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Effect of Fermented Small Soybean Powder Mixed with Mulberry Leaf on Metabolic Improvement and Hexokinase Activity in Streptozotocin-induced Diabetic Rats*

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Beans are well known to be high-protein diets. Bean seeds contain arginine, lysine, or glycine-rich proteins which are effective to maintain lower glucose levels. In this study, the synergistic effect of fermented small soybean (*Chounggukjang*) and mulberry leaf on metabolism and hexokinase activity was investigated in streptozotocin (STZ)-induced diabetic rats. We divided 8 groups as follows: non-diabetic rat group fed with only water diet (NC: control), and STZ-induced diabetic rat groups fed with water (DC), fermented *Rhynchosia Nulubilis* (Bbc), fermented *Glycine max Merr* (Ybc), Bbc and YBc (BYbc), mulberry leaf and Bbc (MBbc), mulberry leaf and Ybc (MYbc), or the mulberry leaf, Bbc, and Ybc (MBYbc). Diabetes mellitus was induced in rats by subcutaneous STZ administration (70 mg/kg of body weight). All diet groups were fed with *Chounggukjang* in a powder form. Three ml of *Chounggukjang* solution (0.75 mg per gram of body weight) dissolved in distilled water was orally administered to all rat groups after STZ administration except for NC rat group. In groups fed with fermented soybeans, the body weight (increased), food efficiency ratio (FER) (increased), glucose level (decreased) and hexokinase (HK) activity (increased) significantly differed to NC. Among them, particularly in the groups fed with both fermented soybeans and mulberry leaf, kidney weight significantly decreased, whereas HK activity significantly increased compared to DC. These results suggest that *Chounggukjang* of both fermented soybeans and mulberry leaf is potentially used as an effective functional food to prevent diabetes complications.

Key words: Small soybean, Mulberry leaf, Food efficiency ratio, Hexokinase

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INTRODUCTION

Diabetes mellitus is one of the most serious metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1)] Glucose (Glc) is essential for central metabolism in virtually all organisms from microbes to humans. Glycolytic metabolism of Glc is a major pathway for the generation of energy (ATP), and glycolytic intermediates also serve as precursors for biosynthesis of other cellular constituents. Metabolism of Glc through the pentose phosphate pathway generates NADPH and precursors required for a variety of anabolic pathways. Alternatively, Glc may be converted to its polymeric forms (glycogen and starch), which are the storage forms of carbohydrates in many organisms. In mammals, these pathways relatively depend on the particular tissue, or cell type within a tissue, but the potential for metabolism of Glc via alternative pathways exists in most mammalian cells.[2)] Glc 6-phosphatase (G6Pase) is a key enzyme in Glc homeostasis, catalyzing the hydrolysis of Glc 6-phosphate (G6P) to Glc and phosphate in the terminal steps of gluconeogenesis and glycogenolysis.[3)] The initial step of the Glc metabolism is phosphorylation to form glucose-6-phosphate (Glc-6-P), which is catalyzed by hexokinase (HK). Therefore, the expression of isozymic forms of HK is likely to be an important factor in determining the pattern of Glc metabolism in mammalian

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cells/tissues.[4] Hyperglycemia and hyperlipidemia are two important characteristics of diabetes mellitus, an endocrine based disease. In modern medicine, no satisfactory effective therapy is available to cure diabetes mellitus.[5] In recent years, traditional and complementary folk medicines have been popular in the treatment with functional food. Black beans are important sources of protein and energy in the diet. They contain "lente" digestion carbohydrates and a high proportion of non-digested carbohydrates that may be fermented in the large intestine. Theses types of carbohydrates are associated with a low glycemic response, low serum cholesterol levels, and a decrease of colon cancer risk factors. Black beans also contain several non-nutritional compounds (enzymatic inhibitors, haemaglutenins, saponins, phytic acid, and etc.).[6] Isoflavones have been proposed to be the active component responsible for the beneficial effects of soybean foods, and appear to work in conjunction with the proteins to protect against cancer, cardiovascular disease, and osteoporosis.[7] Diet supplementation with small soybean showed a significant effect on hyperglycemia, hypoinsulinaemia and glycosylated haemoglobin in STZ-induced diabetic rats. Thus, small black soybean exhibited antidiabetic action in streptozotocin (STZ)-induced diabetic rats. In rats fed small black bean powder, blood glucose level and insulin sensitivity were decreased and increased, respectively, compared to the diabetic rats without any diet.[8] The mulberry leaves are well known to improve the excretion of cholesterol resulting in low blood cholesterol content. The mulberry leaf contains hydrophilic phospholipids such as lecithin, which can be used as a natural emulsifying agent.[9] Thus, the mulberry leaf is expected as a functional food since it contains many nutrients as well as flavonoid components, GABA (aminobutyric acid), DNJ (Deoxynojirimycin)[10] or rutin in high contents. More functional food products with mulberry leaf need to be developed. Lee *et al.*[8] studied the effect of small bean powder on blood glucose reduction and insulin sensibility in STZ-induced diabetes. However, it has not well studied that *Choungggukjang* of small bean has the beneficial effect for the prevention and remedy of diabetes mellitus. Thus, the aim of this study is to investigate beneficial effects of *Chounggukjang* powder of small soybean and mulberry leaf on food efficiency ratio, blood glucose level, and HK activity of liver and kidney in STZ-induced diabetic rats.

