



Antihyperglycemic and Antihyperlipidemic Effects of Fermented *Rhynchosia nulubilis* in Alloxan-induced Diabetic Rats

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Alloxan administration in rats is used as a model for non-insulin dependent diabetes mellitus (NIDDM). NIDDM is a multifactorial disease, characterized by hyperglycemia and lipoprotein abnormalities. In this study, we evaluated the antihyperglycemic and antihyperlipidemic effects of fermented *Rhynchosia nulubilis* (FRN) through the regulation of glucose uptake in alloxan-induced rats. Fermented *R. nulubilis* was administered orally for 28 d at 500 mg/kg of body weight. Body weight and food intake were monitored every day. Biochemical parameters were quantified after 4 week. In the diabetic + FRN group, body weight increased significantly and blood glucose concentrations decreased when compared to those of the diabetic group. After 2 hr of administration, the oral glucose tolerance test (OGTT) indicated a significant reduction in the diabetic + FRN group compared to diabetic group. The diabetic + FRN group experienced a significant reduction in total cholesterol, triglycerides, low density lipoprotein, coronary risk factors, and malondialdehyde concentrations, with significantly increased high density lipoprotein compared to those of diabetic group. These results demonstrate that fermented *R. nulubilis* possesses potent antihyperglycemic and antihyperlipidemic activity in alloxan-induced diabetic rats.

Key words: Alloxan, *Rhynchosia nulubilis*, Hyperglycemic, Hyperlipidemic

INTRODUCTION

The prevalence of diabetes for all age groups worldwide was estimated to be increased from 2.8% in 2000 to 4.4% in 2030. The total number of people with diabetes is expected to rise from 171 million in 2000 to 366 million in 2030 (1).

Diabetes mellitus consists of a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates, proteins and persistent elevations of fasting blood glucose above 200 mg/dl, due to insufficient or complete cessation of insulin synthesis or secretion and/or peripheral resistance to insulin action (2,3). It is generally classified as either type I or type II diabetics. Both types of diabetes are prone to complications such as hyperosmolar hypergly-

cemia state, diabetic ketoacidosis, oxidative stress resulting from elevated glucose concentration, microvascular and impaired immune response (4). Insulin and oral hypoglycemic agent including biguanides, sulfonylureas and thiazolidinediones are the main way to treat diabetics and are effective in controlling hyperglycemia, but these kinds of drugs also have prominent side effects (5).

Currently there is growing interest in herbal remedies due to side effects associated with therapeutic agents (insulin and oral hypoglycaemic agents) for the treatment of diabetes mellitus (6). Black soybean [*Glycine max* (L.) Merr.] known as SoRiTae and *Rhynchosia nulubilis* (Yak-Kong) is a soybean cultivar with a black seed coat. In traditional Chinese medicine, black soybean has been used for detoxification, as an anti-inflammatory agent, and for improving the blood circulation (7). Anthocyanin and other components of black soybeans possess similar biological activities to those of isoflavone, vitamin E and trypsin inhibitor (8). It has been reported that enrichment of bioactive components in black soybean was associated with various therapeutic effects, such as anticancer, antiobesity, antidiabetes, antimutagenic and anti-osteoporosis activities (9-12).

Fermentation has been reported to cause a general improve-

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ment in the natural nutritive values of soybean products, increasing total soluble solids and vitamins, free fatty acids and free amino acids (13). The black soybeans showed the enhanced antioxidant activity and total phenolic content and total flavonoid content from fermentation with *B. subtilis* (14). But no study has focused on antihyperglycemic and antihyperlipidemic activities. Based on the above knowledge, fermented *Rhynchosia nulubilis* is selected to evaluate antihyperglycemic and antihyperlipidemic activities in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Animals. Sprague-Dawley (SD) rats (Female, 130 g~150 g) were supplied by the Korean Experimental Animal Center (KEAC) and acclimated for seven days prior to experiments. All animals were maintained in separate cages, with laboratory chow and tap water provided *ad libitum*. They were housed at $22 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ relative humidity and kept on a 12 hrs light/dark cycle throughout the experimental period. Perform of animal experiments was considered to examine in accordance with the ethical use of laboratory animals and the principles of 3R (reduction, refinement, replacement). Or all protocols were approved by the Silla university IACUC.

Preparation of extract. To make product by the traditional method, *Rhynchosia nulubilis* was washed and put in water for 5 hrs so that it swells. Swelled *R. nulubilis*/water (3 : 1, by w/v) was put in the pot and steamed with rice straw for 5 hrs at 70°C . Steamed *R. nulubilis* water extract was fermented in the pot for 48 hrs at 30°C and sterilized at 120°C . The suspension was filtered through a fine muslin cloth and the extract was lyophilised (yield: 2% w/w, dry weight).

