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HYPOCHOLESTEROLEMIC AND ANTI-OXIDATIVE PROPERTIES OF GERMINATED BROWN RICE (GBR) IN HYPERCHOLESTEROLEMIA-INDUCED RATS

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ABSTRACT

Hypercholesterolemia, as one of the causes of obesity, affects vital organs in the body, such as the liver and kidney, resulting to oxidative stress. Germinated Brown Rice (GBR) as a food-based solution in dealing with this condition is highly recommended. In this study, the effects of GBR on hypercholesterolemia-induced rats were evaluated by measuring and analyzing the changes on body weight, serum lipid profiles (TC, TG, LDL and HDL), liver function (ALT and AST), kidney function (Crea and Urea) and its antioxidant capacity (MDA, SOD, GSH-PX and TAOC). Thirty (30) SD male rats were divided into 5 groups (6 rats per group); Group A was given normal basal diet, Group B (hypercholesterolemic group) was given a high fat diet, while Groups C, D, and E were given 12.5%, 25% and 50% GBR, respectively. Groups C, D and E were fed with high fat diet for 4 weeks, then fed with the GBR feeds, accordingly, for another 5 weeks. Sera and liver samples were collected for testing and evaluation. Hypercholesterolemia was successfully induced in Groups B, C, D, and E after 4 weeks. Noticeable responses were observed in groups fed with GBR after 5 weeks. Group E fed with 50% GBR showed the satisfactory results (significant at $p<0.05$) in weight gain, serum lipid profiles, liver function enzymes, creatinine, urea and oxidative stress markers compared to the hypercholesterolemic group. The hypocholesterolemic and antioxidant properties of GBR were found to have a dose-response effect where higher percentage of GBR showed acceptable results as compared to the normal and hypercholesterolemic groups. GBR showed to effectively lessen TC, TG and LDL while increases HDL. It effectively protects the liver while its kidney protective ability was associated to its hypocholesterolemic properties. Oxidative stress was reduced as shown by a decline in lipid peroxidation and improved antioxidant production. In addition, the abovementioned GBR's properties are combined effects of its bio-active nutrient components making GBR a complete food that not only deals with the basic nutrient needs of the body but also can deal with metabolic disorders the body encounter.

Keywords: Germinated brown rice; hypercholesterolemia; serum lipid profile; antioxidant

INTRODUCTION

With the rapid economic development and continues improvement of living standard, people as well become more aware of eating healthy foods. Hypercholesterolemia, as one of the causes of obesity, has been classified as a lifestyle disease where raised cholesterol is estimated to cause 2.6 million deaths annually that were found to be highest in high-income countries. Hypercholesterolemia has been directly associated with high fat diet that can be exacerbated by the presence of physiological or pathological disorders. It is characterized by high serum low-density lipoprotein (LDL) and total cholesterol (TC) (**Otunola *et al*., 2010**). Hypercholesterolemia increases the risks of heart disease and stroke (**Alwan, 2011**). Furthermore, hypercholesterolemia is generally recognized as a risk factor of atherogenesis, oxidative stress and oxidatively modified LDL play a crucial role (**Ondrejovicova *et al*., 2010**). Prolonged high-saturated fat diet reduces the hepatic enzyme antioxidant system and increases lipid peroxidation products in the liver and plasma, thus inducing oxidative stress (**Marczuk-Krynicka *et al*., 2009**).

It is recommended that first line treatment for hypercholesterolemia entails dietary and 'lifestyle' modification (**Smart *et al*., 2011**). The Food and Agriculture Organization of the United Nations (FAO) recognizes the importance of food-based approaches for the prevention and control of micronutrient deficiencies as well as for the improvement of nutrition in general (**Thompson & Amoroso, 2010**).