MATERIALS AND METHODS

1. Preparation of Fermented Soybeans and Mulberry Leaf Materials

Soybean seeds of two species; Black soybean (Rhynchosia Nulubilis) and Yellow soybean (*Glycine max Merr*) were cultivated and harvested in Imsil, South Korea. For soybean fermentation process, seeds were pilled off and incubated in water for 24 hrs. The seeds were heated under the high pressure for 6 hrs and fermented in a fermentation room. The fermented beans were dried and ground to powder form for *Chounggukjang* preparation. Eight kg of mulberry leaves were boiled in 1000 L water at 115 ℃ for 3 hrs, and leaf extracts were obtained. Six different types of diet materials were prepared for *in vivo* animal experiment: Black soybean *Chounggukjang* (Bbc), Yellow soybean *Chounggukjang* (Ybc), the mixture of Bbc and Ybc (BYbc) with the same ratio, Bbc mixed with mulberry leaf extracts (MBbc), Ybc mixed with mulberry leaf extracts (MYbc), and BYbc mixed with mulberry leaf extracts (MBYbc).

2. Diabetes Induction by Streptozotocin (STZ) and Breeding Condition

Male Spraque-Dawley rats (280.18 g±9.83 g) (Samtaco Co. Ltd, Daejeon, South Korea) were obtained and fed for adaptmaintenance feeds (Samtaco) for 7 days. After adaptation, 0.2 mL [0.1 M sodium citrate buffers (pH 4.5)] of STZ, which provokes hyperglycemia due to insulin deficiency in a pancreatic-cell without influencing other organs[11,12] was subcutaneously administered to the rats to induce diabetes mellitus (70 mg of STZ/kg of rat weight).

Induction of diabetes mellitus in rats was confirmed according to glucose concentration above 300 mg/dL in tail vein on the 3rd day after STZ administration. Eight groups of 8 rats each were divided by randomized complete block design. Among the eight groups, seven groups were STZ-induced and one group was not STZ-induced. One STZ-induced group (DC) and the non- induced group (NC) were fed only distilled water. The six STZ-induced groups were fed as follows: Bbc, Ybc, BYbc, MBbc, MYbc, and MBYbc. Rats were maintained in a controlled environment with temperature at 23±1 ℃, humidity at 55±5%, and noise at below 70 db, a 14 hr and 10 hr photoperiod (lights on at 6 AM, off at 8 PM). Three mL of *Chounggukjang* powder dissolved in distilled water (0.75 mg/g of animal body weight) was administered orally to rats on the 8^{th} day after STZ administration. The oral administration was conducted once a day for four weeks.

3. Amino Acid Analysis

HPLC (AccQ-Tag)[11] analyses were performed to quantify the amounts of amino acid, fat, and water in

100 g of each different *Chounggukjang* mixtures according to the method mentioned by Wang and Murphy[12] with minor modification. One gram of *Chounggukjang* was mixed with 15 mL of 6 N hydrochloric acid and N_2 in an ampule. The mixtures were hydrolyzed at 110 ℃ for 24 hrs and cooled down. After the mixtures were diluted with 50 mL of deionized water in a flask, the diluted mixtures were filtered by using 0.2 μm filtering membrane. The samples were analyzed after derivation by AccQ-Tag method. The standard was the amino acid solution (type H; Wako, Japan). The column was 3.9×150 mm Nova-pak C 18. JASCO FP-920 fluorescence detector (Japan) was applied to detect the sample peaks at Ex. 250 nm, Em. 395 nm. The mobile phase was analyzed by gradient method using 0.14 M sodium acetates and 60% acetonitrile. Analysis interval was 50 mins and dosage was 10 μL. The column temperature was 37 ℃. All analyses were conducted according to the law of food justice.

4. Food Intake and Food Efficiency Ratio (FER)

Food intake was measured every three days. The amount of food intake per day was calculated by the mean value of amount of food intake. Body weight was measured by a weighing machine (Scaltec instruments, Sartorius, AG) in the same order at a fixed time everyday. Food efficiency ratio (FER) was calculated by percentage of body weight gain (g) per amount of food intake (g).

5. Water Intake and Urine Quantity

Amount of water that rats drank was measured in a metabolic cage to check the levels of polydipsia for 24 hrs twice a week during both the adaptation (1 week) and the experiment periods (4 weeks). Amount of rat urines was measured to check the levels of polyuria as mentioned above.

