Cholesterol Lowering and Antioxidative Effect of Pregerminated Brown Rice in Hypercholesterolemic Rats

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Summary
Pregerminated brown rice (GBR) is assumed to be more beneficial than polished white rice (WR), with regard to nutrition and cardiovascular health. To support this with scientific evidence, cholesterol-lowering and antioxidative effects of GBR were studied in the present investigation. The most popular rice variety in Bangladesh BIRI-29 was used to prepare GBR and WR. Initially, we analyzed the proximate composition, antioxidative phytochemicals, in vitro 2, 2-diphenyl-1-picrylhydrazyl (DPPH)-free radical scavenging ability and anti-hemolytic effects of GBR. To examine the dietary impact and possible benefits of the GBR, experimentally-induced hypercholesterolemic (HC, 1% cholesterol) rats were fed with GBR against WR for 12 wk. At the end, plasma total cholesterol (TC), low- and high-density lipoprotein-cholesterol (LDL-C and HDL-C), triglyceride (TG), fecal TC, and hepatic TC, lipid peroxide (LPO) and proinflammatory TNFα levels were determined. Relative to WR, GBR contained higher amounts of total polyphenols, total flavonoids, β-carotene and lycopene, and exhibited a stronger in vitro DPPH-free radical scavenging ability and antihemolytic potentials. Levels of plasma TC, LDL-C, TG, and hepatic TC and TG significantly decreased, while plasma HDL-C and fecal TC levels significantly increased in the GBR-fed HC-rats, indicating dietary GBR demonstrates a stronger antilipidemic effect than WR. The hepatic levels of LPO and TNFα also decreased (p<0.05) to a greater extent in GBR-fed HC-rats than those in the WR-fed rats. It is thus concluded that dietary GBR could be a natural treatment of hypercholesterolemia and related cardiovascular risk factors, and a source of antioxidants to reduce hemolysis and related anemia.

Key Words Pregerminated rice, proximate composition, brown rice, white rice, hypercholesterolemic, lipid peroxide, TNFα

Recently, attention is being paid to demonstrate whether a better health benefit is conceivable directly from the daily-taken staple food, i.e. rice in Bangladesh. In China and Japan, pregerminated brown rice (GBR) are becoming popular. The difference between WR and BR being in their milling system. If the milling process removes the rice’s husk, bran, and germ, leaves only the inner endosperm, then it is WR. On the other hand, BR is a whole grain except it is dehusked and retains bran, germ and endosperm. Eating BR became popular in Japan back in the 1970’s (1). Ordinary brown rice is hard to cook, pre-germination, however, softens its bran layer and makes it quick-cooking. Most importantly, the pregermination processes adds many important nutrients, including gama-aminobuteric acid (GABA), inositol, more fibers, various antioxidants, amino acids, which are missing in the WR and/or even present in less amount in the brown rice (2–7). All these nutrients have immense effects on nutrition, health and diseases of humans. The prevalence of hypercholesterolemia and related incidence of atherosclerosis, atheromatous plaques, ischemia and heart attack are ever increasing in all over the world, including Bangladesh. Therefore, the changes of the traditional dietary habits of carbohydrate intake might be one of the choice to manipulate aforementioned risk factors of cardiovascular diseases. We thus investigated whether the dietary administration of GBR could decrease the hypercholesterolemia and related cardiovascular risk factors in the experimentally-induced hypercholesterolemic rats. We also evaluated whether GBR ameliorates the oxidative and pro-inflammatory stress of the liver tissues both in the in vitro and in vivo conditions.

Materials and Methods
Rice seeds (BIRI-29 variety) were soaked in water at room temperature for 24 h, and then dispersed into a tray, and covered by a thick cloth and germination was carried out for 72 h at 28–30°C. When both the plume and radicle extended to more >1.0 cm, the germination was stopped, seeds were dried, de-husked by milling to prepare rice and/or ground into rice flour to prepare the diets of the experimental rats and conduct in vitro or dietary experiments.
in vitro study

Proximate composition, microelements, antioxidant-phytochemicals and free-radical scavenging ability analyses. The moisture content, ash, crude protein and lipid, carbohydrate and fibers were determined by methods as previously described (8). Sodium (Na), potassium (K), magnesium (Mg) and iron (Fe) were measured by atomic absorption spectroscopy (AAS) by AA-7000 (Shimadzu Corporation, Kyoto, Japan), as previously described (9). In brief, acid digested rice samples after appropriate dilution were aspirated into the AAS, which burnt the samples into atomic components. The atomic components were read at their respective wavelengths with optimized instrumentation. The AA-7000 was coupled with an autosampler ASC 7000. The results were expressed as mg/100 g of rice powder.

