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Hypoglycemic Effects of Germinated Rough Rice Extract in Streptozotocin-induced Diabetic Rats

- Research Note -

Youn Ri Lee[1], In Guk Hwang[2], Koan Sik Woo[3], Hyun Young Kim[4], Dong Sik Park[2], Jae Hyun Kim[2], Yun Bae Kim[5], Junsoo Lee[4], and Heon Sang Jeong[4†]

[1]Department of Food and Nutrition, Daejeon Health Science College, Daejeon 300-711, Korea
[2]Department of Agrofood Resources, NAAS, Gyeonggi 441-857, Korea
[3]Department of Functional Crop, NICC, Gyeongnam 627-803, Korea
[4]Department of Food Science and Technology and [5]College of Veterinary Medicine, Chungbuk National University, Chungbuk 361-763, Korea

Abstract

The hypoglycemic effects of germinated rough rice extract in streptozotocin (STZ)-induced diabetic rats were investigated. Weight gain was significantly lower in the diabetic groups than in the normal control (NC); however, they were higher in the 1% and 3% diabetic groups given germinated Goami2 rough rice extract (DM-3%GGRRE) than in the diabetic control (DC). While food intake in all diabetic groups was significantly higher than that of the NC, there was no significant difference among all diabetic groups. The weight percentages of liver and kidney in all diabetic groups were significantly higher than that of the NC. In terms of blood glucose, the diabetic group showed about a three times larger value than the normal group. Moreover, in the 3% germinated rough rice extract group, the blood glucose level became lowered. The levels of alanine transaminase, aspartate transaminase, blood urea nitrogen, creatinine phosphokinsae, and creatinine increased in general with the induction of diabetes using STZ; however, the 3% GGRRE-treated group displayed a significant decrease in these levels compared to the diabetic group. The results show that the 3% GGRRE, rather than the 1% GGRRE, was considerably more effective at reducing blood glucose and improving impaired glucose tolerance, suggesting the germinated rice extracts may play a role in preventing liver and kidney damage.

Key words: germinated rough rice extract, blood glucose level, serum insulin, liver, kidney

INTRODUCTION

Diabetes mellitus (DM) is considered a major health problem in the world today. DM is a metabolic disorder of carbohydrate-, fat- and protein-metabolism characterized by elevation of both fasting and postprandial blood glucose levels (1). According to World Health Organization projections, the prevalence of diabetes is likely to increase by 35% (2). Currently, there are over 150 million diabetics worldwide, an amount which is likely to increase to 300 million or more by the year 2025 (3).

Rice (*Oryza sativa* L.) is a major cereal food consumed by a large part of the world's population, especially in Asian countries (4). Germinated brown rice, a new cereal diet product, has attracted public attention in Japan and Korea (5). In germinated cereal grains, activated hydrolytic enzymes decompose starch, non-starch polysaccharides, and proteins, which leads to an increase of oligosaccharides and amino acids in barley (6), wheat (7) and oats (8). The decomposition of high molecular weight polymers during germination also leads to the generation of bio-functional substances, as well as improvements in organoleptic qualities due to the softening of texture and increase in flavor for barley (6), finger millet (9), oats (10) and rye (11). Furthermore, germination raises the content of certain essential amino acids such as lysine, methionine, and most drastically γ-aminobutyric acid (12). However, physical damage to the embryo during the process of producing brown rice affects germination. In addition, if unhulled rice is pounded to produce brown rice, the embryo is not protected by the hull, thus exposing it to air and allowing oxidation to occur. Furthermore, because related enzymes are activated and hydrolysis takes place, it loses its germination ability (13). In particular, hydrolysis works on the ester bond of fat and produces free fatty acids in a process involving various enzymes, for example, lipoxidase and lipase, which produces the smell of old rice and increases the acidity (14). This study examines the effects of a

†Corresponding author. E-mail: hsjeong@chungbuk.ac.kr
Phone: +82-43-261-2570, Fax: +82-43-271-441

variety of germinated rough rice extracts on diabetic rats (normal rats that were injected with streptozotocin) to establish foundational data that will be used to find ingredients for anti-diabetic foods as well as other functional foods.

