J Food Sci Nutr
Vol 13, p 276~280 (2008)
DOI: 10.3746/jfn.2008.13.4.276

J Food Science and Nutrition

Lipid Lowering Effect of Anthocyanin-Pigmented Rice Bran in Streptozotocin-Induced Diabetic Male Rats

Hae Kyoung Han[1], Sung Sook Choi[1], Jin Chul Shin[2], and Ha Sook Chung[1†]

[1]*Department of Foods and Nutrition, DukSung Women's University, Seoul 132-714, Korea*
[2]*National Crop Experiment Station, Rural Development Administration, Suwon 441-701, Korea*

Abstract

***Oryza sativa* cv. *Heugjinjubyeo*, an anthocyanin-pigmented rice variety, is well known to contain high levels of bioactive phytochemicals, anthocyanin, quinolone alkaloids and phenolic acids. Here, we studied the inhibitory effect of *Oryza sativa* cv. *Heugjinjubyeo* bran on the absorption of dietary fat in streptozotocin-induced diabetic Sprague-Dawley male rats. For these experiments, experimental animals were divided into four groups: normal, diabetic-control and two experimental groups that were fed 1.0 g or 2.0 g/kg body weight/day of *Oryza sativa* cv. *Heugjinjubyeo* bran supplement for 14 days. As a result, liver glycogen levels increased significantly by 65% and 32% in groups receiving 1.0 g and 2.0 g/kg body weight/day, respectively, compared to diabetic-control. Liver cholesterol levels were significantly lower by 8.3% and 14.5% in the groups fed 1.0 g and 2.0 g of anthocyanin-pigmented rice extracts, respectively.**

Key words: *Oryza sativa* cv. *Heugjinjubyeo*, anthocyanin-pigmented rice, diabetic rats, glycogen, cholesterol, malondialdehyde, hypolipidemic effects

INTRODUCTION

Anthocyanin-pigmented rice (APR; *Oryza sativa* cv. *Heugjinjubyeo*, Gramineae), with its dark purple color, was produced by genetic engineering in the early 1990s in Korea. It has an enriched taste, color and nutritional content. We previously reported the hypoglycemic and hypolipidemic effects of APR in diabetic rats (1). Also, alkaloids and phenolic acids with moderate antioxidant activity, and the anthocyanidins, cyanidin and malvidin, with cytotoxicity against human monocytic leukemia cells were identified in the aleurone layer of APR (2-4).

Diabetes is the most common metabolic disorder, and its prevalence is increasing in developing countries. It gives rise to severe complications, which can cause organ damage, and increases oxygen free radicals by altering anti-oxidative defenses (5,6). Synthetic hypoglycemic agents for diabetes treatment may cause potent side effects including hematological coma and liver and kidney disturbances (7). However, natural plant-derived biocomponents are considered less toxic with fewer side effects (8). For this reason, interest in hypoglycemic agents from natural sources has been increasing.

As a part of our ongoing studies of the biological activities of highly developed rice grains, we investigated the glycogen and lipid-metabolizing effects of *Oryza sativa* cv. *Heugjinjubyeo* in streptozotocin-induced diabetic male rats.

This paper describes the hypolipidemic effects of the ethanolic extract of *Oryza sativa* cv. *Heugjinjubyeo* bran, which increased glycogen levels and reduced liver cholesterol levels in diabetic male rats.

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were purchased from commercial sources and were of the highest purity available.

Sample material

Fully ground bran of anthocyanin-pigmented rice (APR) was supplied by the National Crop Experiment Station, Rural Development Administration (RDA), Suwon, Korea, in October 2006. A voucher specimen (OS-Suwon #415) has been deposited at the RDA.

Preparation of APR extracts

Dried APR bran powder (5 kg) was extracted with 15 L of ethanol for three hrs, five times at room temperature. The supernatants were combined and concentrated under reduced pressure in a rotary evaporator to yield a dark purple ethanolic extract (72.4 g).

†Corresponding author. E-mail: hasook@duksung.ac.kr
Phone: +82-2-901-8593, Fax: +82-2-901-8442

Animals and experimental design

Male Sprague-Dawley rats (220 ~ 230 g) were bred in the Samtaco Animal Research Center, Osan, Korea, and fed a standard pellet laboratory diet and distilled water *ad libitum*. The experimental feeding period was 14 days. The rats were randomized to four groups of 7 male rats each. The dosage of APR ethanolic extract used was based on our earlier study (1). The groups were: a) normal control; b) diabetic control; c) diabetic plus 1.0 g; and d) diabetic plus 2.0 g/kg body weight/day of ethanolic APR extract.

