

Antidiabetic Effect of *Beta vulgaris* Mixture: Regulation of Glycolytic Enzymes and Pancreatic Beta Cells

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Abstract Diabetes is a chronic metabolic disease with a high prevalence worldwide. Beet (*Beta vulgaris*) is a plant that is widely used in many countries and has various biological activities. In this study, we aimed to evaluate the antidiabetic effect of a *B. vulgaris* mixture (BM). In the *in vitro* evaluation, we measured the inhibitory activities of α -amylase and α -glucosidase, performed the oral starch tolerance test (OATT) and oral sucrose tolerance test (OSTT) in Sprague Dawley (SD) rats, and evaluated the clinical symptoms, oral glucose tolerance test (OGTT), number of blood cells, and insulin resistance in *db/db* mice. BM showed an inhibitory effect against α -amylase and α -glucosidase activity and decreased the blood glucose increased in the OATT and OSTT. In the diabetes mouse model, BM alleviated the general symptoms of diabetes and OGTT results showed a decrease in the increased blood sugar level. Regarding diabetes-related tissue weight, BM decreased the reduced pancreatic weight and showed an effect on diabetes-related factors of blood. Histological analysis indicated that BM decreased insulin concentration, insulin resistance, and insulin secretion ability in serum, and increased insulin concentration in the islets of Langerhans. These results demonstrate that BM has an antidiabetic effect through the regulation of glycolytic enzymes and β cell activity in the pancreas.

Keywords: *Beta vulgaris*, diabetes, glycolytic enzymes, pancreas, β cell

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1. Introduction

Diabetes, a chronic metabolic disease with high prevalence, is characterized by high blood sugar and is an endocrine system disease caused by insufficient insulin secretion by the pancreas and is classified as insulin-dependent and insulin-independent [1]. Insulin-dependent diabetes, also known as type 1 diabetes, occurs as an autoimmune disease and is related to genetic factors and viruses [2]. Insulin-independent diabetes, also known as type 2 diabetes, accounts for more than 90% of diabetes and causes impaired glucose metabolism owing to insulin deficiency [3,4]. Insulin deficiency leads to complications such as chronic hyperglycemia and hyperlipidemia, leading to adult mortality [5]. Therefore, prevention of complications and blood glucose management are important in diabetes.

Drugs such as insulin secretion promoters and carbohydrate absorption inhibitors are used as a treatment

method for type 2 diabetes; however, long-term use is difficult because of various side effects [3,6]. Therefore, research is being conducted actively on natural products to find drugs that can alleviate or prevent diabetes. In particular, natural product-related health functional foods that help prevent diabetes are attracting much attention in the diabetes market [7,8]. Therefore, in this study, we evaluated beet (*Beta vulgaris*) mixture (BM), a natural material, for its antidiabetic effects.

Beets are cultivated in the United States, Europe, and Africa, and has many medicinal values; therefore, it is also used in medicine [9,10]. As it is low in calories and rich in minerals and vitamins, it can provide health benefits [10]. It is known that beets have different biological activities depending on their location. Leaves are effective against diuresis, as an anti-inflammatory agent, for alleviation of spleen and liver diseases, and in paralysis, and roots have biological activities such as antioxidant, anti-inflammatory, anti-tumor, and immune regulation [9,10,11,12]. These biological activities are known to be owing to components such as anthocyanins, betanins, phenols, and flavonoids

contained in beets [12]. However, no studies have been conducted on the effects of beets on diabetes.

This study was conducted to evaluate the effect of a beet mixture (BM) on diabetes. For the study, db/db mice were used, and the evaluation demonstrated the antidiabetic effect of the beet mixture by measuring oral starch tolerance test (OATT), oral sucrose tolerance test (OSTT), oral glucose tolerance test (OGTT), organ weight, and the amount of insulin.