Rice is a major staple food and a chief support for domestic food security (**Calpe, 2006**). Rice grain has not only been a main source of carbohydrate but also is a quality source of many bio-active non-nutrient compounds. However, valuable loss of nutrients occurs during the processing of rice where husked rice contains more nutrients than polished rice (**Abbas *et al*., 2011**). Brown rice (BR) is unpolished whole grain that is produced by removing only the husk that is more nutritious than white rice or polished rice, which is devoid of nutrients after milling (**Dinesh Babu *et al*., 2009**). One way of enhancing the nutritional value of grain is through germination as proven in the case of germinated soybeans and grains such as wheat and BR. GBR is a recent rice product, which has been soaked in 37°C water for 24h to initiate germination of sprouts approximately 0.5-1mm long, has gained popularity worldwide.

During germination, concentrations of major bioactive compounds such as dietary fiber (soluble and insoluble), vitamins such as vitamin E, B1, B6, and niacin, amino acids such as lysine and gamma(γ)-aminobutyric acid (GABA) and mineral such as magnesium (**Ohtsubo *et al*., 2005; Kayahara & Tsukahara, 2011**) . Furthermore, germination makes BR soft and grain nutrients much easier to digest and absorb (**Tian *et al*., 2004; Komatsuzaki *et al*., 2007**).

This study evaluated the effects of GBR on hypercholesterolemia-induced rats. Specifically, we measured and analyzed the changes on body weight, serum lipid profile such as LDL, high-density lipoprotein (HDL), TC, total triglyceride (TG), liver functions such as alanine transaminase (ALT), aspartate aminotransferase (AST), kidney functions such as Creatinine (Crea) and Urea and antioxidant capacity such as total antioxidant capacity (TAOC), malondialdhyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX).

MATERIALS AND METHODS

Reagents and instruments

The cholesterol and sodium cholate were bought from Sangon Biotech (Shanghai, PRChina 201611) Co. Ltd. and egg yolk powder was purchased from Saifate Biotech Co. Ltd. (Changsha, PRChina 410000). The reagents for TC, TG, ALT, and AST were obtained from Mindray Biotech Co. Ltd (Shenzhen, PRChina 518057) while T-AOC, MDA and SOD test kits were bought from the

Nanjing Jiancheng Bioengineering Institute (Nanjing, PRChina 210000). The following instruments were used in the experiment; BS-190 automatic biochemical analyzer, Microplate reader (infinite M200PRO), WH-2 Microvortex mixed instrument.

Feed formulation

The high-fat diet was composed of the following ingredients, 1% cholesterol, 10% lard, 0.2% sodium cholate, 10% egg yolk powder, 78.8% basal diet. Germinated brown rice (Zi Yuyuan Jiang ™) was provided by the Hunan Sevoc Ecological Agriculture & Husbandry Technology Co. LTD (Changsha, PRChina 410000). The different ratios (12.5%, 25% and 50%) of feeds together with the basal diet were prepared by the SJA Lab Animal Co. Ltd.

Experimental animals

Thirty (30) Sprague-Dawney (SD) male rats were purchased from the SJA Lab Animal Co. Ltd. The rats were given 1 week to adapt to the laboratory condition. Feeds and water was given ad libitum while the laboratory temperature was maintained at 27 ± 1°C. The rats were divided into 5 groups with 6 animals in each group. The following were the groupings; Groups A was given normal basal diet, Groups B (hypercholesterolemic group) was given a high fat diet, while Groups C, D, and E were given 12.5%, 25% and 50% GBR, respectively.
Groups C, D and E were fed with high fat diet for 4 weeks, then fed with the GBR supplemented feeds, accordingly, for another 5 weeks. On the 9th week, all groups were fasted for 12h before intra-cardiac blood collection and liver sampling were done. This research has been approved by the ethics committee and all experimental rats were treated in accordance to the approved protocol for experimental animal handling and experimentation of the College of Veterinary Medicine, Hunan Agricultural University.