Induction of diabetes

Animals were fasted for 16 hr prior to diabetes induction. Diabetes was induced by single injection of STZ (45 mg/kg body weight in 10 mM citrate buffer, pH 4.5) into the tail vein; normal rats were injected with an equal volume of citrate buffer. After one day, rats with orbital vein blood glucose levels greater than 300 mg/dL were considered diabetic. APR extracts were dissolved in 5% Tween 80, and STZ-diabetic rats ingested the extracts by gavage for 14 days; the normal group received citrate buffer for 14 days. After 14 days, rats were anesthetized with diethyl ether and killed by decapitation. The liver, spleen, pancreas, heart, lung and muscle were quickly excised and weighed and stored at -70^{o}C for ongoing biochemical assays.

Biochemical assays of organs

Tissue samples were digested with hot, concentrated KOH and treated with 2% anthrone reagent. Glycogen content in liver and muscle was determined by a colorimetric method (9). Liver cholesterol and triglyceride levels were determined by an enzymatic method (10) and the Trinder method (11), respectively. Lipid peroxide levels were estimated by the thiobarbituric acid method and expressed as malondialdehyde levels (12). Protein contents were analyzed by the Lowry-Folin test using bovine serum albumin as a standard, and the optical density was measured at 750 nm (13).

Statistical analysis

All data are expressed as mean ± standard deviation (SD) and analyzed statistically by analysis of variance. When F values were considered statistically significant, the L.S.D. procedure was used to determine significant differences between treatment means at $p < 0.05$.

RESULTS AND DISCUSSION

Organ weights

Lung and kidney weights of STZ-diabetic control rats were significantly higher than those of normal control rats; however, these weights were decreased by APR administration. The weights of heart and spleen were not significantly different between normal and STZ-diabetic control rats (Table 1). Previous reports have suggested that STZ may inhibit renal hypertrophy (14,15). It was also reported the kidney size, with elevated kidney-body weight ratio, is remarkably increased in STZ-injected rats compared control group (16). The increase in the kidney weights in diabetic rats could be caused by adaptive changes in response to augmented blood flow, by electrolyte loss caused by osmotic diuresis, or by direct hyperglycemia (17). In the present study, APR administration exerted an anti-hyperglycemic effect (Fig. 1) and prevented renal enlargement, the plasma glucose levels remained well above 300 mg/dL. Thus, it is likely that the mechanism or mechanisms independent of anti-hyperglycemic properties played a role in preventing renal enlargement (18).

Glycogen levels of liver and muscle

Liver glycogen levels increased significantly with administration of 1.0 g of APR extract (Fig. 2). Muscle glycogen levels also increased in APR group, but these increases were not significant between experimental groups. Diabetes mellitus is known to impair the normal capacity of the liver to synthesize glycogen (19-21). Synthase phosphatase activates glycogen synthase resulting in glycogenesis, and this activation appears to be

Table 1. Organ weights in normal and STZ-diabetic rats fed APR ethanolic extracts[1)]

Organs	Normal	STZ-Control	APR extracts	
			1.0 g/kg/day	2.0 g/kg/day
Liver	3.91 ± 0.13[NS2)]	3.98 ± 0.38	4.10 ± 0.19	4.10 ± 0.24
Lung	0.43 ± 0.04[b3)]	0.54 ± 0.07[a]	0.49 ± 0.03[a]	0.50 ± 0.03[a]
Kidney	0.34 ± 0.03[b]	0.52 ± 0.04[a]	0.48 ± 0.07[a]	0.47 ± 0.04[a]
Heart	0.33 ± 0.02[NS]	0.34 ± 0.02	0.34 ± 0.03	0.32 ± 0.02
Spleen	0.28 ± 0.05[NS]	0.24 ± 0.03	0.26 ± 0.04	0.24 ± 0.02
Pancreas	0.31 ± 0.08[a]	0.20 ± 0.09[b]	0.20 ± 0.02[b]	0.18 ± 0.02[b]

[1)]Values are mean ± SD, n=7.
[2)]NS: not significant at $p < 0.05$.
[3)]Values with different superscripts within a row are significantly different at $p < 0.05$.

