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Radical Scavenging and Antihypercholesterolemic Effects of Red Yeast Rice in Cholesterol Fed Rats

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Abstract

This study demonstrates that red yeast rice exhibits radical scavenging and antihypercholesterolemic activities in rats fed cholesterol. Sprague-Dawley male rats were divided to five dietary groups (normal, chol-control; and M-1, M-2 & M-3 administered 150, 300, and 500 mg/kg red yeast rice, respectively) and fed their respective diets for 4 weeks. No significant differences in food efficiency ratio (FER) were found among the five groups. The weight of perirenal fat pads decreased with increasing amounts of red yeast rice supplementation. There was a significant decrease in the levels of cholesterol in M-3 group fed red yeast rice with 500 mg/kg compared to those in the chol-control, M-1 and M-2 groups ($p<0.05$). Among the rats fed the cholesterol-enriched diet, all groups fed red yeast rice showed higher concentration of the HDL cholesterol, but lower concentration of the LDL cholesterol than those of the chol-control group. The scavenging activity of the methanol extract from red yeast rice was increased with increasing amounts of the extract. The glutathione content in the normal group and in the M-3 group were higher than that in the other groups. The M-3 group showed similar hepatic glutathione contents to those of the normal group. These results suggest that red yeast rice may be safe and effective for lowering serum levels of total and LDL-cholesterol, ratio of non-HDL/HDL, and severity of experimental atherosclerosis.

Key words: red yeast rice, radical scavenging, antihypercholesterolemic effects

INTRODUCTION

Red yeast rice is a fermented rice product on which red yeast (*Monascus purpureus*) has been grown. This product has been used in food as a preservative, for maintaining taste and color in fish and meat, and for its medicinal properties (1). Red yeast rice is a dietary staple in many Asian countries with typical consumption ranging from 0.5 to 2 oz/person/day (2).

A pharmacological preparation from *Monascus purpureus* fermented on rice has been in public use in China as well as in many other countries including the United States: *Monascus purpureus* rice or red yeast rice, is composed, in part, of 73.4% starch, 5.8% protein, less than 2% fat and a number of compounds called monacolins, which are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. The total monacolin level of the *Monascus purpureus* rice product is 0.4% (3). There are several other monacolin derivatives (now generally referred to as "statins") which are used to treat hypercholesterolemia. Ma et al. (3) have identified thirteen different monacolins in red yeast rice, of which monacolin K represents about half of the total monacolin yield.

The hypolipidemic and anti-atherogenic effects of lovastatin (commercial name of monacolin) in rabbits have been confirmed by several authors (4,5), and it has also been shown to be anti-atherogenic in hamsters (6).

Increased levels of cholesterol and triglycerides are known risk factors for developing coronary artery disease (7,8). Management of hypercholesterolemia with diet and exercise is successful for certain patients, but for a significant number of patients, pharmacological intervention is required to effectively lower cholesterol levels. The treatment of hypercholesterolemia with a specific drug is highly cost-effective when treating patients in secondary prevention (9), while in primary prevention lifestyle change and dietary habits appear to be more cost-effective than any pharmacological treatment (10).

After an intensive investigation, *Monascus purpureus*

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(red yeast) rice was identified as an effective herb to lower serum cholesterol and triglycerides (TG) in animals, and its hypolipidemic effects were confirmed in pilot clinical studies (11-15). However, research on the antioxidant properties of red yeast rice is limited to a few recent *in vitro* studies. This paper describes the radical scavenging and antihypercholesterolemic effects of red rice yeast on reducing oxidative stress in rats.

MATERIALS AND METHODS

Materials

Red yeast rice powder (Tianjin Tasly Import & Export Trad Co., China) containing 0.24% of monacolin K was provided by Ankook Pharm in Korea. Assay kits for total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL), were purchased from Wako Chemical Co. (Osaka, Japan).

Hypocholesterolemic effects

The experimental protocol was reviewed and approved by the Korea University Animal Care Committee. Sprague-Dawley (SD) male rats (6~8 weeks old) were obtained from Daihan-Biolink Co. (Korea) as experimental animals for the antihypercholesterolemic effect.