6. Collection of Blood and Organs

One drop of blood was collected from tail vein of a rat every three days, and glucose levels were measured using a test machine (An ACCU-CHEK co., Ltd.) according to manufacturer's recommendation. After *Chounggukjang* oral administration for 4 weeks, rat liver and kidney were extracted and blood was washed by cold 0.9% normal saline. The weight of washed liver and kidney was measured. To compare their weights among rats, each weight was converted to the ratio of organ weight per body weight.

7. Hexokinase (HK) Activity

To determine HK activity in liver and kidney, levels of NADPH were measured by ELISA analysis. Rat liver or kidney was incubated in 1 mL of a buffer [50 mM Tris-HCl (pH 7.4), 0.8 mM EDTA, 7.5 mM $MgCl_2$, 1.5 mM KCl, 2.5 mM ATP (2Na), 10mM creatine phosphate (2Na), 0.9 IU/mL creatine phosphokinase, 0.7 IU/mL glucose-6-phosphate dehydrogenase, 0.4 mM NADP] release to HK at 30 ℃ for 15 mins. D-glucose (10 mM) was added to the buffer containing liver or kidney. The buffer was incubated at 30 ℃ for 15 mins. One μL of the incubated buffer was mixed with 999 μL of 1X Bradford reagents (Bio-Rad). 200 μL of the mixture was placed into the well of 96-well Nunc-Immuno Maxisorp surface plate (Nunc). Absorbance was measured at 340 nm by the Bio-Rad reader.

8. Statistical Analysis

All data obtained from this study was statistically analyzed at $p<0.05$, $p<0.01$ and $p<0.001$ levels with two-way ANOVA test to confirm the differences among 8 different treatments using Statview (version 5.0) and GraphPad Prism (version 4.0).

RESULTS AND DISCUSSION

1. Amino Acid Analysis in Various Fermented Bean (*Chounggukjang*)

Amino acids of 6 different *Chounggukjang* (Bbc, Ybc, BYbc, MBbc, MYbc, and MBYbc) were analyzed (Table 1). Amount of most amino acids among 6 different *Chounggukjang* varied. The *Chounggukjang* mixture of both small soybeans and mulberry leaf had different amount of each amino acid compared to the *Chounggukjang* with only small soybeans. It is likely that these differences were affected by different soybean species, addition of mulberry leaf, or variation of fermentation process.[8,13] In this study, it is unlikely that variation of fermentation process did occur since the process was conducted in the same controlled conditions. Many studies showed close correlations between diabetes and amino acids. Mulberry leaves contain volatile and non-volatile ingredients. The volatile ingredients are guaiacol, eugenol, methylsalicylate, benzaldehyde and phenylacetaldehyde. Most non-volatile ingredients are various flavonoids in high concentrations. In addition, mulberry leaves contain rutin, quercetin, isoquercetin, astragalin, quercetin-3,7-diglucoside, quercetin-3-triglucoside,[14-18] and 1-deoxynojirimycin (DNJ)[19] which inhibit the activity of α-glycosidease and N-containing sugar.[20]

In mulberry leaves, 50 inorganic ingredients, 59 organic

Table 1. Amino acids of various fermented beans grown in Imsil, Korea.

(Unit : mg/100 g)

Amino acid	Ybc	BYbc	MBbc	MYbc	MBYbc	Bbc
Aspartic acid	4,510±384	4,605± 20	4,011± 85	4,430± 80	4,701±133	5,119± 72
Serine	1,136± 54	959± 20	1,096±113	1,029± 96	1,306±105	1,254±123
Glutamic acid	7,448±563	7,692± 51	6,754±105	7,162±241	7,680± 80	8,549± 40
Glycine	1,819±119	1,813± 26	1,591± 14	1,735± 39	1,858± 26	1,750±114
Histidine	1,202±134	1,208±108	1,078± 16	1,170± 77	1,170± 88	1,054± 85
Threonine	1,049± 15	878± 55	889± 60	894± 43	1,048± 87	1,334±115
Arginine	2,426±219	2,433±310	2,566± 44	2,372± 78	2,629± 72	3,306±180
Alanine	1,789±143	1,766± 21	1,478± 46	1,640± 29	1,832± 9	1,971± 60
Proline	1,997±265	2,016± 58	1,749± 35	1,852±254	2,175±119	2,247±146
Cystein	337± 11	211± 13	202± 11	257± 44	335± 13	82± 18
Tyrosine	1,307±148	1,200± 23	1,294± 37	1,273± 66	1,507± 12	977± 49
Valine	1,912±167	1,985± 20	1,705± 46	1,900± 23	2,133± 38	2,350±162
Methionine	510± 75	529± 80	455± 94	561± 38	924± 18	378± 46
Lysine	2,236±194	2,246±160	1,936± 28	2,194± 9	2,373± 12	2,684±117
Isoleucine	1,608±145	1,641± 62	1,438± 30	1,595± 11	1,982± 22	2,217±135
Leucine	2,483±189	2,550±110	2,320± 33	2,511± 14	3,083± 15	3,396±178
Phenylalanine	1,913±179	1,988± 23	1,738± 49	1,951± 45	2,033± 1	2,076±136
Total	**35,680.5**	**35,718.7**	**32,296.9**	**34,580.0**	**38,769.4**	**4,0743.2**
Protein (%)	39.8	39.7	35.1	38.7	39.1	37.0
Lipid	22,080±0.02	19,510±0.03	17,760±0.01	20,130±0.08	20,260±0.24	18,260±0.01
Lime powder (g/100 g)	5,580±0.00	5,550±0.02	5,630±0.02	5,630±0.02	5,890±0.01	5,320±0.02
Moisture (mL/100 mL)	7,060±0.03	7,310±0.03	9,100±0.00	7,490±0.00	7,380±0.03	9,780±0.00

