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Effect of the Ethanol Extract from the Aleurone Layer of Anthocyanin-Pigmented Rice on Blood Glucose and Lipid Metabolism in Streptozotocin Induced Diabetic Rats

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Abstract

The present study was designed to investigate the hypoglycemic activity and lipid metabolism of ethanol (EtOH) extract from the aleurone layer of anthocyanin-pigmented (AP) rice in streptozotocin (STZ)-induced diabetic rats. Sprague-Dawley male rats weighing 210~240 g were divided into 4 groups, normal, diabetic control, and two experimental groups, and diabetes in rats was induced by injection of STZ (45 mg/kg, body weight) into tail vein. The EtOH extract of the powdered aleurone layer of AP rice was administered orally in diabetic rats for 14 days. In order to find the hypoglycemic effects in the animal model, the body weight, plasma glucose levels, cholesterol, HDL-cholesterol, triglyceride (TG), free fatty acid (FFA), aspartate aminotransferase (AST) and alanine aminotransferase activities (ALT) were determined. Oral administration of 1.0 g/kg on the EtOH extract for 14 days resulted in a significant reduction in blood glucose, ALT, TG and FFA. However, in the case of 2.0 g/kg, the hypoglycemic effects were not considerable. This results suggest that the EtOH extract might induce hypoglycemic effects in STZ-induced diabetic rats due to some phytochemical components in the aleurone layer of AP rice.

Key words: anthocyanin-pigmented rice, hypoglycemic effects, lipid metabolism, diabetic rats

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food in many Asian countries. Varieties of rice include long-grain white, long-grain brown, glutinous brown, wild, basmati, brown basmati, jasmine and rosotto. Cultivation of pigmented rice through genetic engineering in the early 1970's began a surge in world production of various kinds of rice grains (1). Extraction and industrial use of new bioactive compounds from crops have been accomplished for the added-value of agricultural products. The anthocyanin-pigmented (AP) rice (*Oryza sativa* cv. *Heugjinmi*) having dark purple-colored grains, is broadly known as an enriched rice for taste, color and nutrient improvement.

Diabetes mellitus is a major disease affecting nearly 10% of the population. In spite of the introduction of hypoglycemic agents, diabetes and related complications continue to be a critical medical problem. Clinically, it is a heterogeneous disease, characterized by a low glycemic control, due to impairment of the balance between β-cell secretion of insulin, peripheral insulin action and hepatic glucose production. However, the mechanism which leads to increased plasma glucose level has not been confirmed so far (2). One of the well-known symptoms of diabetes is hyperglycemia, which is associated with an increased incidence of micro- and macro-vascular complications in diabetes (3). Though synthetic drugs can inhibit and reduce the symptoms, synthetic hypoglycemic agents can give rise to serious side effects including hematoma, coma and disturbances of liver and kidney function (4). Therefore, the development of hypoglycemic agents from natural resources has been needed. The plant-derived drugs are frequently considered to be less toxic and more free from side effects than synthetics (5). For these reasons, the potent hypoglycemic effects of some natural plants has been reported (6-8). In addition, many traditional plants and herbs were used throughout the world for the care of diabetes (9-17).

In the present study, the improving effects of EtOH extract from the aleurone layer of AP rice in STZ-induced oxidative damaged and diabetic rats was investigated.

MATERIALS AND METHODS

Plant material

The fully ground aleurone layer of AP rice was supplied by National Crop Experiment Station, Rural Development Administration (RDA), Suwon, Gyeonggi-do, Korea, in February 1999. A voucher specimen has been deposited at the RDA. The ground sample was kept in airtight and deep freeze containers until the time of use.

Extraction of sample

The fully ground aleurone layer of AP rice (5.0 kg) was extracted with 15 L of EtOH in the dark, at room temper-

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ature five times overnight. The supernatant was collected and evaporated to dryness at 40°C under pressure in a rotary evaporator to yield the EtOH extract (35.6 g).