Experimental induction of diabetes. A freshly prepared solution of alloxan monohydrate (150 mg/kg body weight), in sterile normal saline solution, was injected intraperitoneally to overnight fasted rats. Blood glucose was measured after 72 hrs of alloxanisation by one-touch glucometer (Accu-Chek Advantage, Roche, USA), and it was confirmed by testing for glucosuria using glucose indicator sticks. Rats showing fasting blood glucose (FBG) levels > 250 mg/dl were selected for the study. The rats were divided into three groups with seven rats in each group as follows; Normal group: untreated rats received 5 ml/kg of water; Diabetic group: alloxan-induced diabetic rats received 5 ml/kg of water; Diabetic+FRN Group: Diabetic rats received fermented *R. nulubilis* (5 ml/kg of 500 mg/kg body weight) for a period of 28 days orally. After the experimental regimen, the animals were fasted overnight and anesthetized with ether and dissected.

Blood glucose determination. Blood samples from experimental rats were collected from the tail vein after 14~

16 hrs of overnight fast. Fasting blood glucose levels were determined by one-touch glucometer Monitoring system and blood glucose test strips before treatment in day 1, day 7, day 14, day 21 and day 28 after initiation of treatment. Body weights and water intake of all groups of rats were assessed on the same days that blood glucose levels were measured.

Oral glucose tolerance test. Rats were fasted for 16 hrs before being subjected to an oral glucose tolerance test (OGTT) by intragastric gavage with a glucose solution to achieve a glucose load of 2 g/kg. OGTT was carried out 2 hrs at the end of the 4 weeks period. Blood samples were collected from the tail vein at 0, 30, 60, 90 and 120 min for blood glucose determination.

Blood and tissue collection. On day 31, the rats were anesthetized with ether and dissected. Blood samples were centrifuged at $800 \times g$ for 10 min at 4°C to obtain serum samples, which were then stored at -70°C . The livers were rinsed with saline solution and stored at -70°C . Liver tissues were homogenized in 1 : 5 volumes of PBS (pH 7.4), and the homogenate was centrifuged at $800 \times g$ for 10 min. The supernatant was used as the total liver homogenate sample.

Measurement of serum enzyme activities. Asparate aminotransferase, alanine aminotransferase, total cholesterol, triglyceride and high density lipoprotein concentrations were determined with enzymatically-available serum slides using a FUJI Dri-Chem clinical chemistry analyzer (FUJI Dri-Chem 3500, FUJIFILM). LDL [TC-(HDL-TG/5)] and CRF [TC/HDL] levels were calculated using the formula of Friedewald *et al.* (15).

Assay of lipid peroxidation. Lipid peroxidation was assayed by measuring the amount of malondialdehyde that reacted with thiobarbituric acid (TBA), according to a method previously described by Ha *et al.* (16). Briefly, 1 ml of sodium dodecyl sulfate (7% SDS) was added to 0.5 ml of total liver homogenate. Tubes were mixed and incubated for 30 min at 37°C , after which 2 ml of 0.67% TBA (mixed 1 : 1 with acetic acid) was added to tubes. Tubes were mixed and placed in boiling water (100°C) for 50 min, after which 5 ml of butanol was added. The tubes were mixed again and centrifuged at $800 \times g$ for 10 min. The absorbance of the resulting supernatant was then measured at 535 nm. For this experiment, 1,1,3,3-tetraethoxypropane was used as the standard (17,18).

Statistical analysis. The results are expressed as mean \pm S.D [standard error of the mean (SEM)]. Significant difference was set at $p < 0.05$ using the Student's t-tests (SPSS computer software, version 18.0). Statistical significance was represented by $p < 0.01^*$, and $p < 0.001^{**}$.

Table 1. Effects of fermented *Rhynchosia nulubilis* on body weight and food intake in alloxan-induced diabetic rats

Experimental groups	Body weight (g)					Food intake (g/rat/day)
	1 day	7 day	14 day	21 day	28 day	
Normal	168.2 ± 3.9	206.12 ± 3.79	251.93 ± 4.23*	281.63 ± 4.35**	300.55 ± 9.14**	17.28 ± 2.98*
Diabetic	164.33 ± 3.82	201.39 ± 5.19	238.19 ± 4.61	250.13 ± 3.53	270.96 ± 1.05	23.74 ± 2.47
Diabetic + FRN	169.28 ± 5.87	207.26 ± 4.92	253.18 ± 4.14*	282.88 ± 1.55**	311.46 ± 3.45**	20.52 ± 2.08

Values are presented as the means ± S.D (n=7). ** $p < 0.001$ and * $p < 0.01$ significantly different from the value of Diabetic in Student's t-test.

