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Antidiabetic Activity of Mung Bean Extracts in Diabetic KK-A^y Mice

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The antidiabetic effects of Mung bean sprout (MBS) extracts and Mung bean seed coat (MBSC) extracts were investigated in type 2 diabetic mice. Male KK-A^y mice and C57BL/6 mice were used in this study. In KK-A^y mice, the blood glucose, plasma C-peptide, glucagon, total cholesterol, triglyceride, and blood urea nitrogen (BUN) levels were significantly higher than those in the C57BL/6 mice ($P < 0.001$, $P < 0.001$, $P < 0.01$, $P < 0.001$, $P < 0.01$, and $P < 0.01$). In addition, KK-A^y mice showed an obvious decrease in insulin immunoreactivity in pancreas as well. MBS and MBSC were orally administrated to KK-A^y mice for 5 weeks. It was found that MBS (2 g/kg) and MBSC (3 g/kg) lowered blood glucose, plasma C-peptide, glucagon, total cholesterol, triglyceride, and BUN levels and at the same time markedly improved glucose tolerance and increased insulin immunoreactive levels. These results suggest that MBS and MBSC exert an antidiabetic effect in type 2 diabetic mice.

KEYWORDS: Mung bean sprouts; mung bean seed coats; antidiabetic activity; KK-A^y mice

INTRODUCTION

Diabetes mellitus is a serious, complex chronic condition that is a major source of ill health all over the world. This metabolic disorder affects approximately 4% of the population worldwide and is expected to increase to 5.4% in 2025 (*1*). Diabetes mellitus is characterized by hyperglycemia and carbohydrate, protein, and fat metabolism disturbances. The control of diabetes mellitus normally involves exercise, diet, and chemotherapy. The chemical drugs for diabetes have a number of limitations, such as adverse effects and high cost. In contrast, herbal medicine is likely to have a similar degree of efficacy without the troublesome side effects associated with chemical drug treatment. Presently, there is growing interest in herbal remedies for the treatment of diabetes mellitus. More than 400 plants with glucose-lowering effects are known (*2*). Grains and cereals are the main source of energy for Asians, and whole grains and cereals are recommended for diabetes to control blood glucose (*3*).

Mung bean (*Vigna radiatae* L.) is native to the northeastern India-Burma (Myanmar) region of Asia. There is now much interested in it for its physiological functionalities, such as antitumor activity (*4*), angiotensin I-converting enzyme (ACE) inhibitory activity (*5*), and antioxidant activity (*6*). Mung bean sprouts (MBSs) harvested after 5 days of germination are popular consumed food items. The seed coats lost in the germination progress are commonly used as inner pillows or fertilizer in China. Both mung bean seed coats (MBSCs) and mung bean sprouts are traditional medicines for the treatment of several diseases, mainly to reduce fever and to remove toxic materials. Mung bean is an important natural source of D-*chiro*-inositol (DCI). Obendorf and Horbowicz detected free DCI in lupines, pigeon peas, soybeans, chickpeas, mungbeans, and buckwheat; among all of the seeds analyzed, mung bean contained the highest levels of free DCI than others (*7*). A deficiency of the DCI phosphoglycan mediator of the action of insulin may result in the resistance to insulin. Insulin resistance has been linked to decreased urinary excretion of *chiro*-inositol (a component of the putative DCI phosphoglycan mediator) in primates, in humans with impaired glucose tolerance or type 2 diabetes mellitus, and in nondiabetic first-degree relatives of persons with diabetes (*8*−*11*). Mung bean has been suggested as a dietary food for diabetic patients for a long time because of its high fiber and protein contents. Diabetic nephropathy is characterized by a thickening of the basement membrane, expansion of the mesangium, reduced filtration, albuminuria, and ultimately renal failure. Advanced glycation end products (AGEs) have been detected in renal tissues in amounts that correlate with the severity of diabetic nephropathy (*12*).

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Hyperglycaemia and AGEs increase the release of transforming growth factor-b (TGF-b), which in turn stimulates the synthesis of collagen matrix components, and this may account for, at least in part, the thickening of the basement membrane in diabetic nephropathy (*13*). Peng's study confirmed that phenolics extracted from mung bean are effective in inhibiting the formation of AGEs in vitro, and mung bean is a beneficial food choice for diabetics as AGEs, which are important pathogenetic mediators of various diabetic complications and other diseases (*14*).