ingredients, and 21 amino acids exist. For the inorganic ingredients, particularly, Ca, K and Fe contents are high in the leaves. The mulberry leaves contain hydrophilic phospholipids such as lecithin, which is used as a natural emulsifying agent. Thus, the mulberry leaves are expected as a functional food since they contain many nutrients as mentioned above. Mulberry leaves inhibit the activity of α-glycosidease, resulting in the reduction of blood glucose level.[21,22] Other biological activities of mulberry leaf include the reduction of triglyceride and cholesterol degradation in the bloodstream, arteriosclerosis and hyperlipemia.[23-26] A previous study reported[27] that animals fed food mixed with mulberry leaf reduced 20% of blood glucose. These previous studies suggested that fermented black soybean, yellow soybean, and mulberry leaf give a synergistic effect on diabetic remedy in animals.

2. Effect of *Chounggukjang* on Body Weight in Rats

The effect of various *Chounggukjang* (fermented bean powders) on the body weight was analyzed in normal and diabetic rats for 4 weeks (Fig. 1). In normal rat group treated with distilled water (NC), the mean body weight increased up to 397.9 g on 30 day (Fig. 1). In streptozotocin (STZ)-induced diabetic rats treated with distilled water (DC), the mean body weight was steady (263.9 g). The mean body weight of DC (263.9 g) significantly more

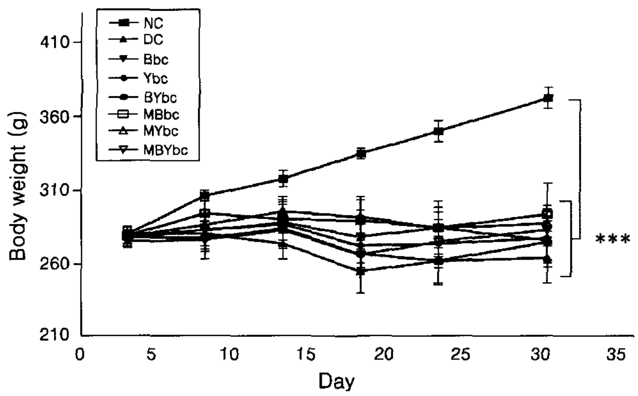

Fig. 1 Changes of body weight in normal and diabetic rats fed various fermented beans powder (*Chounggukjang*).

NC: normal control fed water, DC: diabetic control fed water,
Bbc: fermented *Rhynchosia Nulubilis*, Ybc: fermented *Glycine max Merr*,
BYbc: fermented *Rhynchosia Nulubilis* and *Glycine max Merr*,
MBbc: mulberry leaf and fermented *Rhynchosia Nulubilis*,
MYbc: mulberry leaf and fermented *Glycine max Merr*,
MBYbc: mulberry leaf and fermented *Rhynchosia Nulubilis*, *Glycine max Merr*.
Each column represents mean value of body weight (n = 8).
*** $p<0.001$ vs. normal control group (NC).

decreased ($p<0.001$) than that of NC (397.9 g). Most rat groups with oral *Chounggukjang* administration tended to show higher increase of the body weight than DC. The body weight of Bbc, Ybc, BYbc, MBbc, MYbc, and MBYbc groups was increased with rates of 26.9%, 26.7%, 17.1%, 58.1%, 59.1% and 46.9% compared to that of DC, respectively. However, regardless of different types of *Chounggukjang* administration, in all STZ-induced diabetic rat groups, the body weight significantly decreased compared to NC group. The body weight of STZ-induced diabetic rat decreased due to insulin deficiency and dysfunction of energy metabolism.[28] STZ induced high levels of serum glucose and triglyceride in rats. STZ also caused higher levels of serum calcium and lower levels of serum inorganic phosphorus levels due to the disorder of bone metabolism.[29]