After adaptation to the cholesterol-free diet, 30 SD rats were divided into 5 groups (6 animals/group) fed either the cholesterol free or cholesterol-enriched diet for 28 consecutive days, as shown in Table 1. Red yeast rice was suspended in distilled water and orally administered to the M-1, M-2 and M-3 groups at doses of 150, 300, and 500 mg/kg of body weight, respectively, once every day in addition to the cholesterol-enriched diets. The normal group was fed a cholesterol free diet and the chol-control group was fed a cholesterol enriched diet, and both were administered distilled water alone instead of the sample suspension. Water was given *ad libitum* during the experimental trials. All rats were housed under temperature (about 21°C) and humidity (about 60%) controlled conventional clean conditions.

Table 1. Composition of the experimental diet (%)

Composition	Cholesterol-free diet	Cholesterol-enriched diet
Casein	25.0	25.0
Soybean oil	10.0	10.0
Lard	7.0	7.0
Cellulose	5.0	5.0
Vitamin mixture[1)]	1.0	1.0
Mineral mixture[2)]	3.5	3.5
Cholesterol	-	0.35
Sucrose	25.0	25.0
Corn starch	23.5	23.15

[1)]Vitamin mixture: ICN Vit. mixture (No. 904654, 1999).
[2)]Composition of mineral mixture was as follows (g/kg): $CaPO_4 \cdot 2H_2O$, 145.6; KH_2PO_4, 257.2; NaH_2PO_4, 93.5; NaCl, 46.6; calcium lactate, 350.9; ferric citrate, 31.8; $MgSO_4$, 71.7; $ZnCO_3$, 1.1; $MnSO_4 \cdot 4H_2O$, 1.2; $CuSO_4 \cdot 5H_2O$, 0.3; KI, 0.1.

Food was withheld for 12 hr before death. Blood was collected from the arota ventralis into tubes containing EDTA and was separated by centrifugation at $1{,}100 \times g$ for 20 min at 4°C. Plasma concentrations of TC, HDL cholesterol and TG were measured using enzymatic kits. LDL cholesterol was calculated according to the Friedewald equation, LDL cholesterol (mg/dL) = TC − (HDL cholesterol + TG/5).

Glutathione and lipid peroxides content in hepatic tissue

The liver was promptly excised after decapitation, weighed and chilled in ice-cold 0.9% NaCl. After washing with 0.9% NaCl, the tissue homogenate was prepared in a ratio of 1 g of wet tissue to 4 mL of 0.25 m sucrose buffer by using a glass homogenizer. The homogenate was centrifuged at $2{,}800 \times g$ for 15 min. The supernatant was collected and assayed for glutathione and lipid peroxide contents by the methods of Ellman (16) and Ohkawa et al. (17), respectively.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging effect

Reactions were performed in 1.25 mL of methanol containing 0.5 mmol/L freshly-made DPPH (Sigma-Aldrich, St. Louis, MO) and 10~50 μL of methanol extract of red yeast rice. Reaction mixtures were incubated at 37°C for 30 min, and the absorbance at 517 nm was measured (18).

Statistical analysis

The data were subjected to analysis of variance and expressed as mean ± SD. The significance of differences was compared using Duncan's multiple range test. Values of $p < 0.05$ were considered to indicate statistical significance. Calculations were made with the SPSS software package.

RESULTS AND DISCUSSION

Food intake, body weight gain, food efficiency and hypocholesterolemic effects

Changes in food intake, body weight gain and food efficiency of the rats fed normal, chol-control, M-1, M-2 and M-3 groups for 4 weeks are presented in Table 2. The Food intake of the normal group was significantly higher than the other group, but there were no significant differences in body weight gain among the groups. Therefore, the FER of the normal group was slightly

Table 2. Effect of orally administrated red yeast rice on feed intake, body weight gain and feed efficiency ratio of rats fed cholesterol-free and cholesterol-enriched diets