Sample preparation

Blood samples were allowed to stand for 2h before centrifuging at 3000 rpm for 10 min. The sera were collected and stored at -20°C until used. Each liver sample was rinsed with cold phosphate buffer solution (PBS) to remove blood and dried up using filter paper. Using a handheld Homogenizer, 0.15g of liver tissue from each sample was homogenized in 1.5ml PBS. The homogenates were centrifuged at 3000 rpm for 10-15 minutes. The supernatant was collected and stored at -20°C until used.
Serum LDL, high-density lipoprotein (HDL), TC, total triglyceride (TG), alanine transaminase (ALT), aspartate aminotransferase (AST), Creatinine (Crea), and Urea levels were evaluated using Mindray BS-190 automatic biochemical analyzer while liver total antioxidant capacity (TAOC), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) levels were measured in accordance with the instruction provided in the test kit.

Statistical analyses

The data were presented as the mean ± standard deviation. Statistical analyses of the differences between each group were performed using one-way Analysis of Variance (ANOVA) using SPSS 13.0 software. Differences were regarded as significant at $p < 0.05$.

RESULTS

Weight gain

Final weight was taken on the 9th week and the percentage difference was computed using normal-diet group (Group A) as the baseline weight. As shown in Table 1, highest percentage was seen in rats fed with high lipid diets while descending percentages were observed in Groups C, D, and E, respectively. However, among experimental groups, significant result ($p<0.05$) was only found in Group E in relation to the hypercholesterolemic group (Group B), while only 2.46% weight gain difference with the normal-diet group. A previous study by **Ho *et al*., (2012)** showed the same results in the significant decrease in body weight gain of high fat diet-induced obese mice given with GBR extract.

Table 1 Mean final weight, percentage difference and mean weight gain after 9 weeks

Group	Final Weight (g)	Weight Gain (g) 4th to 9th week	Percentage Difference (%) *
A	452.67±5.50	113.83±3.87^{a}	0
B	514.17±7.24	139.00±3.86^{b}	19.91
C	487.00±12.29	133.67±4.14^{bc}	16.03
D	482.00±12.43	124.00±11.64^{ab}	8.55
E	468.67±7.31	116.67±4.09^{ac}	2.46

A – normal-diet group, B – hypercholesterolemic group, C – fed with 12.5% GBR, D – fed with 25% GBR, E – fed with 50% GBR
Mean followed by different superscript letters (a, b, c) in a column are significantly ($p < 0.05$) different from each other.
*Compared with the mean weight gain of Group A from 4th to 9th week

Serum lipid profile

Consumption of hypercholesterolemic diet can significantly increase the level of serum TC and LDL (**Otunola *et al*., 2010**). In this study, positive results (as shown in Table 2) were observed in Group E which has significantly lower TG (together with Group D) and TC which is comparably similar to the normal-diet group. Furthermore, its HDL level was significantly higher while its LDL level was significantly lower than group C, D and the hypercholesterolemic group. Group D also showed significant results compared with the hypercholesterolemic group with lower TC and LDL, and higher HDL, while Group C only showed acceptable results in LDL, which was lower than the hypercholesterolemic group. These results coincide with previous studies in hypercholesterolemic animals supplemented with GBR where a decrease in TC, LDL and TG, and increase HDL was observed (**Roohinejad *et al*., 2010; Mohd. Esa *et al*., 2011; Ho *et al*., 2012; Imam *et al*., 2012**).

Table 2 Effects of GBR on serum lipid profiles (mmol/L)

Group	TG	TC	HDL	LDL
A	0.5667±0.0423^{a}	1.19±0.09^{a}	1.38±0.02^{a}	0.30±0.03^{a}
B	0.8267±0.0254^{b}	2.85±0.10^{b}	0.96±0.03^{b}	2.73±0.08^{b}
C	0.8167±0.0635^{b}	2.80±0.06^{b}	0.93±0.02^{b}	2.43±0.16^{c}
D	0.6817±0.0595^{ab}	2.25±0.10^{c}	1.05±0.03^{c}	1.91±0.11^{d}
E	0.5583±0.0620^{a}	1.33±0.08^{a}	1.20±0.05^{d}	1.49±0.10^{e}

A – normal-diet group, B – hypercholesterolemic group, C – fed with 12.5% GBR, D – fed with 25% GBR, E – fed with 50% GBR
Mean followed by different superscript letters (a, b, c, d, e) in a column are significantly ($p < 0.05$) different from each other.