Animals and experimental induction for diabetes

Male Sprague-Dawley rats were used for this study. Rats were fed a standard pellet laboratory diet and provided with water ad libitum. They were fasted for 16 hr before STZ injection but were allowed free access to water. Diabetes was induced in experimental diabetic rats by a single STZ (Sigma Chemical Co., 45 mg/kg, in 10 mM sodium citrate buffer, pH 4.5) into the tail vein. The rats with blood glucose levels greater than 300 mg/dL were considered diabetes. Normal rats received citrate buffer alone. The rats were divided into four groups; group 1 (5% tween 80) was served as normal; group 2, which served as a STZ-induced diabetic group, received 5% tween 80 instead of EtOH extract; The rats of groups 3 and 4 received two different concentrations of the EtOH extract of the aleurone layer of AP rice, 1.0 g and 2.0 g/kg, b.w. for 14 days, respectively.

Biochemical analysis

During the experimental period, a small volume of blood was withdrawn periodically from orbital veins for monitoring plasma glucose and cholesterol levels. All rats were decapitated after two weeks, and blood was subsequently collected in heparinized tubes, and separated immediately for plasma processing for further experiments. The heparinized blood was centrifuged to obtain plasma at 3,000 rpm at 4°C for 15 min.

Rat body weights were recorded daily and hematocrit levels measured by the microhematocrit method (18,19). AST and ALT activities were analysed by the Reitman-Frankel method (20), and plasma glucose levels were determined by the glucose oxidase-peroxidase method (21) using a glucose kit (Yeongdong Pharm. Co., Seoul). Plasma cholesterol level was measured using an enzymatic method (22) using a cholesterol kit (Yeongdong Pharm. Co., Seoul). TG and FFA levels were measured using the methods of Giegel et al., (23) and Trimble (24), respectively.

Statistical analysis

The results shown were expressed in mean±S.D. Statistical analysis was done using analysis of variance (ANOVA). As F values obtained were significant values statistically, the L.S.D. procedure was used to determine the significant differences at $p<0.05$ between treatment means (25).

RESULTS AND DISCUSSION

The EtOH extract from the aleurone layer of AP rice was found to exhibit reducing glucose levels on plasma in STZ-induced diabetic rats. STZ, a 2-desoxy-D-glucose derivative of N-methyl-N-nitroso-urea (26), is a diabetogenic compound and is known to specifically destroy β-cells in the pancreas (27-29). STZ-induced hyperglycemia has been described as a useful experimental model to study the activity of anti-diabetic agents with or without insulin (28,30). Our results showed that intravenous administration of STZ (45 mg/kg) induced effectively diabetes in normal fasted rats, as reflected by glycosuria, high glycemia, poly-phagia, polydypsia and body weight loss when compared to the normal group. Table 1 demonstrates the changes in body weight between normal and experimental rats. As expected, STZ-induced diabetic rats showed considerable weight loss compared to the normal group, and no significant differences among the diabetic rats. However, the animal groups treated with EtOH extract from the aleurone layer of AP rice, showed greater increases in body weight than the STZ-control group. It has already been reported that mean body weight in diabetic animals was lower than the non-diabetics by Van Dam et al. (31).

Table 2 also shows the hematocrit levels of normal and experimental rat groups. There were no significant differences in normal, STZ-control and EtOH groups. However, the activities of AST and ALT in plasma in the STZ-control group were elevated in comparison to the normal group. The significant difference was found between the STZ-control group and the EtOH extract 1.0 g group (Table 3).

Table 1. Changes of body weights of normal and diabetic rats fed on EtOH extract from aleurone layer of AP rice

Experimental groups[1)]	Initial BW[2)] (g)	Final BW[3)] (g)	Weight gain[3)] (g/14 day)
Normal (n = 6)	227.4±9.8	267.7±16.3[a]	40.3± 7.4[a]
STZ-control (n = 8)	224.9±6.5	218.3±12.3[b]	-6.6±10.0[b]
EtOH extract 1.0 g (n = 7)	228.5±7.8	230.1±27.2[b]	1.6±20.0[b]
EtOH extract 2.0 g (n = 7)	228.0±7.1	233.4±22.6[b]	5.4±16.8[b]

[1)]Values are mean±SD.
[2)]Not significant at 5% level.
[3)]Values with different superscript within column differ at 5% level.