MATERIALS AND METHODS

Preparation of ground germinated rough rice extracts

The Ilpum and Goami2, rough rice cultivars were grown at the National Institute of Crop Science, Rural Development Administration, and Suwon, Korea during the 2006 growing season. The Ilpum and Goami2 rough rice were soaked in water for 3 days at room temperature, and the soaking water was changed every 24 hr. After 3 days of germination, the rough rice was dried at 60°C for 24 hr and then ground. The germinated rough rice was extracted and wrapped in a pouch with a dried weight of either 1% or 3%. During the four weeks of the experimental period, the experimental substance was stored in the refrigerator, and the package was opened right before the intake.

Animals

Male Sprague-Dawley rats with a mean body weight of 140±10 g were purchased from Hyochang Science (Daejeon, Korea). The rats were fed a standard rodent pellet chow (Purina Co., Seoul, Korea) and acclimatized to their environment for 1 week before commencement of the experiments. Animals were individually housed in stainless steel cages in a room maintained at 20～22 °C and 50±10% relative humidity with a 12-hour light and dark alternate cycle. Body weight and germinated rough rice extract were measured daily and weekly, respectively. The animals were given intraperitoneal (i.p.) injections of freshly prepared streptozotocin (STZ, 60 mg/kg in 0.01 M citrate buffer) for 5 days, while the normal control group was injected with the buffer only. Five days after injection, blood was collected from the tip of the tail vein, and the fasting glucose level was measured. All diabetic rats had a blood glucose concentration higher than 250 mg/dL. Six groups of rats (10 rats per group) were fed for 4 weeks with the following: normal control (NC), diabetic control (DC), 1% diabetic mellitus germinated Ilpum rough rice extract (DM-1% GIRRE), 3%diabetic germinated Ilpum rough rice extract (DM-3% GIRRE), 1% diabetic germinated Goami2 rough rice extract (DM-1%GGRRE), 3% diabetic germinated Goami2 rough rice extract (DM-3%GGRRE). NC and DM drank water while the experimental diabetic group took the germinated rough rice extract instead of water as they needed during the four weeks.

Measurement of fasting blood glucose levels

Fasting blood glucose levels were measured at the beginning of the experiment and at 1-week intervals for 4 weeks. Blood was collected from the tip of the tail vein, and the level of fasting blood glucose was measured using a glucometer (Glucotrend, Mannheim, Germany).

Glucose tolerance test

Glucose (2 g/kg) was administered orally to 16 hr-fasted rats by gastric incubation and blood samples were collected from the tail vein at 15, 30, 60, 90 and 120 min. Blood glucose was measured using a glucometer (Glucotrend) (15).

Measurement of the biochemical parameters

At the end of the experimental period of 4 weeks, the rats were anesthetized with *i.p.* injection of 1% ketamine hydrochloride (2 μL/g) following a 12 hour fast and subsequent weighing. Sacrificed animals were bled with heparin zed syringes from abdominal aorta, and plasma was obtained by centrifugation at 3000 rpm and 4°C for 20 minutes. Liver and kidney were removed and perfused with cold physiological saline. The excised organs were blotted dry, and then weighed. All prepared samples were stored at -70°C until analyzed. Blood samples were centrifuged for 20 min at 3000 rpm at 4°C to obtain plasma. The plasma was stored at -70°C until further analysis. Alanine transaminase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), creatinine phosphokinsae (CPK), and creatinine were measured using an Automated Biochemical Analyzer (Hitachi-7060, Hitachi Medical Co., Tokyo, Japan). Serum insulin was measured by commercially available rat insulin kit (Rat insulin ELISA kit, Shibayagi Co., Shibukawa, Japan)