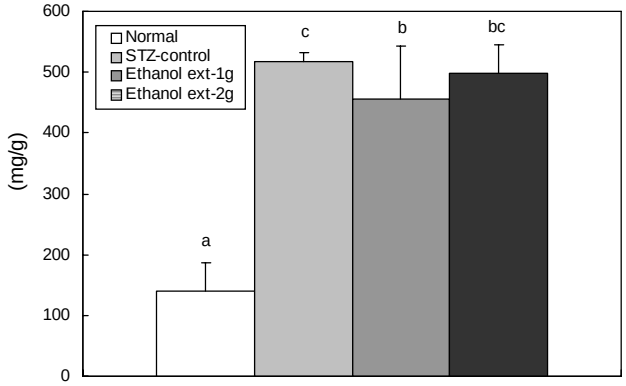

Fig. 1. Effect of APR ethanolic extracts on blood glucose levels in normal and STZ-diabetic rats. Data are mean±SD. Different superscripts indicate a significant difference ($p<0.05$).

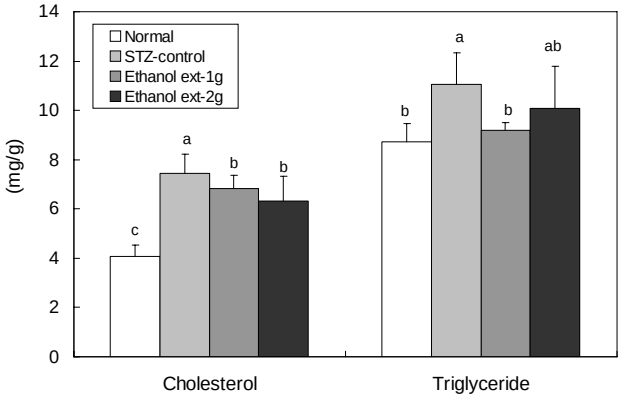

Fig. 3. Effect of APR ethanolic extracts on liver cholesterol and triglyceride levels in normal and STZ-diabetic rats. Data are mean±SD. Different superscripts within groups indicate a significant difference ($p<0.05$).

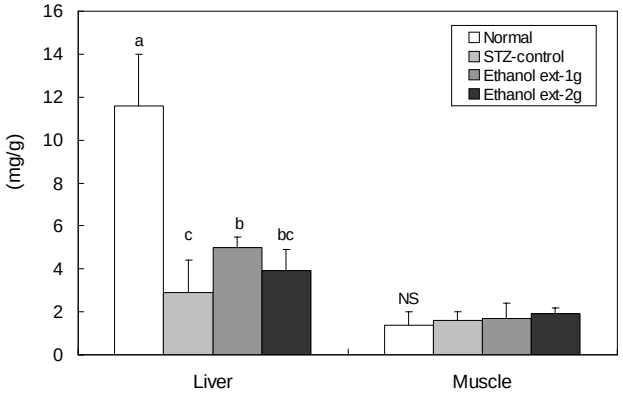

Fig. 2. Effect of APR ethanolic extracts on liver and muscle glycogen levels in normal and STZ-diabetic rats. Data are mean±SD. Different superscripts within groups indicate a significant difference ($p<0.05$). NS: not significant.

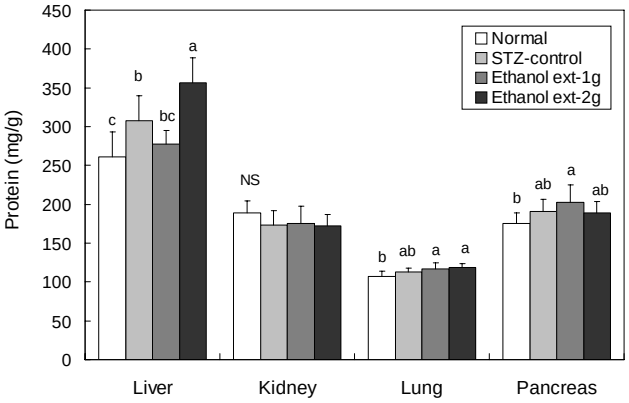

Fig. 4. Effect of APR ethanolic extracts on malondialdehyde levels in liver, kidney, lung, and pancreas in normal and STZ-diabetic rats. Data are mean±SD. Different superscripts within groups indicate a significant difference ($p<0.05$). NS: not significant.

defective in STZ-induced diabetic animals (22-24). Ethanol extracts of *Catharanthus roseus* may promote glycogen synthase activity, which would explain the results (25).