3. Effect of *Chounggukjang* on Blood Glucose Level

Blood glucose levels were measured to investigate the effect of *Chounggukjang* in rats (Fig. 2). In all STZ-induced diabetic rat groups, blood glucose levels increased, resulting in hyperglycemia (>500 mg/dL) in DC for 4 weeks. In STZ-induced diabetic rat groups treated with *Chounggukjang* and DC, the glucose levels rapidly increased up to 8 day. After 8 day, the increase rate was reduced. On 30 day, blood glucose levels of Bbc, Ybc, BYbc, MBbc and MYbc were 445 mg/dL, 448 mg/dL, 480 mg/dL, 434 mg/dL and 422mg/dL, respectively (Fig. 2). MBYbc (380 mg/dL) showed the blood glucose reduction of 26.5% compared to DC (517 mg/dL). Although *Chounggukjang*-treated rat groups didn't show significant reduction of blood glucose levels compared to DC, we observed the tendency of reduction in the *Chounggukjang*- treated rat groups on 30 day. In a previous study, a certain level of mulberry leaf in a diet was required to obtain significant reduction of blood glucose level in STZ- diabetic rats.[30] Thus, in the present study, it is likely that the amount of *Chounggukjang* or mulberry leaf was not enough to give significant reduction of blood glucose level in rats.

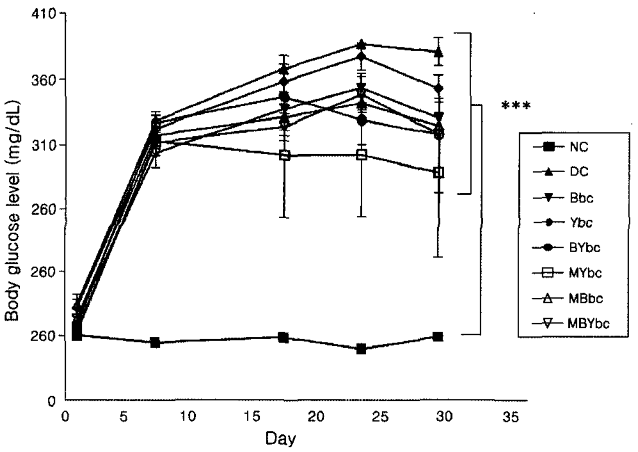

Fig. 2 Fasting blood glucose level in STZ-induced diabetic rats fed various fermented beans (*Chounggukjang*) for 30 days.

NC: normal control fed water, DC: diabetic control fed water,
Bbc: fermented *Rhynchosia Nulubilis*, Ybc: fermented *Glycine max Merr*,
BYbc: fermented *Rhynchosia Nulubilis* and *Glycine max Merr*,
MBbc: mulberry leaf and fermented *Rhynchosia Nulubilis*,
MYbc: mulberry leaf and fermented *Glycine max Merr*,
MBYbc: mulberry leaf and fermented *Rhynchosia Nulubilis*, *Glycine max* Merr.
Each column represents mean value of fasting blood glucose level (n = 8).
*** $p<0.001$ vs. normal control group (NC).

4. Effect of *Chounggukjang* on Kidney and Liver Weights in Rats.

Weight of liver and kidney after diet administration was measured in each experimental group and converted to weight of organs per body weight (Fig. 3). Kidney weights of all diabetic rat groups significantly increased compared to that of NC ($p<0.001$). However, kidney weights of all *Chounggukjang*-treated groups significantly decreased compared to that of DC ($p<0.01$ or 0.05). The volume of the glomerular tuft and its components was estimated in juvenile diabetics at onset and after one to six year's duration of the disease. An enlargement of the glomerular tuft was demonstrated in the newly diagnosed diabetics. The volume of the individual capillary lumina and of the individual glomerular cells was enlarged, whereas the number of cells was unchanged.[31,32] Diabetes-induced renal hypertrophy produces enhanced dimensions of renal cells along with increased kidney weight.[33]

The liver weight of Bbc, Ybc, BYbc, MBbc, DC ($p<$ 0.001), MYbc and BYbc ($p<0.01$) significantly increased compared to that of NC. However, compared to DC, the liver weight of MYbc and MBYbc decreased at the rate of 8.9% and 12.4%, respectively. STZ-induced diabetes suffered from abnormal immunity in liver. Hypertrophied

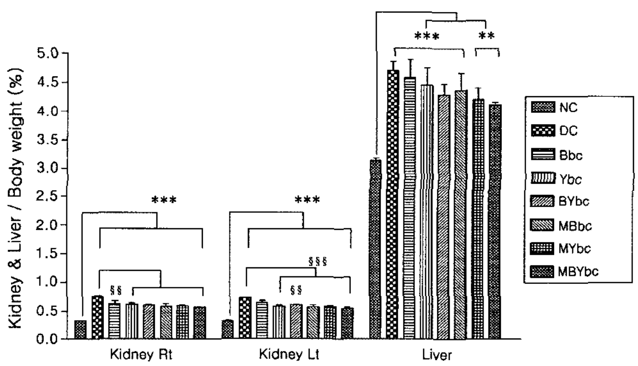

Fig. 3 The liver and kidney weight in STZ-induced diabetic rats fed various fermented beans (*Chounggukjang*) for30days.