Statistical analysis

The results were expressed as mean±standard deviation (SE) of the 10 animals. Statistical comparison of differences between the different groups was carried out one-way ANOVA test followed by Duncan's multiple range tests using SPSS statistical software package (Version 12.0, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Body weight gain, food intake, germinated rough rice extract intake and organ weight

After the induction of diabetes, animals were fed with an experimental diet for 4 weeks, and body weight gains, the amount of food intake and the germinated rough rice extract intake were subsequently examined (Table 1). In the STZ-treatment experiments, the body weight gains of STZ-diabetic rats were significantly lower than the

Table 1. Body weight gains, food intake, germinated rough rice extract intake and organ weight of rats fed experimental diet for 4 weeks

Group	Body weight gains (g/day)	Food intake (g/day)	Germinated rough rice extract (mL/day)	Liver (g/100 g)	Kidney (g/100 g)
Normal control	81.4±11.0[1)c2)]	27.4±2.5[a]	35.6±3.8[a]	3.87±0.36[a]	0.32±0.02[a]
Diabetic control	45.1±9.8[ab]	38.2±3.4[b]	98.5±10.3[ab]	4.87±0.53[b]	0.49±0.06[b]
DM-1%GIRRE[3)]	14.7±5.3[a]	33.0±2.9[ab]	114.9±18.0[b]	4.42±0.27[b]	0.49±0.15[b]
DM-3%GIRRE[4)]	38.0±10.4[ab]	29.17±2.7[ab]	97.0±34.6[ab]	4.39±0.32[b]	0.47±0.05[b]
DM-1%GGRRE[5)]	48.2±15.2[ab]	34.1±1.8[ab]	102.5±20.6[ab]	4.45±0.33[b]	0.50±0.06[c]
DM-3%GGRRE[6)]	59.0±2.6[bc]	32.6±2.4[ab]	85.4±20.5[ab]	4.34±0.42[b]	0.44±0.07[b]

[1)]Each value is expressed as mean±SE.
[2)]Means with different kind of superscripts in a same column are significantly different at $p<0.05$ level by method of one-way ANOVA.
[3)]1% diabetic mellitus germinated Ilpum rough rice extract.
[4)]3% diabetic germinated Ilpum rough rice extract.
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[6)]3% diabetic germinated Goami2 rough rice extract.

normal rats, in agreement with the previous study (15). It is thought that the reason for weight loss in diabetes is a decrease of glucose disposal in cells, which leads to fat and protein from the adipose tissue, liver, and/or muscle producing the necessary energy, eventually resulting in reduced body weight (16,17). The feed intake measurements indicate that the diabetic group ate more than the NC group, and the DC and 1% and 3% germinated rough rice extract groups did not display any significant differences in the amount of food ingested. This observation corresponds to the results of other studies that report that the diabetic group shows significantly higher water and feed intake than the normal group. While, germinated rough rice extract intake in all diabetic groups were significantly higher than that of the NC group, there was no significant difference among all diabetic groups. This study measured the weights of critical organs, such as the liver and kidney, according to the intake of the germinated rough rice extract and converted them to values per 100 g of body weight. Generally, liver, kidney, heart and testes in rats with diabetes induced by STZ are enlarged because of abnormal glucose metabolism and the accumulation of lipids caused by reduced insulin formation and insulin resistance (18). Every diabetic group showed a significantly higher weight of their liver and kidney than the normal group did, which conforms to the results of other reports that state STZ-induced diabetic rats have fatter livers and kidneys than normal ones. It is assumed that the hypertrophic phenomenon of the liver results from decreased insulin secretion and abnormal glucose metabolism, such that lipids accumulate in the liver (19-23). Although there was not a significant difference, the 3% germinated rough rice extract group displayed a lower liver weight than the 1% germinated rough rice extract group. These data suggest that 3% GIRRE and 3% GGRRE might reduce the burden on the kidney of diabetic rats and also contribute to improving glucose metabolism.