Group	Body weight gain (g/day)	Food intake (g/day)	FER[1)]
Normal	4.23±0.11[2)NS3)]	63.49±4.23[b4)]	0.067±0.002[NS]
Chol-control	4.16±0.38	38.72±4.16[a]	0.107±0.010
M-1	3.69±0.35	38.61±3.69[a]	0.096±0.009
M-2	3.81±0.52	37.48±3.81[a]	0.102±0.014
M-3	3.76±0.64	38.08±3.76[a]	0.099±0.017

[1)]FER: feed efficiency ratio=body weight (g/day)/feed intake (g/day).
[2)]Values are mean±SD for 6 rats.
[3)]Not significant.
[4)]Values with the same superscripts in the column are not significantly different at $p<0.05$.

lower than for the other groups, but there were no significant differences among the groups.

Changes in the liver, and other internal organ weights of the rats after 28-days administration with red yeast rice are presented in Table 3. Liver weights of the normal group were significantly lower than those of the others ($p<0.05$). Spleen weight of M-3 group was significantly lower than that of the M-1 group ($p<0.05$). Perirenal fat pad weights were lower with increased doses of red yeast rice, and M-2 and M-3 (oral administration of 300 and 500 mg/kg, respectively) were significantly lower than those of chol-normal and M-1 groups ($p<0.05$).

The changes in plasma lipid concentrations are summarized in Table 4. There were no significantly differences in TG concentration among the groups. However, TC concentrations of the red rice fed groups (M-1, M-2, M-3 groups) were significantly lower than the chol-control group. Decreases in cholesterol concentrations consequently decreased the atherogenic values. The results presented here show that red yeast rice decreased the high plasma cholesterol concentrations in rats fed a high cholesterol diet, with the greatest effect realized in the group fed the highest dose of red yeast rice (M-3). Consequently, red yeast rice affected the plasma lipid metabolism in rats, and this effect appeared to decrease cholesterol concentrations. There were no significant differences in HDL-cholesterol concentration between the chol-control and the red rice fed groups (M-1, M-2 and M-3), but LDL-cholesterol of the red yeast rice fed groups was significantly lower than the chol-control group.

The atherogenic value for rats fed red yeast rice with the cholesterol-enriched diet was significantly decreased in this experiment. The atherogenic value is an indicator of cardiovascular disease risk. Several studies have demonstrated the protective effect of HDL in atherosclerosis and cardiovascular disease, while high concentrations of LDL constitute a risk factor. The addition of red yeast rice to cholesterol-added diets tended to decrease atherogenic values (Table 4), which may be beneficial in the

Table 3. Effect of orally administrated red yeast rice on the relative organ weights of rats fed cholesterol-free and cholesterol-enriched diets (g/100 g of body weight)

Group	Liver	Kidney	Spleen	Perirenal fat pad
Normal	3.24±0.24[1)a2)]	0.87±0.13[NS3)]	0.21±0.02[ab]	0.63±0.08[a]
Chol-control	3.90±0.40[b]	0.84±0.12	0.22±0.04[ab]	0.89±0.05[b]
M-1	3.98±0.12[b]	0.85±0.07	0.24±0.02[b]	0.82±0.03[b]
M-2	4.20±0.43[b]	0.84±0.08	0.22±0.02[ab]	0.73±0.05[a]
M-3	4.20±0.19[b]	0.81±0.13	0.20±0.01[a]	0.70±0.04[a]

[1)]Values are mean±SD for 6 rats.
[2)]Values with the same superscripts in the column are not significantly different at $p<0.05$.
[3)]Not significant.

Table 4. Effect of orally administrated red yeast rice on plasma lipids of rats fed cholesterol-free and cholesterol-enriched diets

Group	TG[1)] (mg/dL)	TC[2)] (mg/dL)	LDL[3)] (mg/dL)	HDL[4)] (mg/dL)	AI[5)]
Normal	58.4±16.9[6)NS7)]	62.2±5.2[a8)]	24.9±11.7[a]	30.4±3.4[b]	1.1±0.3[a]
Chol-control	47.2±8.5	275.2±19.5[e]	220.6±15.3[d]	17.8±4.8[a]	15.4±4.3[c]
M-1	58.8±13.1	226.3±13.2[d]	187.4±14.5[c]	24.5±11.9[ab]	9.6±3.6[b]
M-2	68.1±26.2	210.6±4.2[c]	181.4±20.0[c]	23.0±6.9[ab]	8.9±3.0[b]
M-3	58.0±24.0	194.4±15.4[b]	121.6±30.2[b]	22.4±5.0[ab]	7.7±1.4[b]