Liver enzymes, urea and creatinine

Serum AST and ALT are enzymes that elevate during hepatocellur injury and are thus used in evaluating liver conditions. Serum creatinine and urea are good markers for evaluating the condition of the kidney where high level indicates kidney damage (**Imam *et al*., 2013**). Evaluation of the liver enzymes, urea and creatinine shows significant differences ($p<0.05$) between Group B and GBR-supplemented groups. As shown in Table 3, AST (except in Group C) and ALT of GBR supplemented groups were significantly lower than hypercholesterolemic group but incomparable with the normal-diet group. Same observation was seen in urea and creatinine of GBR-supplemented groups, except group C creatinine which is comparable with the hypercholesterolemic group, and Group E urea which is comparable with the normal-diet group. In the study conducted by **Imam *et al*.,(2012)** same results were obtained in type 2 diabetic rats fed GBR for 4 weeks where reduction of the liver enzymes (ALT and AST) and serum creatinine were seen, while serum urea was slightly elevated compared to the normal group.

Table 3 Effects of GBR on liver Enzymes, urea and creatinine

Group	ALT (V/L)	AST (V/L)	Urea (µmol/L)	Creatinine (µmol/L)
A	36.93±1.25^{a}	140.98±5.31^{a}	4.51±0.14^{a}	82.17±2.13^{a}
B	121.50±8.43^{b}	329.22±19.77^{b}	6.76±0.14^{b}	118.00±2.63^{b}
C	113.85±7.59^{b}	283.02±15.61^{c}	6.24±0.10^{c}	113.03±3.47^{bc}
D	94.97±5.10^{c}	246.10±5.38^{cd}	5.59±0.08^{d}	108.87±1.41^{c}
E	68.58±6.31^{d}	213.98±16.23^{d}	4.68±0.14^{a}	99.57±0.96^{d}

A – normal-diet group, B – hypercholesterolemic group, C – fed with 12.5% GBR, D – fed with 25% GBR, E – fed with 50% GBR
Mean followed by different superscript letters (a,b,c,d) in a column are significantly ($p < 0.05$) different from each other.

Oxidative stress markers

High saturated fat diets are found to cause oxidative stress negatively affecting the hepatic enzyme antioxidant system (**Marczuk-Krynicka *et al*., 2009**). Oxidative stress in the liver can be evaluated by assay of enzymes such as MDA, SOD and GSH-PX (**Marczuk-Krynicka *et al*., 2009; Noeman *et al*., 2011**). Furthermore, TAOC is used as biomarker with its advantage of measuring antioxidant capacity of all antioxidants in a biological sample (**Kusano & Ferrari, 2008**). As shown in Table 4, Group E showed the most satisfactory results where all of its oxidative markers were significantly different ($p<0.05$) with the hypercholesterolemic group while comparable to the normal-diet group.

Group D showed significantly lower MDA and significantly higher TAOC compared with the hypercholesterolemic group. Group B only showed a significant result in MDA while other oxidative markers are comparable with the hypercholesterolemic group. In a previous study (**Mohd. Esa *et al*., 2011**), a reduction of the MDA level was demonstrated in rabbits fed with GBR. While elevated total antioxidant was observed in GBR supplemented type 2 diabetic rats and elevated expression of SOD gene in HEPG2 treated with GBR extracts (**Imam *et al*., 2012**). Also, several studies already explained that GSH-PX can decrease in hypercholesterolemic-animal model (**Wresdiyati *et al*., 2008; Noeman *et al*., 2011; Prasanna & Purnima, 2011**).