NC: normal control fed water, DC: diabetic control fed water,
Bbc: fed fermented *Rhynchosia Nulubilis*, Ybc: fermented *Glycine max Merr*,
BYbc: fermented *Rhynchosia Nulubilis* and *Glycine max Merr*,
MBbc: mulberry leaf and fermented *Rhynchosia Nulubilis*,
MYbc: fermented *Glycine max Merr* and mulberry leaf,
MBYbc: fermented *Rhynchosia Nulubilis* and *Glycine max Merr* and mulberry leaf.
Each column represents mean value of liver or kidney weight (n = 8).
** $p<0.01$ vs. normal control group (NC),*** $p<0.001$ vs. normal control group (NC), §§ $p<0.01$ vs. diabetic control group DC), §§§ $p<0.001$ vs. diabetic control group (DC).

liver was often observed in the STZ-induced diabetes because lipid component was accumulated in the liver where gluconeogenesis normally was not active.[34,35] Diabetes is also associated with hyperlipidemia.[36]

5. Water Intake and Urine Quantity

After STZ-induction in rats, polyuria and polydipsia, distinctive symptoms of diabetes mellitus gradually increased. Water intake and urine quantity were measured twice a week for 30 days after STZ administration (Fig. 4).

Water intake and urine quantity of STZ-induced diabetes groups with different diet administration significantly increased compared to NC. Urine quantity of DC, Ybc, and BYbc significantly increased ($p<0.001$), and that of Bbc, MBbc, MYbc and MBYbc significantly increased ($p<0.01$) compared to NC. As compared to DC, the urine quantity of Bbc, MBbc, MYbc, and MBYbc decreased with the rate of 17.1%, 20.8%, 27.0% and 15.8%, respectively. In all diabetes groups, water intake significantly increased compared to NC. Except for MBbc, water intake of DC and all diet groups significantly increased compared to NC ($p<0.001$). Compared to DC, the water intake of MBbc decreased at the rate of 31.8%.

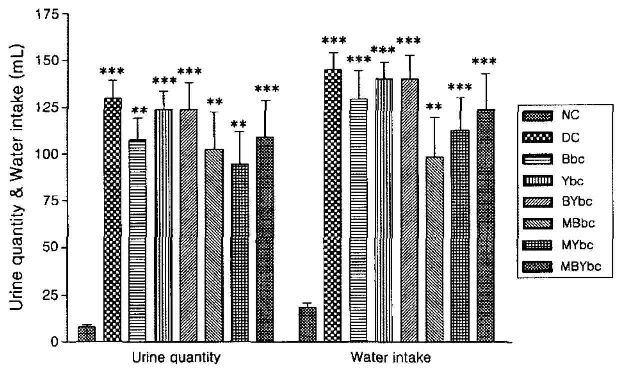

Fig. 4 Water intake level and urine quantity in STZ-induced diabetic rats fed various fermented beans (*Chounggukjang*) for 30 days.
NC: normal control fed water, DC: diabetic control fed water,
Bbc: fermented *Rhynchosia Nulubilis*, Ybc: fermented *Glycine max Merr*,
BYbc: fermented *Rhynchosia Nulubilis* and *Glycine max Merr*,
MBbc: mulberry leaf and fermented *Rhynchosia Nulubilis*,
MYbc: mulberry leaf and fermented *Glycine max Merr*,
MBYbc: mulberry leaf and fermented *Rhynchosia Nulubilis, Glycine max Merr.*
Each column represents mean value of water intake or urine quantity (n = 8).
** $p<0.01$ vs. normal control group (NC), *** $p<0.001$ vs. normal control group (NC).

6. Food Intake Quantity and Food Efficiency Ratio (FER)

Food intake, food efficiency ratio (FER) and changed body weight in STZ-induced diabetic rats fed various beans were observed for 4 weeks (Table 2). Food intake of all diabetes groups significantly increased compared to DC ($p<0.001$). These results agree with Lau and Failla's report.[32] The food intake quantity was not significantly different between DC and all diet groups. These results are not surprising because diabetes induces gluttony.