[1)]Triacylglycerols. [2)]Total cholesterol. [3)]Low-density lipoprotein. [4)]High-density lipoprotein. [5)]Athrogenic index=(TC−HDL)/HDL.
[6)]Values are mean±SD for 6 rats.
[7)]Not significant.
[8)]Values with the same superscripts in the column are not significantly different at $p<0.05$.

prevention and treatment of cardiovascular disease. This could be due to a reduction in the activity of the liver enzyme HMG-CoA reductase.

Pharmaceutical agents which lower cholesterol by interfering with cholesterol synthesis have been studied for several decades and the most successful agents are compounds which inhibit HMG-CoA reductase, one of the key steps in cholesterol synthesis. There are several other monacolin derivatives (now generally referred to as "statins") which are used to treat hypercholesterolemia. Statins have profound positive effects on the outcome of cholesterol lowering trials (19). It has been suggested that the statin family of drugs exert beneficial health effects beyond their effects on hyperlipidemia (20-22). In addition to the previous report (23), serum total cholesterol was reduced by 25% and 43% ($p<0.05$) on the lower and higher doses of Cholestin (*Monascus purpureus* rice; red yeast rice) and 47% ($p<0.05$) when treated with lovastatin.

We believe that red yeast rice is not merely an impure form of statin drug and that chemical components other than monacolins may be responsible for this observation.

DPPH radical scavenging effect and glutathione and lipid peroxide content in hepatic tissue

It is well known that antioxidants can seize the free-radical chain of oxidation and form stable free radicals, which do not initiate or propagate further oxidation. DPPH has been used extensively as a free radical to evaluate reducing substances. The scavenging ability of the methanol extract from red yeast rice was increased with higher dosages of the extract of the red yeast rice (Fig. 1). IC_{50} values of red yeast rice was 350.9 μg/mL (data was not shown).

At 750 μg/mL, scavenging ability on DPPH radicals was 78.2%. However, Chang reported that the scavenging abilities were 6.2%~20.9% for methanolic extracts from various red yeast rice at 1 mg/mL (24). Similarly, at 1~10 mg/mL, the scavenging abilities increased rapidly, up to the range of 81.1~94.1%, for four methanolic extracts (24).

Naturally occurring antioxidant components, including ascorbic acid, tocopherols, and total phenols, have been found in methanolic extracts from various rice products (25). β-Carotene was not found whereas the contents of ascorbic acid and tocopherols were in the range of 0.05~0.25 mg/g. However, total phenols were the major naturally occurring antioxidant components and methanolic extracts from red yeast rice products contained more total phenols than those from uninoculated rice products. The Folin-Ciocalteau method does not exclusively react with phenolic substances, but any reductive substance present, some unidentified substances other than ascorbic acid and tocopherols contribute to the high total phenols determined in the extracts. Phenols such as BHT and gallate are known to be effective antioxidants (26). Yen et al. (27) found that the antioxidant activity of the methanolic extract from peanut hulls correlated with its content of total phenols. Therefore, the high content of total phenols in all methanolic extracts might explain the high antioxidant properties in various rice products.

Glutathione and lipid peroxide contents of hepatic tissue are shown in Fig. 2. Glutathione concentrations were highest in the M-3 group among the cholesterol-enriched dietary groups, and significantly higher than the chol-control group. The M-3 group showed a similar glutathione content as that of the normal group.