Levels of blood glucose and serum insulin

The results of the blood glucose analyses (Table 2)

Table 2. Effect of germinated rough rice extract in supplementation of blood glucose and serum insulin in STZ-induced diabetic rats

Group	Blood glucose (mg/dL)			Serum insulin (μIU/mL)
	0 week	2 weeks	4 weeks	
Normal control	100.83±7.49[1)a2)]	104.83±6.49[a]	107.17±14.0[a]	2.00±1.27[d]
Diabetic control	348.50±25.71[b]	366.17±44.77[c]	398.15±84.6[d]	1.37±0.59[a]
DM-1%GIRRE[3)]	354.67±30.88[b]	300.00±49.71[b]	327.5±22.5[c]	1.47±0.54[b]
DM-3%GIRRE[4)]	354.33±30.03[b]	305.50±43.04[b]	269.8±21.4[b]	1.55±0.36[b]
DM-1%GGRRE[5)]	351.17±28.38[b]	307.67±27.08[b]	317.5±54.5[bc]	1.48±0.55[b]
DM-3%GGRRE[6)]	354.14±35.44[b]	295.50±29.32[b]	239.6±31.9[b]	1.65±0.78[c]

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[6)]3% diabetic germinated Goami2 rough rice extract.

show that the diabetic group had about a three times higher blood glucose level than the normal group. This hyperglycemia did not show a significant decrease until the fourth week with 1% GGRRE; however, the level of hyperglycemia was greatly reduced in 3% GGRRE after only one week. In 1% and 3% GIRRE, hyperglycemic levels showed a noticeable improvement after three weeks. Moreover, in the 3% germinated rough rice extract group, the blood glucose level was also lowered. The result of this experiment implies that the diabetic animals' intake of 3% GGRRE should activate glucose metabolism and reduce diabetic symptoms to some extent. The blood insulin concentration results showed that the diabetic group had a decrease of insulin generation compared with the normal group. Previous research has shown that streptozotocin causes a great reduction in insulin release through the selective destruction of β-cells of the islets of Langerhans (24). The groups ingesting germinated rough rice extracts showed an increase in their blood insulin level, in particular, the 3% GGRRE supplemented group.

Oral glucose tolerance test

For the glucose tolerance test, animals were fasted for sixteen hours and were then made to take glucose orally. The change of their glucose level was measured over time (Table 3). As a result, the glucose level of normal animals showed a slight increase of about 10% up to 90 minutes after the administration of glucose, which then recovered to a normal level. On the contrary, the diabetes-induced group had blood glucose levels that increased of about 51% up to the initial level which then decreased 30 to 90 minutes after the administration of glucose. However, the glucose levels showed a higher concentration at about 330 mg/dL up to 120 minutes, indicating a major impairment to glucose tolerance. This impaired glucose tolerance tended to improve to some degree with the intake of the germinated rough rice extract intake, and especially when 3% GGRRE and 3% GIRRE were ingested, with improvements up to 30% to 35% or so. The control of postprandial hyperglycemia is critical in the early therapy for diabetes. Controlling postprandial glucose levels is an also important strategy in the prevention of type 2 diabetes (25).

Biochemical parameters

This research also examined the effect of the germinated rough rice extract on the function of diabetic rats'

Table 3. Blood glucose levels during oral glucose tolerance test in normal and diabetic rats (Unit: mg/dL)

Group	Fasting	30 min	60 min	90 min	120 min
Normal control	98.75±8.06[1)a2)]	133.75±13.72[a]	149.25±3.66[a]	155.00±6.48[a]	116.75±7.85[a]
Diabetic control	145.20±12.9[b]	351.00±56.34[b]	397.40±64.92[b]	400.80±86.01[b]	318.20±72.8[c]
DM-1%GIRRE[3)]	156.80±30.48[b]	328.40±70.88[b]	396.20±52.75[b]	402.40±85.86[b]	297.00±67.60[bc]
DM-3%GIRRE[4)]	153.00±32.66[b]	286.40±80.55[b]	329.00±36.08[b]	301.00±60.27[b]	260.40±53.17[bc]
DM-1%GGRRE[5)]	178.40±32.88[b]	257.80±64.87[b]	388.20±53.63[b]	374.80±52.39[b]	251.20±19.20[bc]
DM-3%GGRRE[6)]	153.20±24.10[b]	288.80±90.86[b]	323.20±72.68[b]	318.60±90.59[b]	226.40±43.33[c]