Body weight of NC (47.5 g±3.8 g) increased after 4 weeks, whereas that of all diabetes groups decreased. Bbc showed the highest weight loss (-40.2 g±4.6 g), and MBbc showed the lowest loss (-10.0 g±3.5 g). Thus, FER in all diabetes groups was significantly lower than NC ($p<0.001$). FER of all diet groups tended to show higher values than NC. Despite lots of food intake in diabetes groups compared to NC, the weight loss and decreased FER phenomena were resulted from a degenerative change of *in vivo* metabolism by diabetes.[37] The FER in MBbc and MBYbc significantly increased compared to other

Table 2. The food intake and food efficiency ratio (FER) in STZ-induced diabetic rats fed various fermented soybeans (*Chounggukjang*) for 4 weeks.

Group[1)]	Body weight change (g/4 Weeks)	Food in take 1 Week	2 Week	3 Week	4 Week	Means	FER[2)]
NC (n=8)	47.5± 3.8[3)]	11.0± 1.41[4)]	11.5±2.1	13.0± 1.4	12.0± 5.7	11.9± 2.5	101.2±37.1
DC (n=8)	-62.9±15.9***	21.0± 4.2***	27.0±4.2*	32.5± 5.0	27.0± 0.0	26.9± 5.3	57.1±58.1***
Bbc (n=8)	-40.2± 4.6***	29.0± 4.2***	29.5±0.7*	29.5± 3.5	27.0± 2.8	28.8± 2.6	34.9±15.6***
Ybc (n=8)	-23.4± 6.5**	25.0± 4.2***	22.5±2.1	35.5± 2.1	26.5± 3.5	27.4± 5.8	19.3±21.7**†
BYbc (n=8)	-16.3± 7.0**	14.0±11.3***	27.5±0.7*	33.5± 0.7	35.5± 2.1*	27.6±10.0	24.6±39.6***
MBbc (n=8)	-10.0± 3.5*	21.5±13.4***	26.5±2.1*	19.5±12.3	18.5± 6.4	21.5±11.0	10.9±16.5**†
MYbc (n=8)	-13.8± 7.9**	25.0± 9.9***	17.5±7.8	29.0± 5.7	25.5± 3.5	24.3± 7.0	20.4±42.9***
MBYbc (n=8)	-20.8± 5.1**	25.0± 8.5***	28.5±2.1*	26.5± 9.2	22.0±12.7	25.5± 7.3	19.4±18.6**†

1) NC: normal control fed water, DC: diabetic control fed water, Bbc: fermented *Rhynchosia Nulubilis*, Ybc: fermented *Glycine max Merr*, BYbc: fermented *Rhynchosia Nulubilis* and *Glycine max Merr*, MBbc: mulberry leaf and fermented *Rhynchosia Nulubilis*, MYbc: mulberry leaf and fermented *Glycine max Merr*, MBYbc: mulberry leaf and fermented *Rhynchosia Nulubilis* and *Glycine max Merr*.
2) FER: [Body weigh gain (g/4 weeks) / Food intake (g/4 weeks)]×100.
3) Each column represents mean value±S.D. (n=8).
4) Values with different superscripts within the same column are significantly different at p<0.05 level (Two-way ANOVA test).
* $p<0.05$ vs. normal control group (NC), ** $p<0.01$ vs. normal control group (NC), *** $p<0.001$ vs. normal control group (NC), † $p<0.05$ vs. diabetic control group (DC).

groups. These results suggest that small black soybean is effective to increase FER.

7. Hexokinase Activity

The HK activities of liver and kidney (right, left) were observed in rats (Fig. 5). The initial step in glucose (Glc) metabolism through most common pathways is phophorylation to form Glc-6-phosphate (G-6-P), the reaction is catalyzed by hexokinase (HK).[38] Inactivation of HK was induced by the diabetes mellitus.[39-41] By this mechanism, HK is maintained low, which permits facilitated Glc entry into cells. The HK initiates all major pathways of Glc utilization, including the glycolytic, pentose phosphate, and uronic acid pathways.[42-44] The deficiency of HK activity in cultured glomerular mesangial cells increased by factors associated with renal injury[45,46] or altered injury susceptibility.[47] Diabetes mellitus causes complication and functional abnormality in kidney because intracellular Glc-6-phosphate dehydrogenase (G6PD)-deficient cells have an increased propensity for hydrogen peroxide (H_2O_2)-induced senescence.[48] The HK activity of liver did not significantly increased in all *Chounggukjang*-treated groups compared to DC and NC. The HK activity of kidney in DC decreased by 51.9% compared to that of NC. MBbc, MYbc and MBYbc treated groups showed increasing trend of the HK activity of kidney compared to NC. In detail, the HK activity of left kidney in MYbc and MBYbc groups significantly increased with the rate 55.5% and 53.2%, respectively (p<0.05). The HK activity of right kidney in MBYbc group significantly increased with the rate 41.5% compared to DC (p<0.05).