RESULTS AND DISCUSSION

Effects of fermented *R. nulubilis* on body weight and food intake. Dehydration and loss of body weight have been associated with diabetes mellitus (19). The body weight and food intake were measured and represented in Table 1. The initial body weights were similar in normal and diabetic groups, whereas alloxan-induced diabetic rats showed a significant reduction in body weight on days 14, 21 and 28 when compared to the normal rats. Fermented *Rhynchosia nulubilis* at 500 mg/kg body weight dose given to a diabetic rats caused significant increase in body weight when compared to diabetic group. Food intake amount was higher in diabetic group than the normal. Oral administration of fermented *R. nulubilis* for 4 weeks to diabetic rats decreased food consumption and improved body weight.

Effect of fermented *R. nulubilis* on fasting blood glucose level. Induction of diabetes in the experimental rats was confirmed by the presences of a high fasting glucose level when compared with normal group. The effect of fermented *R. nulubilis* on fasting blood glucose levels was pre-

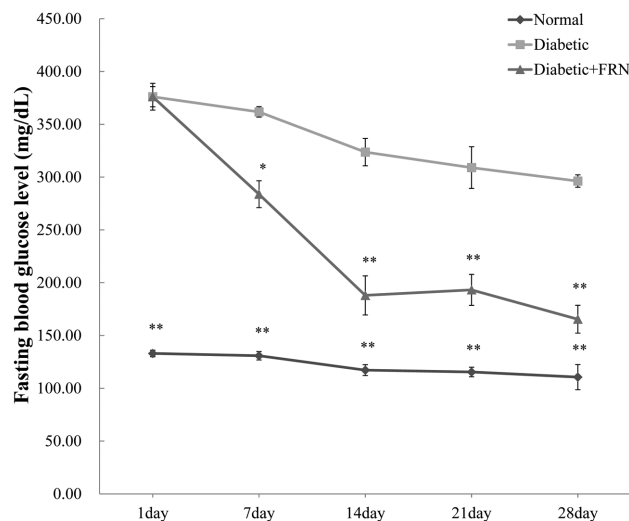


Fig. 1. Effect of fermented *Rhynchosia nulubilis* on fasting blood glucose concentration in alloxan-induced diabetic rats. Each bars are presented as the means ± S.D (n = 7). ** $p < 0.001$ and * $p < 0.01$ significantly different from the value of Diabetic in Student's t-test.

sented in Fig. 1. Treatment of diabetic rats with fermented *R. nulubilis* for 4 weeks significantly reduced their blood glucose levels when compared with those of diabetic group. The observed hypoglycemic effect of fermented *R. nulubilis* is an indication that fermented *R. nulubilis* contains active principles with potent hypoglycemic property.

Oral glucose tolerance test. The oral glucose tolerance test (OGTT) is the most commonly used method to evaluate whole body glucose tolerance, β -cell function and insulin resistance in vivo. It has been difficult to derive meaningful information about whole-body, peripheral tissue, or hepatic sensitivity to insulin from the results of the OGTT (20,21). The blood glucose level was lowered significantly with the extract, at different time intervals (30, 60, 90 and 120 mins). The supplementation of fermented *R. nulubilis* extract improved the glucose tolerance in the extract treated rats (Fig. 2).

Liver dysfunction parameters. Serum AST and ALT levels were measured as markers of liver damage to predict serious clinical liver injury and cell damage (22). The hepatoprotective effects of the fermented *R. nulubilis*

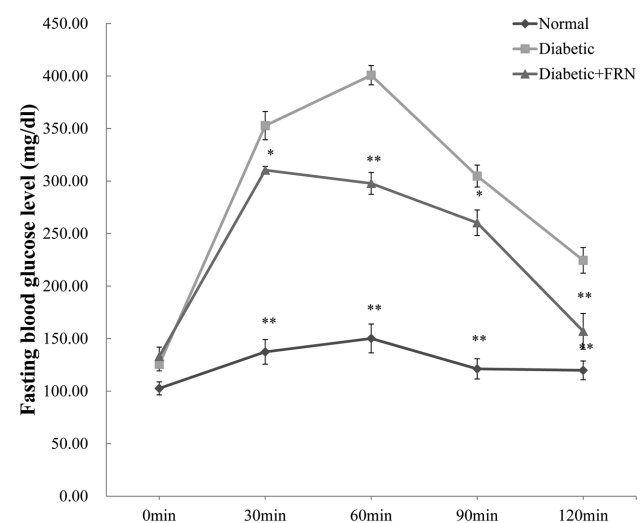


Fig. 2. Effect of fermented *Rhynchosia nulubilis* on oral glucose tolerance test in alloxan-induced diabetic rats. Each bars are presented as the means ± S.D (n = 7). ** $p < 0.001$ and * $p < 0.01$ significantly different from the value of Diabetic in Student's t-test.