In order to determine total polyphenol contents (TPC), total flavonoid contents (TFC), β-carotene, lycopene, and in vitro DPPH-free radical scavenging potential and/or anti-hemolytic effect, rice flour was extracted with 50% ethanol for overnight with continuous shaking. Extracts were then filtered, dried, and suspended in water, subjected to a bath sonicator, aliquoted, and stored at −20˚C. Antioxidant phytochemicals or radical scavenging potential of rice extract was determined, as previously described (10).

Erythrocyte preparation and in vitro hemolysis assay

After deep anesthesia with pentobarbital, blood from a fresh set of rats was collected from the inferior vena cava with a heparinized syringe. Erythrocytes were washed with normal saline, and then subjected to hemolysis assay, as described previously but with slide modification (10, 11). Briefly, erythrocyte suspensions (at 2% hematocrit) were incubated with or without rice (WR/GBR) extract in the absence or presence freshly prepared Fenton’s reagents [H2O2 (45 mM)+FeSO4 (2 mM)] at 37˚C for 1 h. At the end of incubation, erythrocytes were gently pelleted down by centrifuging the samples at 300 × g for 10 min. Then, the supernatant was aspirated and the degree of hemolysis was quantified by determining the amounts of released hemoglobin (Hb) in the supernatant at 540 nm against hemoglobin standard.

in vivo study

In bred male Wistar rats were randomly divided initially into two groups: the normocholesterolemic (NC, n=10) and hypercholesterolemic groups [High cholesterol (1%) (HC)-fed group, n=30)]. The HC rats were again subdivided into 3 groups: HC rats, WR-fed HC rats (HC+WR) and pregerminated brown rice-fed HC rats (HC+GBR) (Fig. 1). Feeding of the diets (Table 1) was continued for 12 wk. Water was supplied ad libitum. The rats were cared for and killed in accordance with the guidelines of laboratory animals and approved by the Institutional Animal Ethical Committee at Jahangirnagar University, Savar, Dhaka, Bangladesh. Body weight was measured at every alternate day. Food intake was measured every day.

Plasma lipid profile (TC, TG, HDL-C, and TG) was determined by commercially available reagent kits. To determine the liver TC and TG or fecal TC levels, total lipids were extracted with chloroform/methanol (2 : 1, v/v) according to the method of Folch et al. (1957) (12). The extract was then dried and resuspended in H2O to measure TC/TG. Liver TC, TG and fecal TC was analyzed in vivo study

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Statistical analysis

Results are expressed as mean±SE (standard error of mean). Data were analyzed by one-way ANOVA, followed by Fisher’s PLSD test for post hoc comparisons.
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Results

Proximate composition and antioxidant phytochemicals of WR and GBR

The content of total dietary fiber and the levels of K and Mg were significantly higher in the GBR than those in the WR. In contrast, Na content was significantly lower in the GBR than those in the WR. Total polyphenol contents, total flavonoid contents, beta-carotene and lycopene contents were significantly (p<0.05) higher in the GBR than those in the WR (Table 2). The IC50 of GBR and WR extract for the DPPH were, respectively, 4.46±1.76 mg/mL and 9.25±2.2 mg/mL, indicating the GBR was more effective than WR extract in scavenging the DPPH free radicals. Effect of GBR on in vitro hemolysis of erythrocytes

Oxidative stress (OS) induced by Fenton’s reagents caused a breakdown of the erythrocyte membranes. However, when OS was induced in the rice extract-treated RBCs, hemolysis occurred to a lesser extent. Again the GBR-extract induced a lesser hemolysis (Fig. 2).

Effect of dietary GBR on plasma lipid profile

The levels of plasma TC and TG significantly increased (p<0.05) in the HC rats, when compared to those in the NC rats. The levels of plasma TC, however, decreased significantly only in the HC+GBR rats, but not in the HC+WR rats. Plasma LDL-C levels decreased in the GBR-fed rats only (Table 4). HDL-C levels also increased significantly in the GBR-fed rats only (Table 4). HDL-C levels also increased significantly only in the HC+GBR rats, but not in the NC rats. However, the weight gain was significantly lower in the GBR-fed HC (HC+GBR) rats. The feeding of WR did not have an effect on the body weight gain of the HC+WR rats (Table 3).