To our knowledge, this is the first biochemical investigation on the effect of mung bean products, MBSs and MBSCs, on diabetic animals. This report is on the effect of MBSs and MBSCs on elements of diabetes mellitus including blood glucose, lipid profiles, and immunohistochemical evaluation in KK-A^y mice.

MATERIALS AND METHODS

Materials. MBSs and its coats were purchased from a local market in Beijing, China. They were dried at 40 °C, ground with a laboratory mill, and passed through a 80 mesh screen sieve. DCI standard (99%), vitexine standard (≥96%), and isovitexine standard (≥98%) were purchased from Sigma-Aldrich (Shanghai, China). All analytical and high-performance liquid chromatography (HPLC) grade solvents used were purchased from Fisher Chemicals (Shanghai, China).

Preparation of Crude Extracts. MBS and MBSC samples were individually extracted with 80% ethanol for 4 h at room temperature. The crude extracts were obtained by filtration, and the extracts were evaporated until dryness with a rotary evaporator, under reduced pressure at 40 °C. After dehydration, the dried powder extracts were stored at 4°.

Determination of Antidiabetic Compounds. *DCI Extraction.* The DCI content in the extracts was determined according to the method established by Nan Yang and Guixing Ren in our laboratory (*15, 16*). In brief, the extraction was performed by mixing 1 g of sample with 20 mL of ethanol:water (1:1, v/v) solution in one conical flask and incubating in the water bath shaker under room temperature for 30 min. The extract was filtered through Whatman #4 filter paper, with 1 mL of supernatant being transferred into a little vial equipped with a cover. The vial was then put into an oven to dry and was then redissolved by 1 mL of methanol. This solution was filtered through 0.45 μm Milex-HN syringe filters (13 mm) (Bedford, MA) and transferred into HPLC autosampler vials for immediate HPLC-ELSD (evaporative light scattering detector) analysis.

Vitexin and Isovitexin Extraction. The extraction was accomplished with 70% methanol (3 × 10 L) in one conical flask and was incubated in the water bath shaker under 30 °C for 2 h. The extracts were combined and filtered through Whatman #4 filter paper, with 1 mL of supernatant being diluted to 25 mL in methanol. Then, 1 mL of the diluted solution was filtered through 0.45 μm Milex-HN syringe filters (13 mm) (Bedford, MA) before injection into the HPLC.

HPLC Analysis. The HPLC system consisted of two Shimadzu LC-20A pumps, a Shimadzu LC-20A autosampler, and a SPD-20A UV/vis detector (Tokyo, Japan).

DCI HPLC Analysis. An Alltech Prevail Carbohydrates ES 5 μm column (4.6 mm × 250 mm, Alltech, Deerfield, IL) was used. The mobile phase was composed of acetonitrile and distilled water (70:30, v/v), the flow rate was set at 1 mL/min, and the elute after the column was sent to an ELSD (Alltech). ELSD conditions were optimized to achieve maximum sensitivity, the temperature of the drift tube was set at 95 °C, the nebulizing gas flow rate was set at 2.2 L/min, and the gain was set at 1.10 μL, and DCI extract was injected for each analysis.

Vitexin and Isovitexin HPLC Analysis. An Alltima C_{18} column (4.6 mm × 250 mm, Metachem Technologies Inc., Torrance, CA) was used. The wavelength of the UV detector was set at 360 nm. The mobile phases were water with 0.2% acetic acid (solvent A) and acetonitrile (solvent B). The elution started with 5% B with a linear gradient to 20% B in 18 min. Then, it was 25−90% B from 18 to 30 min. The flow rate was set at 0.8 mL/min, and the injection volume was 10 μL.

Animals. Diabetic KK-A^y mice, frequently used as an animal model for noninsulin-dependent diabetes, were used in this study (*17−20*). Fifty male KK-A^y mice and 10 male C57BL/6 mice were purchased from the Department of Laboratory Animal Science Center (Beijing, China). The diabetic mice were classified into five groups according to their weights and blood glucose levels to make the average weights and blood glucose levels similar among the groups: group I, control diabetic animals ($n = 10$); group II, diabetic animals given MBS, 3 g/kg ($n = 10$); group III, diabetic animals MBS, 2 g/kg ($n = 10$); group IV, diabetic animals given MBS, 1 g/kg ($n = 10$); and group V, diabetic animals given MBSC, 3 g/kg ($n = 10$). The C57BL/6 mice were in group VI. Body weights were measured during the MBS and MBSC treatments. All mice were housed individually in stainless steel wire-bottom cages in an air-conditioned room kept at controlled ambient temperature (22 ± 1 °C), humidity (50 ± 10%), and a 12 h light/dark cycle. The C57BL/6 mouse diet was conducted according to the general quality standard for formula feeds of laboratory animals in China (GB 14924.1, 2001). The composition of the diets fed to KK-A^y mice was as follows: standard diet, 70%; sucrose, 10%; lard, 10%; and yolk powder, 10%. They were allowed free access to the diet and water. Blood glucose levels were determined for each mouse throughout the experiment. The experiment was carried out according to the European Community guidelines for the use of experimental animals and approved by the Peking University Committee on Animal Care and Use.

