are few mechanisms that could be suggested in relation to this hypocholesterolemic action of BR and GBR. Chemical analysis of each type of rice shows that dietary fiber, oryzanol, vitamin E, and minerals are the distinctive components found abundantly in BR and GBR (Tables 2−4), suggesting their potential roles as the active components of BR and GBR in hypocholesterolemic effect. Increased intake of fiber could reduce postprandial secretion of insulin, which induces the synthesis of TC[23] and TG. In vitro studies also have shown high adsorption of bile acid by rice bran, a component that is still present in BR and GBR.[24] Lee et al.[10] further reported that the level of cholesterol decreased in rats fed germinated giant embryonic rice by increasing the secretion of steroid and bile acid. However, the ability of the GBR diet to suppress hypercholesterolemia and enhance bile acid excretion does not affect cholesterol synthesis in the host liver of hepatoma-bearing rats.[11] In our study, the amount of fiber in all six groups was standardized. Therefore, fiber was not the compound that gave the beneficial effect on reducing the level of TC and TG in the BR and GBR diets. A previous study by Kahlon and Chow[25] also reported that rice bran specifically binds more bile acids than other cereal brans. This suggested an important role of rice bran components other than dietary fiber, probably γ-oryzanol and vitamin E. Both compounds have been reported to have strong antioxidant activities, which protect cells from oxidative damage, cellular protein and DNA impairment, and membrane degeneration.[26] This antioxidant activity could also reduce reactive oxygen species (ROS) level as indicated by decrement in the plasma MDA level in our study. γ-Oryzanol possesses a cholesterol-lowering property when fed to humans[27] and animals[28] by decreasing cholesterol absorption and enhancing fecal sterol excretion.[29,30] Besides that, oryzanol also reduced aortic cholesterol ester accumulation in hypercholesterolemic hamsters.[31] In this study, the content of γ-oryzanol and tocopherol in GBR was found to be significantly higher than in BR; therefore, we suspect that these two compounds might contribute to the greater hypocholesterolemic effect of GBR when compared to BR. In addition to that, results from previous study[9] also showed that by prolonging the pregermination time of BR, the content of GABA also increased, which in turn was shown to have the ability to further reduce the levels of TC, LDL, and TG in hypercholesterolemic rats.

A high mineral content such as Mg in BR and GBR is also believed to help in lowering LDL and increasing HDL because Mg is a necessary cofactor for many enzymes such as those involved in lipid metabolism.[32] Lack of Mg results in lipolysis increment, and subsequent elevation of plasma free fatty acid levels may result in increased hepatic very low density lipoprotein (VLDL), TG synthesis, and TG concentration.[33] Besides Mg, Zn has also been reported to inversely correlate with serum lipid concentration and to positively correlate with serum HDL level.[34] A study conducted by Farvid et al.[35] shows that supplementation of vitamins C and E, Mg, and Zn for at least 3 months can increase HDL-cholesterol by 24% and apolipoprotein A1 by 8.8% in type 2 diabetic patients. However, the exact mechanism is still unknown.

Apart from dietary content and other nutrients, it is well-known that various fatty acids in the diet exert different effects on plasma lipid and lipoprotein concentration. Saturated fatty acid (SFA) are believed to increase CVD by elevating plasma TC and LDL, whereas a diet rich in monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) is able to reduce plasma TC level and LDL level apart from elevating HDL level in normolipidemic subjects and in mice with atherosclerosis.[36] The preventive roles of the GBR in the risk of hypercholesterolemia are undeniably contributed by its MUFA content. MUFA is also believed to be able to suppress LDL's susceptibility to oxidation, oxidative stress, and thrombogenicity.[37]

Therefore, from findings gathered in this study, it can be concluded that GBR performed much better than BR in reducing the cholesterol level and thus reducing the risk of CVD. The possible components that might contribute to this difference were MUFA, GABA, tocopherol, and γ-oryzanol, which were found at higher levels in GBR than in BR.

AUTHOR INFORMATION

Corresponding Author

*E-mail: nhaizan@medic.upm.edu.my. Phone: +603-89472427. Fax: +603-89426769.

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