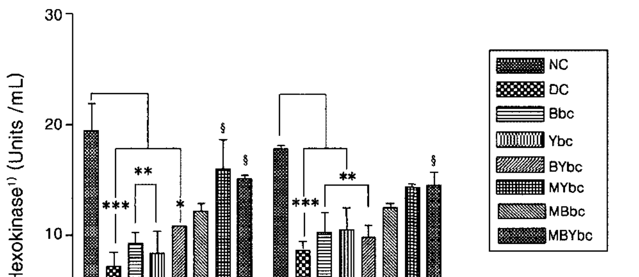

Fig. 5 Hexokinase activity in STZ-induced diabetic rats fed various fermented beans (*Chounggukjang*) for 30 days.

NC: normal control fed water, DC: diabetic control fed water,
Bbc: fermented *Rhynchosia Nulubilis,* Ybc: fermented *Glycine max Merr,*
BYbc: fermented *Rhynchosia Nulubilis* and *Glycine max Merr,*
MBbc: mulberry leaf and fermented *Rhynchosia Nulubilis,*
MYbc: mulberry leaf and fermented *Glycine max Merr,*
MBYbc: mulberry leaf and fermented *Rhynchosia Nulubilis* and *Glycine max Merr.*
Each column represents mean value of hexokinase activity (n=8).

1) Hexokinase (Units/mL) = [(A340 nm/min test - A340 nm/min blank)×Total Volume (mL) of assay]/(millimolar extinction coefficient of β-NADPH at 340 nm×Volume (mL) of enzyme used×Protein)

* p<0.01 vs. normal control group (NC), ** p<0.01 vs. normal control group (NC), *** p<0.001 vs. normal control group (NC), † p<0.05 vs. diabetic control group (DC).

In the present study, HK activities in liver were not significantly different between NC and DC. In contrast, HK activities in the kidney of some *Chounggukjang*-treated groups significantly increased compared to DC. In kidney, our results are consistent with the previous study showing increased HK activity in kidney through exogenous substance.[49] However, in liver, our results were inconsistent with the previous study.[49] No significant improvement of HK activity in liver was observed (Fig. 5). Compared to kidney [7 to 20 (Unit/mL)], the detection sensitivity of HK activity in liver [1 to 3 (Unit/mL)] was almost 10 times less. It is speculated that the released amount of HK enzymes from liver samples was not high enough to be detected at the significant level by the current analytical method. Indeed, only 10 % portion of liver tissue was sampled to release total protein to the buffer for analysis. In the future, the highly concentrated total protein from liver sample should be tested to confirm whether *Chounggukjang* and mulberry leaf improve HK enzyme activity in STZ-diabetic rats. Although the kidney HK activities of Bbc, Ybc, and BYbc significantly differed from that of NC, the promising effect of diabetic remedy on the improvement of HK activity was observed compared to DC. The results suggest that *Chounggukjang* with mulberry and small black soybean powder are highly effective to increase HK activity in kidney of STZ-induced diabetic rats for remedy of diabetes milletus.[50]

SUMMARY & CONCLUSION

This study was conducted to investigate the effect of *Chounggukjang* (fermented small soybean powder) with mulberry leaf on metabolic improvement and HK activity in Streptozotocin (STZ)-induced diabetic rats for diabetic remedy. Most of composition of amino acids of Bbc, which is effective for diabetes remedy was higher than those of Ybc except glycine, cystein, tyrosine and methionine. STZ (70 mg/kg of rat body weight) was subcutaneously administered to rats. The induction of diabetes mellitus in rats was confirmed with the blood glucose levels (>300 mg /dL). Eight experimental groups of 8 rats each were as follows: control group (NC) fed water, diabetic group (DC) fed water, Bbc, Ybc, BYbc, MBbc, MYbc and MBYbc as described in Materials and Methods. Body weight of NC (47.5 g±3.8 g) increased 4 weeks after *Chounggukjang* diet administration. However, all diabetes groups showed

body weight loss. The body weight loss of Bbc was the highest (-40.2 g±4.6 g), and that of MBbc was the lowest (-10.0 g±3.5 g). Food efficiency ratios (FER) of all diabetes groups were significantly lower than NC ($p<0.001$). The FER of Ybc, MBbc and MBYbc was significantly higher than DC ($p<0.001$). In the rat groups fed fermented soybeans, the body weight, FER, glucose level, and HK activity significantly differed as compared to DC. Among them, the mixture of fermented soybeans and mulberry leaf significantly inhibited the blood glucose level and urine quantity, whereas increased weight of kidney and liver, food intake, and HK activity. The initial step in Glc metabolism is phosphorylation to form Glc-6-phosphate (Glc-6-P), which is catalyzed by HK. In this study, HK activity of kidney in all diet groups significantly increased compared to NC and DC ($p<0.05$). These results suggested that the functional food for remedy of diabetes mellitus using *Chounggukjang* with mulberry leaf and small black soybean are highly effective to recover HK activity of kidney in STZ-induced diabetic rats.

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