Fasting Blood Glucose Levels and Oral Glucose Tolerance Test (OGTT). Fasting animals were food-restricted and given only water to drink the night before the experiment. Blood glucose levels were determined using blood samples from the tail vein by a glucose analyzer (ACCU-CHEK Active, Roche, Shanghai, China) every week. On the morning of OGTT, fasting animals were used for oral glucose (2 g/kg). Blood glucose levels were taken at 0 (before oral glucose), 30, 60, and 120 min after glucose administration.

Analysis of Plasma Samples. At the end of the experimental period, the animals were sacrificed after an overnight fast, and blood samples were collected. The blood samples were kept at room temperature for 4 h and then centrifuged at 3000*g* for 20 min. C-peptide was determined by an enzyme-linked immunosorbent assay kit (ADL, San Diego, CA). Glucagon was determined by an enzyme-linked immunosorbent assay kit (RapidBio Laboratory, Calabasas, CA). Total cholesterol, triglycerides, and blood urea nitrogen (BUN) were measured in plasma from all animals, using an autobiochemical analyzer (Hitachi 7600, Japan).

Immunohistochemical Evaluation on Pancreas. The pancreas was removed immediately from the animals after sacrificing and rinsed in ice-cold saline. The tissue samples were fixed in paraformalclehyde, dehydrated in a graded series of ethanol, and embedded in paraffin wax before sectioning. Sections were dewaxed and rehydrated. After the step of washing in phosphate-buffered saline, sections were immersed in a solution of 3% H_2O_2 for 10 min. The sections were then preincubated with nonimmune serum for 15 min and subsequently replaced with the mouse anti-insulin antibody (1:200, SP-9000, ZYMED, CA) for incubation at 4 °C for 16 h. Biotinylated goat antimouse immunoglobulin was used as a secondary antibody. They were labeled with streptavidin peroxidase following incubation with the secondary antibody at 37 °C for 30 min. The localization of the antigen was indicated by a brown color obtained with 3-amino-9-ethylcarbazole (AEC) as a chromogenic substrate for peroxidase activity. Slides were counterstained with hematoxylin for microscopic observation. The specificity of the immunohistochemical staining was checked by omission of the primary antibody or by using an inappropriate antibody (antigastrin).

RESULTS

Antidiabetic Compounds Content. The contents of DCI, vitexin, and isovitexin in MBSs extracts are 24.16, 11.68, and 5.40 mg/g, and in MBSC extracts, the contents are 0.0053, 15.22, and 11.42 mg/g, respectively.

Body Weight Gains of KK-A^y and C57BL/6 Mice. As shown in **Table 1**, the KK-A^ymice body weights were significantly higher than age-matched C57BL/6 mice. Initial body

Table 1. Effect of MBS and MBSC on Body Weight in Diabetic KK-A^y and C57BL/6 Mice

	group I[a]	group II[b]	group III[c]	group IV[d]	group V[e]	group VI[f]
initial body weight (g)	36.06 ± 1.20	36.70 ± 1.08	35.77 ± 1.04	35.71 ± 1.75	35.80 ± 1.68	16.70 ± 0.54
final body weight (g)	37.53 ± 1.06	37.74 ± 1.87	36.86 ± 1.35	35.84 ± 1.67	36.91 ± 1.82	16.80 ± 0.42

[a] As control diabetic KK-A^y mice. [b] As diabetic KK-A^y mice given MBSs, 3 g/kg. [c] As diabetic KK-A^y mice given MBSs, 2 g/kg. [d] As diabetic KK-A^y mice given MBSs, 1 g/kg. [e] As diabetic KK-A^y mice given mung bean coats, 3 g/kg. [f] As C57BL/6 mice.