Effect of dietary GBR on food intake and body weight gain

Food intake was not significantly different among the rat groups. Weight gain was significantly higher in the hypercholesterolemic (HC) rats, when compared with that of the NC rats. However, the weight gain was significantly lower in the GBR-fed HC (HC+GBR) rats. The feeding of WR did not have an effect on the body weight gain of the HC+WR rats (Table 3).

Fig. 2. in vitro anti-hemolytic effects of GBR and WR were evaluated by their ability to withstand the Fenton’s reagents (FeSO4+H2O2)-induced hemolysis. Results are mean±SE (n=5), each with duplicate determinations. Bars with different letters are significantly different at p<0.05. Data were analyzed with one-way ANOVA followed by Fisher’s PLSD for post hoc comparisons. WR: White rice extract alone-incubated RBCs. GBR: Pregerminated brown rice extract alone-incubated RBCs. OS: Oxidative stress (OS) in RBC suspensions was induced by Fenton’s reagents. OS+WR: OS in RBC suspensions was induced in the presence of WR extract. OS+GBR: OS in RBC suspensions was induced in the presence of GBR extract. RBCs without any added reagents and/or extract did not have any appreciable hemolysis (data not shown).

Table 2. Proximate composition (as%), minerals (as mg%) and antioxidant components of GBR.

| Parameters          | WR          | GBR         |
|---------------------|-------------|-------------|
| Proximate composition |             |             |
| Carbohydrate        | 75.8±3.5    | 78.0±4.50   |
| Fiber               | 6.0±0.03    | 7.20±0.04*  |
| Protein             | 13.6±0.50   | 12.20±0.04* |
| Total fat           | 0.50±0.02   | 0.60±0.02   |
| Ash                 | 0.60±0.01   | 0.90±0.03*  |
| Moisture            | 9.50±0.03   | 8.30±0.03   |
| Element             |             |             |
| Na (mg%)            | 121.3±3.9   | 89.3±5.30*  |
| Mg (mg%)            | 117.5±5.0   | 383.10±10.0*|
| K (mg%)             | 2.85±21.1   | 3.18±14.0*  |
| Fe (mg%)            | 1.563±8.0   | 1.503±11    |
| Antioxidant         |             |             |
| TPC                 | 53.7±5.6    | 119.3±3.7*  |
| TFC                 | 62.3±11.0   | 77.58±4.2*  |
| β-carotene          | 65.0±7.04   | 94.5±8.4*   |
| Lycopene            | 213±8.88    | 238±11.2*   |

Results are mean±SE of three independent experiments. *p<0.05 (student’s t-test).

Table 3. Effect of GBR on food intake and body weight gains of the hypercholesterolemic rats.

| Parameter       | NC          | HC          | HC+WR       | HC+GBR      |
|-----------------|-------------|-------------|-------------|-------------|
| Food intake (g/d)| 18.5±1.5    | 21.5±1.5    | 19.0±2.0    | 20.5±1.6    |
| Initial weight (g)| 108±3.0   | 109±3.1     | 110±3.0     | 112±3.1     |
| Final weight (g) | 180±2.8*    | 204±6.4*    | 202±5.2*    | 192±6.9*    |
| Weight gain (g)  | 72±2.40*    | 95±3.7*     | 92±2.8*     | 80±4.0*     |

Results are mean±SE. Values in the same row with different alphabets are significantly different at *p<0.05. NC: Normocholesterolemic rats (n=8). HC: High cholesterol (1%)-fed hypercholesterolemic rats (n=8). HC+WR: White rice-fed HC rats (n=8). HC+GBR: GBR-fed HC rats (n=8). Data were analyzed by one-way ANOVA, followed by Fisher’s PLSD test for post-hoc comparisons.
Effect of dietary GBR on liver TC/TG and fecal TC
The levels of liver total cholesterol (Fig. 3A) and triacylglycerol (Fig. 3B) increased by 2-folds in the HC rats, as compared to those of the NC rats. The levels liver TC and TG decreased significantly in the GBR-fed rats. The levels fecal TC but not the TG decreased in the WR-fed rats. The levels of hepatic TC but not the TG decreased in the WR-fed rats. The levels fecal TC increased both in the WR- or GBR-fed rats, yet the levels of fecal cholesterol were higher in the GBR-fed rats (Fig. 3C), as compared to those in the HC rats.