Cholesterol and triglyceride levels of liver

Liver cholesterol and triglyceride levels (TG) in STZ-diabetic rats were significantly suppressed by APR administration (Fig. 3). This result can be explained as the increase of hydroxymethyl glutaryl-CoA in liver and intestine (26). Hyperlipidemia, a complication caused by diabetes mellitus, is characterized by elevated cholesterol and TG levels (27-29). It has been already reported that components from natural food sources can be associated with lipid lowering properties in several experiments (30-33).

Malondialdehyde levels of liver, kidney, lung and pancreas

The elevated malondialdehyde (MDA) concentrations in the lungs of STZ-diabetic rats decreased significantly with dietary supplementation of APR extract (Fig. 4). Liver MDA levels were higher in STZ-diabetic rats, in agreement with an earlier study (34), and were not suppressed by APR. The decreased MDA concentrations in the kidney by 2.0 g of APR and in the lung by 1.0 g and 2.0 g of APR may be caused by antioxidant phytochemicals (2) in the extract that can lower oxidative stress in diabetic organs. The elevated levels of blood glucose in diabetes produce oxygen-free radicals, which cause membrane damage due to peroxidation of membrane lipids and protein glycation (35). The increased concentration of MDA suggests an increase in oxygen free radicals that could be because of either their increased production or decreased destruction (36). Therefore, the increase in oxidative stress was thought to be prevented by the supplementation of APR, and the beneficial effect of APR on diabetic renal damage might

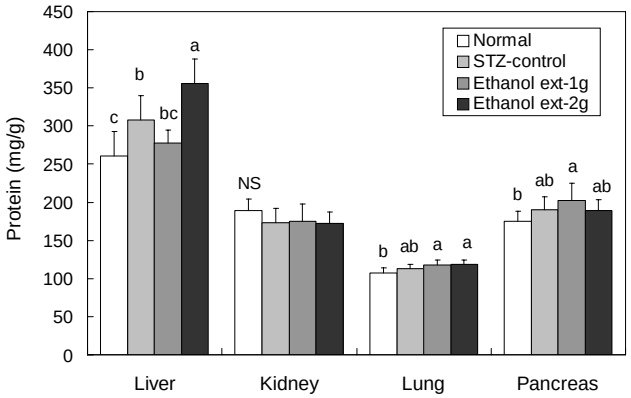

Fig. 5. Effect of APR ethanolic extracts on protein levels in liver, kidney, lung, and pancreas in normal and STZ-diabetic rats. Data are mean±SD. Different superscripts within groups indicate a significant difference ($p<0.05$). NS: not significant.

be related to this inhibitory effect on oxidative stress rather than on advance glycation end product.

Protein levels in liver, kidney, lung, and pancreas

Protein levels in liver, lung, and pancreas were elevated in STZ-diabetic rats (Fig. 5). As shown in Figure, protein concentrations were significantly different in the liver of rats receiving 2.0 g of APR and in the lung of those receiving 1.0 g and 2.0 g of APR extracts; there were no significant differences in either kidney or pancreas. Diabetes mellitus is characterized by a derangement in metabolism not only of glucose and fat but also of protein (37). However, protein has always received less attention that fat and glucose, both for alterations in its metabolism and in its nutritional implications.

CONCLUSION

We investigated the hypolipidemic effects of anthocyanin-pigmented rice bran in STZ-induced diabetic rats using four experimental groups: normal, STZ-control, 1.0 g and 2.0 g/kg body weight/day of APR ethanolic extract supplement. The liver glycogen levels were significantly higher in rats receiving APR extract. Also, liver cholesterol and TG concentrations were significantly lower in APR bran supplemented experimental groups. These results suggest that the lipid lowering effect is associated with decreased cholesterol and triglyceride absorption. Eide et al previously reported that oral administration of garlic extract for 14 days significantly decreased serum glucose, triglycerides, urea, uric acid, creatinine, AST and ALT levels (38). In conclusion, the present study indicates that lipid metabolism in STZ-diabetic male rats can be improved by APR bran supplementation, which may also be beneficial for prevention of diabetic complications caused by lipid peroxidation.

ACKNOWLEDGMENTS

This study was supported by Specific Research-promoting Join Projects, National Crop Experiment Station, Rural Development Administration, Korea.

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(Received September 1, 2008; Accepted September 29, 2008)