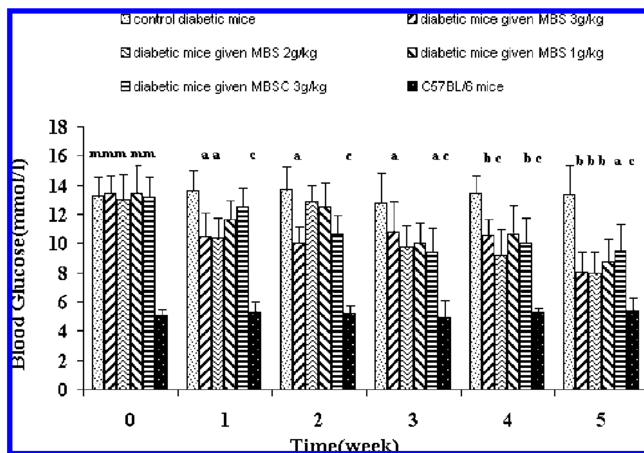

Figure 1. Changes of blood glucose levels in different experimental groups. Each column represents the mean ± SD of 10 animals. ^{m}P < 0.001, as compared with C57BL/6 mice. ^{a}P < 0.05, ^{b}P < 0.01, and ^{c}P < 0.001, as compared with control.

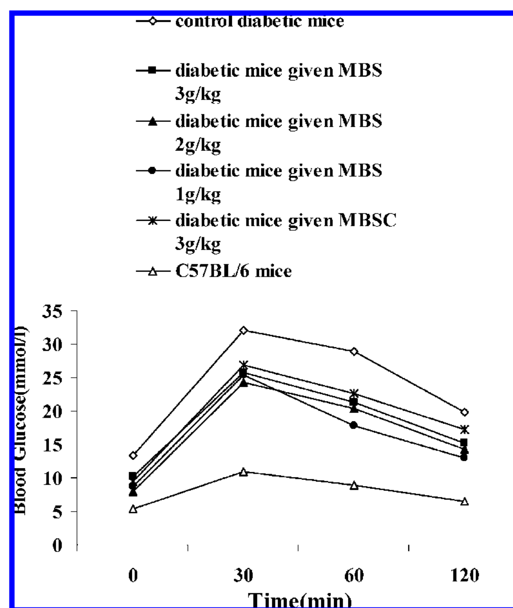

Figure 2. OGTT at the end of the 5 weeks of treatment. Each column indicates the mean ± SD of 10 animals.

weights in KK-A^y mice were similar. There were trends of increase in the weights during supplementation of MBS and MBSC for 5 weeks.

Changes in Blood Glucose Level. Figure 1 shows the changes in blood glucose levels of different experimental groups over the experimental period. The KK-A^y mice (groups I−V) showed a significant increase in the level of blood glucose as compared with C57BL/6 mice (group VI) at the beginning. Oral administration of MBS (3 and 2 g/kg) and MBSC (3 g/kg) lowered the observed blood glucose level.

Blood Glucose Tolerance. Figure 2 shows the blood glucose levels of each group of mice after oral administration of glucose. The blood glucose level in the C57BL/6 mice (groupVI) rose to a peak value 30 min after glucose load and decreased to normal levels at 120 min. In KK-A^y control mice (group I), the higher peak value was observed after 60 min and remained high over 60 min. MBS- and MBSC-treated KK-A^y mice showed significant decreases in blood glucose levels at 30 and 120 min as compared with control KK-A^y mice.

Plasma Biomarkers. Plasma C-peptide and glucagon levels were significantly higher in the control KK-A^y mice than in the C57BL/6 mice. The supplementation of MBS (2 g/kg) and MBSC (3 g/kg) reversed the blood C-peptide and glucagon levels in type 2 diabetic animals as compared with the control KK-A^y mice (**Table 2**).

The triglyceride and total cholesterol concentrations in the serum were significantly higher in the control KK-A^y mice than those in the C57BL/6 mice (**Table 2**). In KK-A^y mice fed with MBSC, the increases in triglycerides and total cholesterol were eliminated. The supplementation of MBS in group II and group III suppressed the increase in the triglyceride and total cholesterol levels in the serum of the diabetic mice, separately.

The plasma levels of BUN were also significantly higher in the control KK-A^y mice than in C57BL/6 mice; yet, MBS (2 g/kg) dramatically lowered it as compared with the diabetes mice. However, the MBSC group has no significant differences as compared to the control KK-A^y mice.