Table 2. Effect of EtOH extract from aleurone layer of AP rice on hematocrit levels in normal and diabetic rats

Experimental groups[1)]	Hematocrit levels (%)[2)]
Normal (n = 6)	41.8±6.4
STZ-control (n = 8)	44.7±2.7
EtOH extract 1.0 g (n = 7)	45.0±1.4
EtOH extract 2.0 g (n = 7)	45.3±1.4

[1)]Values are mean±SD.
[2)]Not significant at 5% level.

Table 3. Effect of EtOH extract from aleurone layer of AP rice on the plasma AST and ALT activities in normal and diabetic rats

Experimental groups[1)]	AST[2)] (KA unit/L)	ALT[2)] (KA unit/L)
Normal (n = 6)	162.8±12.9[a]	35.3± 3.5[a]
STZ-control (n = 8)	215.5±24.3[b]	61.5± 9.6[b]
EtOH extract 1.0 g (n = 7)	193.2±23.1[ab]	51.6±10.3[c]
EtOH extract 2.0 g (n = 7)	300.1±73.7[c]	49.5± 5.8[c]

[1)]Values are mean±SD.
[2)]Values with different superscript within the column are significantly different at 5% level.

Fig. 1 shows the blood glucose levels in normal and STZ-control rats. The blood glucose level was significantly higher by as much as four times in diabetic rats compared to normal rats. In case of the administration of EtOH extract (1.0 g/kg) from the aleurone layer of AP rice, the blood glucose level was significantly lowered compared with STZ-control group. However, at higher doses (2.0 g/kg), the significant effect was not found. It is assumed that the hypoglycemic effects of EtOH extract might not be a dose-dependent mechanism. In general, the possible mechanism that brings on hypoglycemic activity may be the increasing insulin secretion from β-cell of islets in Langerhans by its release from the bound form (32).

Fig. 2 shows the effect of EtOH extract on plasma cholesterol in normal and diabetic rats. The concentration of plasma cholesterol was significantly higher in STZ-diabetic than normal rats. This is the result of the decreased hydroxymethyl glutaryl-CoA in the liver and increased hydroxymethyl glutaryl-CoA in the intestine (33). In this study, cholesterol values were decreased in the EtOH extract of 1.0 g/kg compared with the STZ-control group.

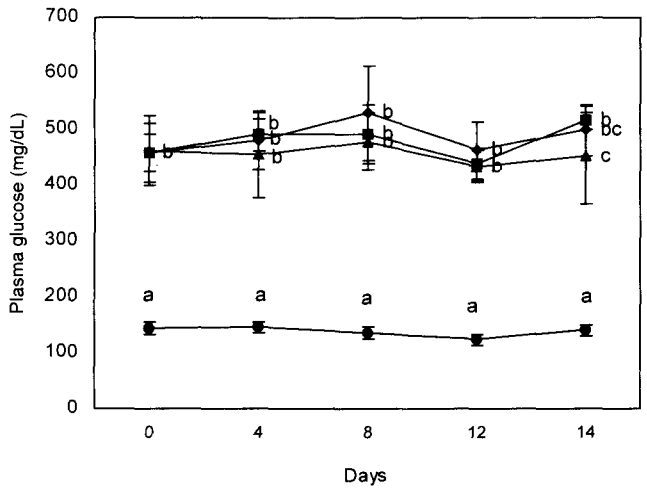

Fig. 1. Plasma glucose levels in normal and diabetic rats fed on EtOH extract of aleurone layer of AP rice (-●- : Normal; -■- : STZ-control; -▲- : EtO Hextract 1.0 g; -◆- : EtOH extract 2.0 g).