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Table 4. Plasma level of alanine transaminase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), creatinine, creatinine phosphokinsae (CPK) in rats fed experimental diet for 4 weeks

Group	ALT (IU/L)	AST (IU/L)	BUN (mg/dL)	Creatinine (mg/dL)	CPK (IU/L)
Normal control	42.18±4.71[1)b2)]	115.4±9.21[c]	19.6±1.86[a]	0.52±0.05[c]	0.71±0.06[b]
Diabetic control	96.32±7.49[c]	130.5±10.7[d]	28.0±4.59[c]	0.57±0.07[c]	0.81±0.47[c]
DM-1%GIRRE[3)]	35.23±2.60[a]	82.8±8.71[b]	28.0±4.41[c]	0.38±0.03[a]	0.67±0.13[ab]
DM-3%GIRRE[4)]	41.12±9.05[b]	84.6±9.77[b]	19.2±2.24[a]	0.35±0.03[a]	0.63±0.13[a]
DM-1%GGRRE[5)]	38.48±1.53[a]	85.5±4.98[b]	27.0±3.54[c]	0.40±0.05[b]	0.67±0.11[ab]
DM-3%GGRRE[6)]	31.12±2.98[a]	71.5±5.10[a]	23.8±4.06[b]	0.34±0.03[a]	0.62±0.11[a]

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[6)]3% diabetic germinated Goami2 rough rice extract.

liver and kidney functions by measuring the contents of ALT, AST, BUN, creatinine, and CPK in blood plasma. AST and ALT are the enzymes that synthesize amino acids in the body. They exist in various kinds of organic cells, but most of them are in the liver (22). Serum ALT and AST activities in diabetes were significantly higher in the DC group than the NC group (Table 4). STZ leads to liver damage by causing light degeneration of lipids in the liver, and ALT and AST activities are used as an index for liver damage (22). The ALT and AST activity in the 3% GGRRE group was significantly decreased in comparison with the DC group, which indicates germinated rough rice extract intake can prevent liver damage from diabetes. BUN is ammonia produced from the amination of amino acids and is mainly generated from passing through the urea cycle in the liver (23). When BUN is higher than normal, it reveals a condition called azotemia. Although BUN mostly increases as a result of diabetes, the 3% GIRRE and 3% GGRRE groups showed a significant decrease in BUN levels than the control diabetic group. Creatinine results from proteins being broken down in the body (22). The blood creatinine level is important to determine kidney function, and its increase means that the kidney is not functioning properly. Thus, this research measured the creatinine level in blood plasma to determine the effect of the germinated rough rice extract on kidney function. The diabetic group showed a significant increase in creatinine compared to the normal group. The creatinine levels decreased significantly in the groups ingesting the germinated rough rice extracts. The intake of 3% GGRRE appeared particularly effective at reducing creatinine levels.

The germinated rough rice extract group tended to show a lower CPK value than the diabetic group, and in particular, 3% GIRRE and 3% GGRRE displayed a CPK level similar to that of the normal group. Previous research suggests that the increase of CPK can cause central nervous system diseases (22). The above results show that 3% GGRRE and 3% GIRRE, rather than the 1% germinated rough rice extracts, were considerably more helpful in reducing blood glucose levels and improving glucose tolerance. The data also suggests this level of supplementation may prevent the liver and kidney from getting damaged. Studies that identify active components with therapeutic effects for diabetes in the germinated rough rice extract and their modes of action are the next steps in this research effort.

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