2. Materials and Methods

2.1. Manufacturing Process of BM

The manufacturing method is divided into two stages, and the contents of the first stage are as follows. Jeju beets, Jeju carrots and domestic apples are being used and, Peel beets and carrots, then remove seeds from apples. Juice and blend all. The ratio of beets: carrots: apples are 1.5 ~ 3.5 : 1.5 ~ 2.5 : 4 ~ 7. At this step, adjust the mixing ratio for the acidity to attain to about 4 ~ 5.5 ph. Ripen this mixture at a low temperature of about -3 ~ 10°C for more than a day. In this way, the following new microorganisms and yeasts will appear naturally in the mixture. *Brevibacterium ravenburgense* BRM-1, *Acinetobacter septicus* AK001. *Branchyobacterium huguanmaarensis* BRM-3, *Kwoniella mangrovensis* CBS 8507. *Torulaspora delbrueckii* R97210, *wickerhamomyces anomalus* E25978. *Pseudomonas koreensis* ps 9-14, *Arthrobaacter crystallopoietes* BRM-9. With only the sample from stage 1, the blood content of nitrite could be increased (see attached).

The contents of the second stage are as follows: Boil tomatoes, broccoli and cabbage in a ratio of 0.2 ~ 0.5: 1 ~ 2: 1 ~ 2 at 100 deg C for 5 ~ 15 min. The error of the ingredients ratio is variable depending on the fructose content of the ingredients. For example, carrots contain more than twice the content of fructose than glucose. On the other hand, broccoli does not contain a lot of fructose. Increasing fructose will increase sucrose. The important point is to increase sucrose in order to increase the body's digestion and absorption rate. Juice the boiled ingredients from step 1. Mix the boiled juice from step 1 with 100% ~ 200% of the juice from stage 1 (beets, carrots and apples). Let the mixture from step 3 be aged at -3 ~ 10c for 1 ~ 8 h. At this point, it is important to provide enough space in the storage container to take into account the circulation of oxygen. Otherwise, you have to make holes in the container.

2.2. In vitro Assay

Measurement of α -amylase inhibition activity was performed using 1% starch (Sigma Aldrich, MO, USA). The iodine solution was prepared by adding 0.0317 g of iodine, 0.1 g of potassium iodide, and 50 ml of 10% HCl to 1 l of distilled water. Then, the sample was added to 1% starch and coenzyme solution and reacted at 37°C for 30 min. Next, an iodine solution was added, and absorbance was measured at 660 nm with a multi detection reader (Infinite 200, TECAN Group Ltd., Switzerland).

To measure the α -glucosidase inhibition activity, α -glucosidase obtained from yeast (Sigma Aldrich) and p-nitrophenyl- α -D-glucopyranoside (Sigma Aldrich) were used. α -Glucosidase was used as an enzyme solution by dissolving 0.7 units in 100 mM phosphate buffer (pH 7.0) containing 0.2% bovine serum albumin and 0.02% NaN₃, and 10 mM p-nitrophenyl- α -D-glucopyranoside in 100 mM phosphate buffer (pH 7.0) was used as a substrate solution. Next, 50 μ l of sample was added to each well of a microplate and 100 μ l of α -glucosidase enzyme was collected, incubated for 5 min at 25°C, and absorbance was measured at 405 nm with a multi detection reader (TECAN Group Ltd.). Finally, 50 μ l of the substrate solution was added, and after 2 min, the absorbance was measured at 405 nm with a multi detection reader to calculate the enzyme inhibition rate.

2.3. Animal Experiments

Sprague Dawley (SD) rats (Specific-pathogen-free, male, 4-week-old) were purchased from Samtaco. (Osan, Korea) and db/db mice (B6. BKS(D)-Lepr^{bd}/K, Specific-pathogen-free, male, 5-week-old), a model of type 2 diabetes mellitus, and age-matched heterozygote non-diabetic mice (*db/+*) were purchased from the Central Laboratory Animal Inc. and acclimatized for one week. During the breeding period, a constant temperature and humidity (23 \pm 1°C and 50 \pm 5%, respectively) were maintained, and all animal experiments were conducted in compliance with the INVIVO Co., Ltd., Experimental Ethics Committee's prior deliberation and ethics regulations.