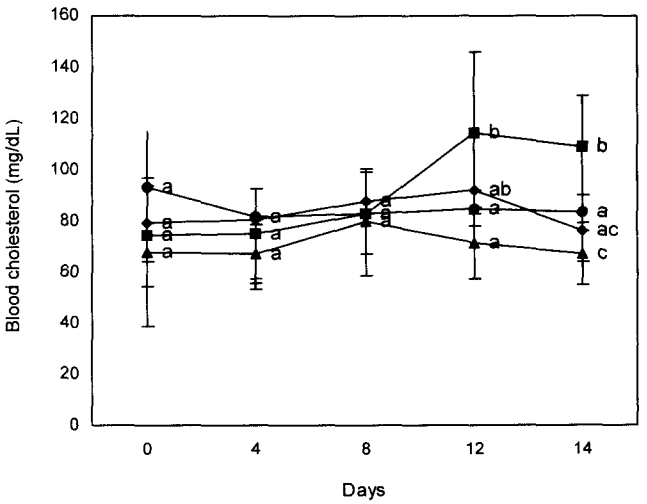

Fig. 2. Plasma cholesterol levels in normal and diabetic rats fed on EtOH extract of aleurone layer of AP rice (-●- : Normal; -■- : STZ-control; -▲- : EtOH extract 1.0 g; -◆- : EtOH extract 2.0 g).

Table 4 gives the concentrations of HDL-cholesterol, TG and FFA in plasma. They noticed a slight increase of HDL-cholesterol in STZ-diabetic rats compared with the normal group. The concentration of plasma TG was elevated as much as five times in diabetic compared to normal rats. The EtOH extract, 1.0 g/kg from the aleurone layer of AP rice reduced plasma TG levels significantly, to give the values to near normal. In the EtOH extract at a dose of 2.0 g/kg, any TG lowering effects as well as reduced blood glucose levels were not evident. There was also a significant elevation in plasma FFA during diabetes compared with normal rats. Oral administration of EtOH extract, 1.0 g/kg, decreased the FFA level significantly. In this study, we have observed higher levels of both cholesterol and FFA in the plasma of the STZ-control group. Accordingly, it has been deduced that there were higher lipid levels in plasma in the STZ-control group. The level of plasma lipids is usually raised in diabetes and coronary heart disease (34). On the contrary, the lowering of lipid levels in plasma through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease (35). The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of FFAs from the peripheral depots, since insulin inhibits the hormone sensitive lipase (34). As a result, it shows that EtOH extract administration suppresses the degree of tissue damage occurring in STZ-induced diabetic rats.

Thus the study shows that EtOH extract of the aleurone layer of AP might have a hypoglycemic effect. And the levels of TG and FFA decreased in the EtOH extract group. The activity was more pronounced in the administration of 1.0 g/kg. For all the parameters studied, in conclusion, EtOH extract from the aleurone layer of AP rice at a dose of 1.0 g/kg can be indicated to regressed therapeutic effect. Further studies are needed to identify the active nutraceuticals for the hypoglycemic effect and evaluate its mechanism. Ultimately, information pertaining to these active phytochemicals from the AP rice may be useful in terms of diet selection

Table 4. Effect of EtOH extract from aleurone layer of AP rice on HDL-cholesterol, triglyceride and free fatty acid (FFA) in plasma of normal and diabetic rats

Experimental groups[1)]	HDL-cholesterol[2)] (mg/dL)	Triglyceride[2)] (mg/dL)	FFA[2)] (μEq/L)
Normal (n = 6)	45.1±5.1[ab]	141.9± 69.8[a]	330.8± 63.6[a]
STZ-control (n = 8)	49.8±4.5[b]	780.5±427.5[b]	1039.0±297.8[b]
EtOH extract 1.0 g (n = 7)	40.0±4.9[a]	176.8±121.8[a]	425.6±175.8[a]
EtOH extract 2.0 g (n = 7)	42.7±7.9[a]	607.6±464.6[b]	744.8±391.9[b]

[1)]Values are mean±SD.
[2)]Values with different superscript within the column are significantly different at 5% level.

and/or their use as dietary supplements for diabetes treatment.