Glutathione (l-gammaglutamyll-cysteinylglycine) is a tripeptide composed of the amino acids, cysteine, glycine and glutamic acid. Glutathione is the major endogenous antioxidant produced by the cell. Glutathione participates directly in the neutralization of free radicals, reactive oxygen compounds and maintains exogenous antioxidants

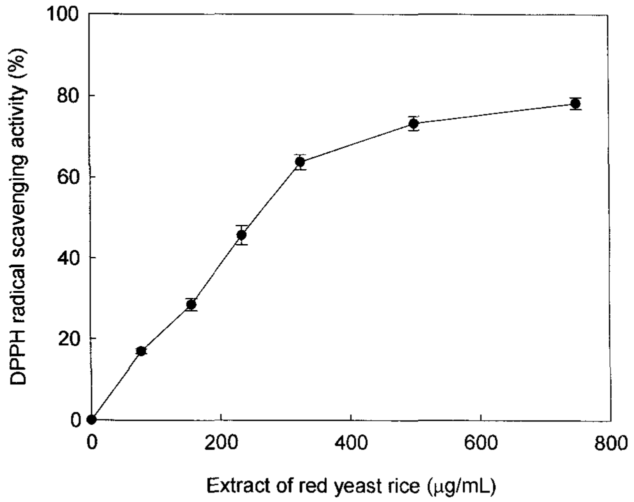

Fig. 1. DPPH free radical-scavenging effects of the extracts of red yeast rice. Each value represents means±SD (n=3).

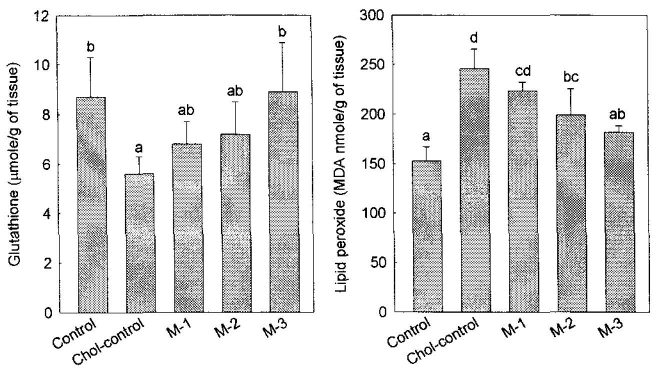

Fig. 2. Hepatic glutathione and lipid peroxides contents in rats fed cholesterol-free and cholesterol-enriched diets.

such as vitamins C and E in their reduced (active) forms. In addition, through direct conjugation, glutathione plays a role in the detoxification of many xenobiotics (foreign compounds) both organic and inorganic (28).

Lipid peroxides are the products of the chemical damage done by oxygen free radicals to the lipid components of cell membranes. This oxidative damage, caused by free radical pathology, is thought to be a basic mechanism underlying many diverse pathological conditions-arteriosclerosis, cancer, aging, rheumatic diseases, allergic inflammation, cardiac and cerebral ischaemia, respiratory distress syndrome, various liver disorders, irradiation and thermal injury, and toxicity induced by certain metals, solvents, pesticides and drugs. Therefore, the lipid peroxide level provides the potential for oxygen free radical pathology, the risk for degenerative processes, and the need for compensatory antioxidant supplementation. High lipid peroxide levels indicate excessive oxygen free radical lipid peroxidation (29). In the chol-control group, the lipid peroxide level was 243.8 nmol/g, but the normal and M-3 groups had lower lipid peroxide levels (151.2 and 188.3 nmol/g, respectively). The low lipid peroxide level of the M-3 group caused a suppression of lipid oxidation. The red yeast rice might have suppressed the lipid peroxidation because of the high glutathione content which resulted in a low lipid peroxide content in the group administered 500 mg/kg.

Because BHA and vitamins C and/or E are good inhibitors of peroxidation, have substantial reducing power, and effectively scavenge DPPH radicals; and EDTA is excellent for chelating ferrous ions, they are additives and used in mg levels in foods. Red yeast rice products could be used in g or hundreds of g levels as food or a food ingredient. Therefore, red yeast rice products in human diets might serve as possible protective agents to help humans reduce oxidative damage.

In summary, the data presented in this paper show that administration of red yeast rice, is a safe and effective way of lowering serum total and LDL cholesterol, the ratio of non-HDL/HDL, and severity of experimental atherosclerosis.

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