Effect of dietary GBR on liver LPO and TNF-α
The levels of hepatic LPO (Fig. 4A) and TNF-α (Fig. 4B) significantly increased in the HC rats, when compared with those of the NC rats. However, the levels of LPO and TNF-α decreased significantly only in the HC+GBR rats but not in the HC+WR rats, when compared with those of the HC rats (Fig. 4A and 4B).

Discussion
This study is aimed to evaluate the effect of pregerminated brown rice (GBR) on cardiovascular risk factors, including hyperlipidemia, and oxidative- and proinflammatory stress in the hypercholesterolemic rats. The body weight gain, as seen generally high in obesity and hyperlipidemia, is implicated in the cardiovascular disease risk factors. In the present study, dietary administration of GBR caused a greater decrease \((p<0.05)\) in the body weight gain in the HC+GBR rats than that in the HC+WR rats. Our results are consistent with those of Lim et al. \((13)\). Dietary administration of GBR significantly decreased the plasma levels of TC, TG and LDL-C, and increased the plasma levels of HDL-C in the hypercholesterolemic rats. Therefore, we suggest that GBR has a stronger hypcholesterolemic effect than WR in hypercholesterolemia. The decreased plasma levels of TC, TG or LDL-C were accompanied with decreases in the levels of hepatic TC and TG in the GBR-fed rats. It is thus conceivable that decreased levels of plasma/hepatic TC is related either to increased excretion of cholesterol from the liver to the gut lumen and/or decreased intestinal absorption of dietary cholesterol or to both. Our speculation is consistent with the increased levels of TC in the feces of the HC+GBR rats. The present findings also support earlier reports that GBR ameliorates lipid

Table 4. Effect of GBR on plasma lipid profile of hypercholesterolemic rats.

| Parameter     | NC     | HC     | HC+WR  | HC+GBR |
|---------------|--------|--------|--------|--------|
| TC (mg/dL)    | 87±2.30 | 161±6.00 | 155±13.00 | 135±3.50 |
| TG (mg/dL)    | 127±2.00 | 158±9.00 | 152±4.20 | 139±5.00 |
| LDL-C (mg/dL) | 16.0±1.50 | 9.3±3.00 | 88.1±2.10 | 67.2±2.80 |
| HDL-C (mg/dL) | 45±0.70  | 36±1.10  | 36±2.10  | 40±2.01  |

Results are mean±SE. Values in the same row with different alphabets are significantly different \((p<0.05)\). TC: Total cholesterol. TG: triglyceride. LDL-C: Low density lipoprotein-cholesterol. HDL-C: High density lipoprotein-cholesterol. Data were analyzed with one-way ANOVA, followed by Fisher’s PLSD test for post-hoc comparisons. NC: Norcholsterolemic rats \((n=8)\). HC: Hypercholesterolemic rats \((n=8)\). HC+WR: WR-fed HC rats \((n=8)\). HC+GBR: GBR-fed HC rats \((n=8)\).
profile and therefore provides protection against cardiovascular diseases (13, 14). On the contrary, the results of decreased levels of plasma or hepatic TC might also be related to GBR-induced inhibition of endogenous cholesterol biosynthesis in the liver of the GBR-fed HC rats. We believe that neither of the above-mentioned mechanisms of the hypocholesterolemic effects of GBR are mutually exclusive. In this line, multiple mechanisms of hypocholesterolemic effect(s) of the bioactive compounds of GBR have been proposed by other investigators. The hepatic LDL-R (receptor) clears up LDL-C from the blood circulation, thus indicating an increased expression of LDL-R would be favorable for the increased elimination of LDL-C. The bioactive compounds of GBR, such as phytosterols, GABA, oryzanol and phenolics have previously been ascribed to the up-regulation of LDL-R gene in HEPG2 cells (15, 16). Other reports also support that GBR causes upregulation of both apoA-1 and LDL-R genes (17). Elevated levels of HDL-C and its carrier protein apoA1 are anti-atherogenic, and reduce the cardiovascular risk. The GBR in our study increased the plasma levels of HDL-C, indicating that GBR may reduce the risk(s) of cardiovascular diseases. The increases in the concentration of bioactive compounds through germination is one of the ways to enhance the benefits of GBR. In the current study, GBR contained a greater concentrations of bioactive compounds, including total polyphenols, total flavonoid compounds, lycopene and beta-carotene. Consistent with other reports (18), GBR displayed a stronger free-radical scavenging effect than WR.