Immunohistochemical Evaluation on Pancreas. In the pancreatic islets of the control diabetic group, a significant decrease in insulin immunoreactivity was observed in comparison with the C57BL/6 group (**Figure 3A**,**B**). In the pancreatic islets of the diabetic mice given MBS (2 g/kg) and MBSC (3 g/kg) groups, a significant increase in insulin immunoreactivity was observed as compared with untreated diabetic mice (**Figure 3C**,**D**).

DISCUSSION

Mung bean has been shown to play protective roles against cancer and oxidant activity, but no report has been issued on the antidiabetic effect of it. In this study, we demonstrated that the supplementation of MBS and MBSC for 5 weeks exerts an antidiabetic effect in type 2 diabetic mice, at least in part, by enhancing the glucose and lipid metabolism.

Diabetic KK-A^y mice have high fasting blood glucose levels similar to those of diabetic patients. These animals, which are homozygous for the mutation, exhibit metabolic abnormalities such as hyperglycemia and glucose intolerance that phenotypically resemble human type 2 diabetes. In the present study, the fasting blood glucose levels of the control KK-A^y mice significantly increased by 163% as compared to the C57BL/6 mice. However, their blood glucose levels in the treatment of MBS (2 g/kg) and MBSC (3 g/kg) reduced by 40 and 28%, respectively, in comparison with the diabetic group after 5 weeks of treatment. These results are consistent with those reported by Kawa et al., who reported that DCI was primarily responsible for the lowering serum glucose effects, and similar results were obtained when chemically synthesized DCI was administrated to STZ rats (*21*). The MBS and MBSC supplementations improved glucose tolerance in diabetic rats. Fonteles et al.

Table 2. Plasma Parameters in Diabetic KK-A^y and C57BL/6 Mice

	group I[a]	group II[b]	group III[c]	group IV[d]	group V[e]	group VI[f]
C-peptide (ng/mL)	1.40 ± 0.04	0.69 ± 0.03[h]	0.64 ± 0.05[h]	1.08 ± 0.05	0.58 ± 0.04[h]	0.48 ± 0.04[i]
glucagon (pg/mL)	108.87 ± 3.54	103.71 ± 6.98	87.72 ± 37.02[g]	99.99 ± 3.39	93.05 ± 6.63[g]	60.07 ± 5.15[h]
triglycerides (mmol/L)	1.32 ± 0.03	1.02 ± 0.02[g]	1.03 ± 0.02	1.06 ± 0.02	0.99 ± 0.04[g]	0.88 ± 0.02[h]
total cholesterol (mmol/L)	5.50 ± 0.3 5	5.08 ± 0.10	4.98 ± 0.12[g]	5.24 ± 0.12	4.63 ± 0.61[g]	2.83 ± 0.08[i]
BUN (mmol/L)	10.09 ± 0.28	8.20 ± 0.37	7.48 ± 0.45[g]	8.75 ± 0.57	9.23 ± 0.27	5.48 ± 0.52[h]

[a] As diabetic KK-A^y mice. [b] As diabetic KK-A^y mice given MBSs, 3 g/kg. [c] As diabetic KK-A^y mice given MBSs, 2 g/kg. [d] As diabetic KK-A^y mice given MBSs, 1 g/kg. [e] As diabetic KK-A^y mice given mung bean coats, 3 g/kg. [f] As C57BL/6 mice. [g] $P < 0.05$. [h] $P < 0.01$. [i] $P < 0.001$.