Table 4 Effects of GBR on oxidative stress markers

Group	MDA (nmol/mgprot)	SOD (U/mgprot)	GSH-PX (U/mgprot)	TAOC (U/mgprot)
A	1.48±0.07^{a}	0.1279±0.0119^{a}	1902.57±67.75^{a}	0.2679±0.0112^{a}
B	2.47±0.16^{b}	0.1050±0.0021^{b}	1253.62±88.45^{b}	0.2695±0.0255^{a}
C	1.98±0.11^{c}	0.1071±0.0046^{b}	1261.86±76.00^{b}	0.3319±0.0261^{ab}
D	1.85±0.10^{c}	0.1087±0.0051^{b}	1324.32±94.78^{b}	0.4224±0.0173^{b}
E	1.36±0.07^{a}	0.1110±0.0025^{ab}	1661.96±88.40^{a}	0.7879±0.650^{c}

A – normal-diet group, B – hypercholesterolemic group, C – fed with 12.5% GBR, D – fed with 25% GBR, E – fed with 50% GBR
Mean followed by different superscript letters (a,b,c) in a column are significantly ($p < 0.05$) different from each other.

DISCUSSION

The above results suggest a dose-response relationship where a higher percentage of GBR resulted to more satisfactory results that were significantly different from hypercholesterolemic group. The decrease in weight gain can be attributed to the high dietary fiber content of GBR that improves upon germination. Suggested mechanism of dietary fibers, in general, on weight regulation were, firstly, production of gut hormones that can induce satiety upon fermentation of soluble fibers, secondly, decrease in energy intake and, lastly, decrease in metabolizable energy (**Lattimer & Haub, 2010**). In addition, lower weight gain can be due to reduction of body fats. GBR was shown to reduce body fat formation by decreasing adipocyte size through down regulation of lipoprotein lipase (LPL) and up regulation of hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) (**Ho *et al*., 2012**).

The above results coincide with previous studies in hypercholesterolemic animals supplemented with GBR where the decrease in TC, LDL and TG, and increase HDL was associated with the dietary fiber, γ-oryzanol, vitamin E and GABA found in GBR (**Roohinejad *et al*., 2010; Mohd. Esa *et al*., 2011**). In addition, **Imam *et al*. (2013)** showed that the hypocholesterolemic effects of GBR involve the upregulation of apolipoprotein A1 (Apo A1) and LDL receptor genes. Apo A1 is an amino acid synthesized in the liver and then secreted into the plasma and lymph (**Irshad & Dubey, 2005**). Apo A1 is the primary component of HDL. It defines the size and shape of HDL solubilizes its lipid components and removes cholesterol from peripheral cells and involved in the activation of lecithin cholesterol acytransferase (LCAT) (**Philips *et al*., 1997**). While, LDL receptors are involve in the cellular uptake of cholesterol-laden lipoprotein from circulation (**Beglova *et al*., 2004**) . Inhibition of LDL receptor synthesis is one of the metabolic effects of oversupply of cholesterol supply. **Trapani *et al*.(2012)** mentioned that one of the aims of hypercholesterolemia treatment for cardiovascular disease, caused by increase plasma cholesterol, is by regulating the LDL receptor-cholesterol biosynthesis. In this study, the improvement of HDL and reduction of LDL in GBR fed rats can be linked to the regulation of Apo-A1 and LDL receptor genes, respectively, as explained by **Imam *et al*. (2013)** where bioactive compounds in GBR such as acylated steryl glycoside (ASG), GABA, oryzanol, and phenolics contributed to the upregulation of these genes.