In our investigation, GBR contained a higher amount of fiber than the WR (Table 2). Dietary fibers decrease the plasma lipids, including cholesterol and triacylglyceride, though an exact mechanism of anti-lipidemic/anti-hypercholesterolemic effect of fibers remains to be known. Originally, it was proposed that fiber increases the size of the bile acid pool (19), and binding and fecal loss of bile acids (20), which in turn co-effluxes cholesterol (hence this pool is known as biliary cholesterol) from the liver to the gut to emulsify the intestinal lipids. This is one of the ways through which hepatic cholesterol is secreted into the feces and/or dietary cholesterol, as it is, is excreted via the feces, and ultimately contributes to the decreases in the plasma and hepatic TC levels. Thus, GBR in these mechanisms, via its high contents of fibers, might have decreased the plasma or hepatic TC in the present investigation.

The contents of bioactive compounds, including oryzanol, GABA, phytosterols, increase during the germination of brown rice (16). Oryzanol has been reported to have strong hypocholesterolemic effects (21). Roohinejad et al. suggested that GABA possesses hypocholesterolemic effects (22). Phytosterol glycosides, via their competitive inhibition and regulation of cholesterol absorption (23), lowers cardiovascular disease risks (24), also have been shown to have hypocholesterolemic effects. Furthermore, phenolic compounds have hypocholesterolemic effects in addition to their antioxidant effects (25). Phenolic concentration was higher in the GBR than WR. It is likely that the synergistic effects of these multiple bioactive compounds produce the overall hypocholesterolemic effects of GBR.

Oxidative stress (OS) is the imbalance between reactive oxygen species (ROS) and the antioxidant defense system (26). Oxidative stress is one of the risk factors and it can be generated by other cardiovascular risk factors, including hypercholesterolemia and triglyceride-bound unsaturated fatty acids (27, 28). On the other hand, pro-inflammatory TNFα is also a lipid metabolism regulator, and an elevated level of TNFα occurs with hyperlipidemia (29, 30). Consistent with these reports, the levels of LPO, as an indicator of oxidative stress (31), and TNFα, as an indicator of proinflammatory stress, significantly increased in the HC rats. Dietary GBR, however, decreased the levels of both the LPO and TNFα in the liver tissues of HC+GBR rats to a greater extent than that decreased by the WR rice, thus demonstrating that GBR dominates over the WR in preventing both the oxidative stress and proinflammatory stress.

Oxidative stress is also related to hemolysis and different forms of anemia (32), diabetes (33) and neurodegenerative diseases (34), including ageing-related Alzheimer’s disease (AD) (35, 36). Erythrocytes membranes, which are exposed to free radicals both intracellularly or extracellularly, are very vulnerable to oxidative stress, and eventually may rupture with a concomitant release of hemoglobin (37). GBR exhibited a better protective effect against hemolysis. A direct evidence was reflected by an increased protective ability of GBR extract against Fenton’s reagent-induced hemolysis than that of the WR rice (Fig. 2). With regard to increased prevalence of anemia in the third world countries, the effects of GBR against hemolysis have immense implications. Anemia, which is a global health problem both in the developed and developing countries, including Bangladesh, is the world’s second leading cause of disability (38, 39). Thus, anemia could be decreased by supplementation of GBR, at least, to some extent, if it occurs as a scarcity of defense against oxidative state, which otherwise breaks the erythrocyte membranes and releases of Hb, and eventually causes anemia. Finally, GBR also contained an increased amount of K and Mg, and a decreased amount of Na than that of the WR, which are well suited for the reductions of blood pressure, i.e. hypertension by balancing out the negative effects of sodium salt. The application of GBR on nutrition standpoint will be very promising in Bangladesh, which is continuously fighting against malnutrition. Moreover, brown rice has not yet received any attention in academics and commercial stages, it will also be very interesting to generate awareness among the consumers and study the properties of brown rice in more details in Bangladesh.

**Conclusion**

The GBR decreases the levels of plasma cholesterol in hypercholesterolemia. It also provides a stronger protection against the oxidative stress and pro-inflammatory potentials via its antioxidative compounds, which could be used to withstand hemolytic anemia, if it happens,
at least due to oxidative stress and related hemolytic diseases.

Disclosure of State of COI
No conflicts of interest to be declared.

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