Table 2. Effects of fermented *Rhynchosia nulubilis* on the activities of serum AST and ALT in alloxan-induced diabetic rats

Experimental groups	Aspartate aminotransferase (AST) unit/L	Alanine aminotransferase (ALT) unit/L
Normal	47.53 ± 3.76**	22.13 ± 3.60
Diabetic	61.92 ± 4.64	26.42 ± 4.36
Diabetic + FRN	54.6 ± 5.58*	23.47 ± 2.59

Values are presented as the means ± S.D (n = 7). ***p* < 0.001 and **p* < 0.01 significantly different from the value of Diabetic in Student's t-test.

extract were observed with the inhibitory activity on the increase of AST and ALT induced by alloxan in serum (Table 2). The activity of AST significantly increased in diabetic rats compared to normal. Because diabetes impairs the normal capacity of the liver to synthesize glycogen (2). These activities decreased significantly after treatment with fermented *R. nulubilis* extract.

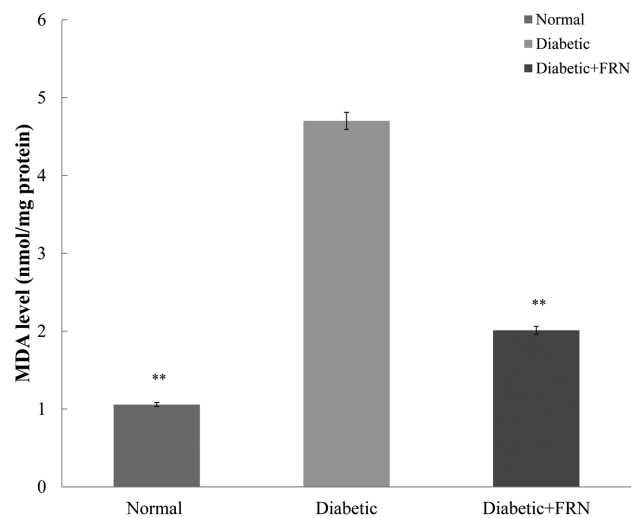
Lipid profile in serum. Table 3 depicts the levels of TC, TG, HDL, LDL and CRF in the serum of control and experimental groups of rats. The levels of TC, TG, LDL and CRF in diabetic group were significantly increased compared to normal group. However, HDL was significantly decreased in diabetic control rats compared to normal group. Oral administration of extract significantly reversed all these changes. Thus, the normalization of lipids in diabetic rats treated with extract may be due to its stimulatory effect on insulin secretion from pancreatic β cells (23).

Lipids peroxidation level in liver. MDA is one of several low-molecular-weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products in oxidative stress (24). The levels of MDA in total liver homogenate were presented in Fig. 3. MDA levels in total liver homogenate were higher in the diabetic group than in the normal group. It was found to be associated with hyperglycemia. After 28 days of treatment with fermented *R. nulubilis* extract, MDA level in liver of diabetic+FRN group was significantly different when compared to diabetic groups. It was reported that black soybean seed coat extract significantly could inhibit LDL oxidation by reduced formation of MDA (25).

Table 3. Effect of fermented *Rhynchosia nulubilis* on lipid profile in alloxan-induced diabetic rats

Experimental groups	Total cholesterol (TC) mg/dl	Triglyceride (TG) mg/dl	High density lipoprotein (HDL) mg/dl	Low density lipoprotein (LDL) mg/dl	Coronary risk factors (CRF)
Normal	71.73 ± 5.53	56.13 ± 6.84**	56.80 ± 2.68**	29.4 ± 3.97**	1.27 ± 0.13**
Diabetic	79.58 ± 3.67	96.67 ± 5.71	44.17 ± 5.45	69.90 ± 3.59	2.02 ± 0.33
Diabetic + FRN	71.20 ± 4.42	68.80 ± 2.53**	51.53 ± 5.21*	33.43 ± 5.22**	1.39 ± 0.11**

Values are presented as the means ± S.D (n = 7). ***p* < 0.001 and **p* < 0.01 significantly different from the value of Diabetic in Student's t-test.

**Fig. 3.** Effect of fermented *Rhynchosia nulubilis* on lipid peroxidation in liver homogenates. Each bars are presented as the means ± S.D (n = 7). ***p* < 0.001 significantly different from the value of Diabetic in Student's t-test.

In conclusion, fermented *R. nulubilis* extract possesses potent antihyperglycemic and antihyperlipidemic activities in alloxan induced diabetic rats and further study is needed to identify the compounds responsible for its promising *in vivo* antidiabetic activity.

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