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REFERENCES

1. Chang, T.T. and Li, C.C. : Genetics and breeding. In "*Rice : Production and Utilization*" Luh, B.B. (ed.), Avi Bool, Westport, Conneticut, USA, p. 87 (1990)
2. Flatt, P.R., Abdel-Wahab, Y.H.A., Boyd, A.C., Barnett, C.R. and O'Harte, F.P. : Pancreatic B-cell dysfunction and glucose toxicity in non-insulin-dependent diabetes. *Proc. Nutr. Soc.*, **56**, 243 (1997)
3. Townsend, R.R. and Kapoor, S.C. : The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N. Eng. J. Med.*, **329**, 977 (1993)
4. Larner, J. : Insulin and oral hypoglycaemic drugs, glucagon. In "*The Pharmacological Basic of Therapeutics*" Gilman, A.G., Goodman, L.S., Rall, T.W. and Murad, F. (eds.), 7th ed., Macmillan, New York, p.1490 (1985)
5. Monin, A. : Role of indigenous medicine in primary healthcare. In "*Proceedings of first international seminar on Unani Medicine*" New Delhi, India, p.54 (1987)
6. Stanely Mainzen Prince, P. and Menon, V.P. : Antioxidant activity of *Tinospora cordifolia* roots in experimental diabetes. *J. Ethnopharmacol.*, **65**, 277 (1999)
7. Indira, G. and Mohan Ram, M. : *Fruits*. National Institute of Nutrition, Indian Council of Medical Res., Hyderabad, India, p.34 (1999)
8. Mahapatra, P.K., Pal, M., Chaudhuri, A.K.N., Chakraborty, D. and Basu, A. : Preliminary studies on glycaemic effects of *Sysigium cumini* seeds. *IRCS Medical Sci. Biochem.*, **13**, 631 (1985)
9. Nadkarni, A.K. : *Indian material medica*. Popular Prakashan, Bombay, Vol. 1, p.157 (1992)
10. Twaij, H.A.A. and Al-Badr, A.A. : Hypoglycaemic activity of *Artemisia* herba alba. *J. Ethnopharmacol.*, **24**, 123 (1988)
11. Gupta, M.P., Solis, N.G., Avella, M.E. and Sanchez, C. : Hypoglycaemic activity of Neurolaena lobata. *J. Ethnopharmacol.*, **10**, 323 (1984)
12. Rajasekharan, S. and Tuli, S.N. : Vijaysar (*Pterocarpus marsupium*) in the treatment of Madhymeha (diabetes mellitus)-a clinical trial. *J. Res. Ind. Med., Yoga Homeopathy*, **9**, 76 (1976)
13. Bhaskaran, N.S. and Santhakumari, G. : Antidiabetic activity of the seed kernal of syzygium. *Cumini. Linn. Ancient Sci. Life*, **6**, 80 (1985)
14. Nagarajan, S., Jain, H.C. and Anlakh, G.S. : Indigenous plants used in the control of diabetes. Publication and information Directorate CSIR, New Delhi, p.586 (1987)
15. Cihattopadhyay, R.R., Medd, C., Das, S., Basu, T.K. and Poddar, G. : Hypoglycaemic and anti-hyperglycaemic effect of *Gymnema sylvestre* leaf extract in rats. *Fitoterapia*, **64**, 450 (1993)
16. Ponnachan, P.T.C., Paulose, C.S. and Pannikkar, K.R. : Effect of leaf extracts of *Aegle marmelose* in diabetic rats. *Ind. J. Exp. Biol.*, **31**, 345 (1993)
17. Subramoniam, A., Pushpangadan, P., Rajasekharan, S., Evans, D.A., Latha, P.G. and Valsaraj, R. : Effects of *Artemisia pallens* wall on blood glucose levels in normal and alloxan-induced diabetic rats. *J. Ethnopharmacol.*, **50**, 13 (1996)
18. Rakel, R.E. : *Medical laboratory technology and clinical pathology*. 2th ed., WB Saunders Co., Philadelphia (1969)