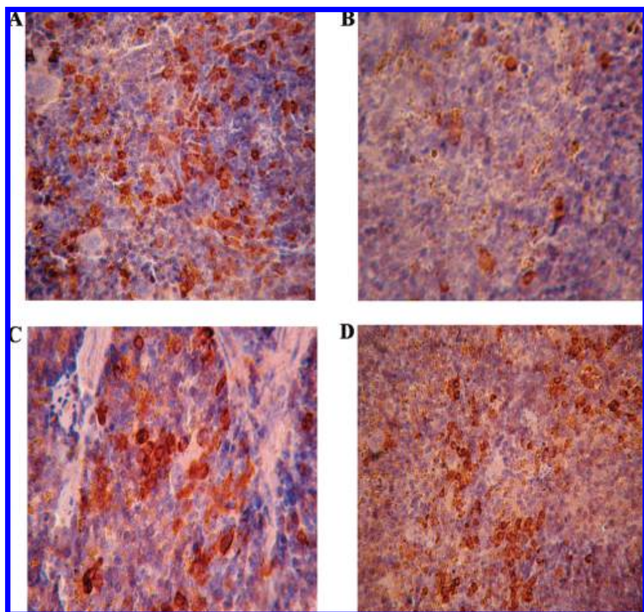

Figure 3. Immunohistochemical evaluation on pancreas (400×): (**A**) C57BL/6 mice showing the normal insulin immunoreactivity, (**B**) control diabetic KK-A^y mice showing the decrease of insulin immunoreactivity, (**C**) diabetic KK-A^y mice treated with MBS showing the increase of insulin immunoreactivity, and (**D**) diabetic KK-A^y mice treated with MBSC showing the increase of insulin immunoreactivity.

reported that a single dose of DCI (15 mg/kg) injected into the jugular vein promoted a 21% decrease in plasma glucose of STZ rats, which was different from the control rats at 120 min after administration (*22*). Ortmeyer et al. reported that intravenously administrated single dose DCI (100 mg/kg) increased the glucose disappearance rates by 129% (*23*). The glucose lowering effect of the MBS demonstrated in the present study is of similar magnitude to that of synthesized DCI, suggesting that DCI in the MBS is primarily responsible for the observed effects. It is likely that more than one functional component is responsible for the antidiabetic property of MBSC, for the content of DCI is too low. Thus, vitexin (45.66 mg/kg) and isovitexin (34.26 mg/kg) may have a synergistic effect on decreasing the blood glucose levels in this test. Further studies should be carried out to evaluate those two phenolics antidiabetic activities, respectively.

Furthermore, MBS and MBSC significantly lowered the levels of plasma C-peptide, which is a byproduct of insulin production. In this study, the KK-A^y mice displayed hyperglucagonemia, which was significantly higher than that of the C57BL/6 mice. However, MBS and MBSC supplementation significantly lowered the plasma glucagons level as compared with the control diabetic mice. Glucagon is one of several hormones that possess antagonistic action against insulin that can exacerbate the metabolic consequences of insulin deficiency. The suppression of endogenous glucose production has been reported not as being a simple response to insulin but rather a complex interplay between the action of glucagons and insulin. Thus, the improvement in hyperglycemia by MBS and MBSC could be partly attributed to the amelioration of hyperglucagonemia.

Lipids play a vital role in the pathogenesis of diabetes mellitus. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. In our study, we have noticed elevated levels of serum lipids such as cholesterol and triglycerides in diabetic mice. The levels of increased serum lipids in diabetes represent a risk factor for coronary heart disease (*24*). Under normal circumstances, insulin activates lipoprotein lipase and hydrolyzes triglycerides (*25*). The diabetic hyperglycemia induces the elevation of plasma levels of urea, which is considered a significant marker of renal dysfunction (*26*). In this study, plasma urea in the diabetic group was 45.7% higher than the control level. After the supplement of MBS to the diabetic mice, the level of urea was significantly ($P < 0.05$) decreased in plasma by 25.9%, as compared with the diabetic control group. This result indicates that MBS is capable of ameliorating the impaired diabetic kidney function. Peng's study confirmed that vitexin and isovitexin extracted from mung bean seeds are effective in inhibiting the formation of AGEs in vitro (*14*), which may have positive effects in diabetic nephropathy. The renal symptom improvement of the MBS in this study is of a similar role to that of vitexin and isovitexin, suggesting that vitexin and isovitexin in the MBS are primarily responsible for the observed effects.

The present study confirms that pancreatic β-cells are destroyed in KK-A^y mice (*27*). In the control diabetic mice, a significant decrease in insulin immunoreactivity was observed as compared with the control of C57BL/6. However, after the treatment of MBS and MBSC, the insulin immunoreactivity was improved in comparison to the diabetic group. According to the immunohistochemical results obtained, MBS and MBSC may have the ability to enhance insulin sensitivity. The protective effect of them could be due to its direct influence on the endocrine pancreatic function in diabetic animals (*28*).

In conclusion, the administration of MBS and MBSC exerts an antidiabetic action in type 2 diabetic KK-A^y mice, at least in part, by improving glucose tolerance and insulin response to glucose metabolism, without increasing body weight. Thus, it seems likely that MBS and MBSC are promising antidiabetic functional food sources. However, further studies should be carried out to develop MBS and MBSC as novel therapies for type 2 diabetes.

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