Liver and kidneys play critical roles in the body and are often targets of insults involving metabolic disorders. ALT and AST are intracellular enzymes found primarily in the liver and their elevated serum level can be mostly associated to hepatocellular injury or apoptosis. Serum ALT and AST has been found to be significantly elevated in hypercholesterolemic diets as a result of damage to the liver and heart, and has been used as indicators for evaluating cardiovascular disease (**Otunola *et al*., 2010**). **Li *et al*.(2009)** conducted an experiment on hepatic L02 cells where cholesterol overloading due to high LDL level and inhibition of acyl-CoA: cholesterol acytransferase (ACAT) leads to apoptosis. As mentioned previously, GBR can upregulates LDL receptors thereby preventing elevation of LDL level. With the increase uptake of LDL by the cells, GBR may also enhance the activity of ACAT in exporting cholesterol esters thus preventing intracellular overload. On the other hand, hypercholesterolemia has been reported to cause renal dysfunction by reduced renal blood flow, increased renal vascular resistance and impairment of glomerular filtration rate (**Balarini *et al*., 2011**). In the present study, reduction in serum creatinine and urea shows a decrease in renal injury as associated with the property of GBR to reduce cholesterol level.

Oxidative stress is caused by an imbalance between reactive oxygen species (ROS) and body's antioxidant to neutralize the reactive intermediates or to repair the resulting damage. Hypercholesterolemia has been reported in previous studies (**Wresdiyati *et al*., 2008; Prasanna & Purnima, 2011**) to cause oxidative stress. Hypercholesterolemia can induce lipid peroxidation (**Das et al., 2000**) that can be measured using MDA, a product of fatty acid peroxidation that accumulates upon increase lipid peroxidation (**Kaur *et al*., 2008**). In the present study, GBR fed rats showed positive antioxidant activities with lower MDA and higher SOD, GSH-PX and TAOC compared with the hypercholesterolemic group. GBR was also found to reduce ROS level as indicated by decrement on the plasma MDA level (**Mohd. Esa *et al*., 2011**). Decrease in MDA, consequently, can be associated to decrease in lipid peroxidation. **Trapani et al. (2012)** mentioned that ROS-preventing antioxidant activity could play significant role in HMGR activity-induced hypercholesterolemia. SOD and GSH-PX are intracellular antioxidant enzymes that provide protection against intracellular superoxide anion radical. Hypercholesterolemia causes decrease in SOD and GSH-PX as reported in previous studies (**Wresdiyati *et al*., 2008; Noeman *et al*., 2011; Prasanna & Purnima, 2011**). In the present study, GBR fed rats showed higher SOD and GSH-PX compared with hypercholesterolemic group. **Imam *et al*. (2012)** reported that GBR upregulates the SOD 2 gene expression in HEPG2 cells and its antioxidant effect was attributed to its high GABA content and antioxidant potentials. Furthermore, the antioxidant activity of GBR could also also be attributed to its vitamin E content where **Musalmah *et al*.(2012)** reported that vitamin E, specifically alpha-tocopherol, reduced plasma MDA levels and increased GSH-PX activity in rats. In general, a decrease in total antioxidant

capacity could be associated with oxidative stress conditions or susceptibility to oxidative damage (**Young, 2001**). Elevated lipid peroxidation in hypercholesterolemia has been correlated to reduced serum total antioxidant level (**Das *et al*., 2000**). Based on **Imam, *et al*.(2012)** improvement in total antioxidant status in GBR fed rats can be associated with low glucose level in the blood causing an improvement of the animal to produce more antioxidants.

CONCLUSIONS

The hypocholesterolemic and antioxidant properties of GBR were found to have a dose-response effect where a higher percentage of GBR showed acceptable results as compared to the normal and hypercholesterolemic groups. GBR showed to effectively lessen TC, TG and LDL while increases HDL. It also effectively protects the liver as shown by lower ALT and AST while its kidney protective ability, as shown by lower urea and creatinine was associated to its hypocholesterolemic properties. Reduction of oxidative stress due to high cholesterol was shown by lower MDA, indicating a decline lipid peroxidation. Furthermore, improved antioxidant production to counter ROS damage was shown by higher SOD, GSH-PX and TAOC. In addition, the abovementioned GBR's properties are combined effects of its bio-active nutrient components making GBR a complete food that not only deals with the basic nutrient needs of the body but also can deal with metabolic disorders the body encounter.

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