19. Bauer, J.D. : *Clinical laboratory methods*. 9th ed., Mosby Co., St. Louis, USA (1982)
20. Reitman, S. and Frankel, S.A. : A colorimetric method for the determination of serum glutamin oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.*, **28**, 58 (1957)
21. Rabbo, E. and Terkildsen, T.C. : On the enzymatic determination of blood glucose. *Scand. J. Lab. Invest.*, **12**, 402 (1960)
22. Richimond, W. : Preparation and properties of a cholesterol oxidase from *Nocardia* sp. and its application to the enzymatic assay of total cholesterol in serum. *Clin. Chem.*, **20**, 1350 (1973)
23. Giegel, J.L., Ham, S.B. and Clema, W. : Serum triglyceride determined colorimetry with an enzyme that produces hydrogen peroxide. *J. Clin. Chem.*, **21**, 1575 (1975)
24. Trimble, M.E. : Effect of free fatty acids on renal metabolism of very low density lipoprotein triglyceride. *Ren. Physiol.*, **8**, 1 (1985)
25. Rao, M., Blane, K. and Wonnenberg, M. : *PC-STAT*. Dept. Food Sci. Univ., Georgia (1985)
26. Laure, S.A. and Parid, R.R. : Sur les polyphenols d'Anacardiaces Africaines et Malgaches. Poupartic birrea Aub., Poupartia caffra H. Peer. et *Anacardium occidentale* L. *Plantes Medicinales et Phytotherapie*, **2**, 16 (1977)
27. Weaver, D.C., McDaniel, M.L., Naber, S.P., Barry, C.D. and Lacy, P.E. : Alloxan stimulation and inhibition of insulin release from isolated rat islets of Langerhans. *Diabetes*, **27**, 1205 (1978)
28. Junod, A., Lambert, A.E., Stauffacher, W. and Renold, A.E. : Diabetogenic action of streptozotocin : Relationship of dose to metabolic response. *J. Clin. Invest.*, **48**, 2129 (1969)
29. Brosky, G. and Logothetopoulos, J. : Streptozotocin diabetes in the mouse ad guinea pig. *Diabetes*, **18**, 606 (1969)
30. Ledoux, S.P., Woodley, S.E., Patton, N.J. and Wilson, L.G. : Mechanism of nitrosourea-induce β-cell damage-alterations in DNA. *Diabetes*, **35**, 866 (1986)
31. van Dam, P.S., van Asbeck, B.S., Bravenboer, B., van Oirschot, J.F., Gispen, W.H. and Marx, J.J. : Nerve function and oxidative stress in diabetic and vitamin E-deficient rats. *Free Radical Biol. Med.*, **24**, 18 (1998)
32. Stanely Mainzen Prince, P., Menon, V.P. and Pari, L. : Hypoglycaemic activity of *Syzigium cumni* seeds : Effect on lipid peroxidation in alloxan diabetic rats. *J. Ethnopharmacol.*, **61**, 1 (1998)
33. Omeara, N.M.G., Devery, R.A.M., Owens, D., Collins, P.B., Johnson, A.H. and Tomkin, G.H. : Cholesterol metabolism in alloxan-induced diabetic rabbits. *Diabetes*, **39**, 629 (1990)
34. Al-Shamaony, L., Al-Khazraji, S.M. and Twaij, H.A. : Hypoglycemic effect of *Artemisia* herba alba II. Effect of a valuable extract on some blood parameters in diabetic animals. *J. Ethnopharmacol.*, **43**, 167 (1994)
35. Rhoads, G.G., Gulbrandsen, C.L. and Kagan, A. : Serum lipoproteins and coronary heart disease in a population study of Hawaii Japanese men. *N. Engl. J. Med.*, **294**, 293 (1976)

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