SD rats were divided into groups (n = 8) according to individual body weight after the acclimation period, and the group separation was as follows: normal group (NC), control group (Control), beet mixture 30 mg/kg (BM30), beet mixture 50 mg/kg (BM50), beet mixture 100 mg/kg (BM100), and positive control (Acar, Acarbose, 3 mg/kg).

db/db mice were grouped according to individual body weight after the acclimation period (n = 8), and group separation was as follows: non-diabetic group (*db/+*), control group (*db/db*), beet mixture 10 mg/kg (BM10), beet mixture 30 mg/kg (BM30), beet mixture 50 mg/kg (BM50), and positive control (Met, metformin, 300 mg/kg). The BM and Met were administered orally five times a week for 3 weeks, and body weight, diet and water volume, and blood glucose were measured once a week for clinical measurement.

2.4. OATT, OSTT, and OGTT

After fasting SD rats for more than 12 h, blood was collected from the caudal vein, and fasting blood sugar was measured using a blood glucose meter (Autocheck, Diatech Korea Co., Ltd.). After administering a blood glucose raiser (OATT, starch; OSTT, sucrose) to all groups, blood glucose was measured at 30-min intervals for up to 120 min, and the area under the curve (AUC) was calculated to determine the change in blood glucose.

After fasting *db/db* mice for 8 h, blood was collected from the caudal vein, fasting blood sugar was measured, and the BM and Met were administered orally. Thirty minutes after BM administration, blood was collected again from the caudal vein to measure blood glucose.

Then, glucose was administered at 2 g/kg to all groups except the normal group, and blood glucose was measured every 30 min.

2.5. Sample Collection

After inhalation of an anesthetic, blood was collected from the abdominal vena cava, and for euthanasia, the abdominal vena cava and artery were cut to bleed. Thereafter, the liver, epididymis fat, pancreas, and kidneys were obtained and weighed, and the pancreas was fixed in 10% formalin solution.

Blood collected for hematological analysis was coagulated at room temperature for 30 min, centrifuged at 3000 rpm for 10 min at 4°C, and the supernatant was collected. Fructosamine, total cholesterol (TC), Triglyceride (TG), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C) in serum were analyzed at KPNT Co. (Oson, Korea), and insulin analysis was performed using an enzyme-linked immunosorbent assay kit.

2.6. Histological Analysis

Analysis of the islets of Langerhans in the pancreatic tissue was performed by immunostaining. The fixed tissue was embedded in paraffin, sectioned at a thickness of 4 μ m, and the deparaffinized tissue was bathed for 20 min at 60°C with an antigen reagent (Vector, Stuttgart,

Germany). After cooling for 20 min, the sections were treated with BLOXALL™ Blocking Solution (Vector, Germany) for 10 min. After washing twice with TBST (tris-buffered saline buffer with Tween20) for 5 min, samples were reacted with 2.5% normal goat serum for blocking (Vector, Germany) for 20 min, after which the blocking reagent and working insulin antibody (Cell Signaling, MA, USA) solution (2–10 μ l of antibody to 1 ml of Ready-To-Use 2.5% normal goat serum for blocking) was reacted at 4°C overnight. Next, the tissue was reacted for 1 h at room temperature using an ImmPRESS™ horseradish peroxidase anti-rabbit IgG (peroxidase) polymer detection kit (Vector, Germany), and then washed twice with TBST buffer for 5 min, and stained using chromogen AEC (Vector, Germany) and hematoxylin. The stained pancreatic tissue was observed and photographed using an optical microscope (Olympus BX50 F4; Olympus, Tokyo, Japan).

2.7. Statistical Analysis

All experimental results were calculated as mean \pm standard error (S.E.) using SPSS ver. 12.0 (SPSS Inc., Chicago, IL, USA). Statistically significant differences between each experimental group were tested using a one-way analysis of variance test (ANOVA), and in case of significance ($p < 0.05$), a post-test was performed with the Duncan's multiple range test.

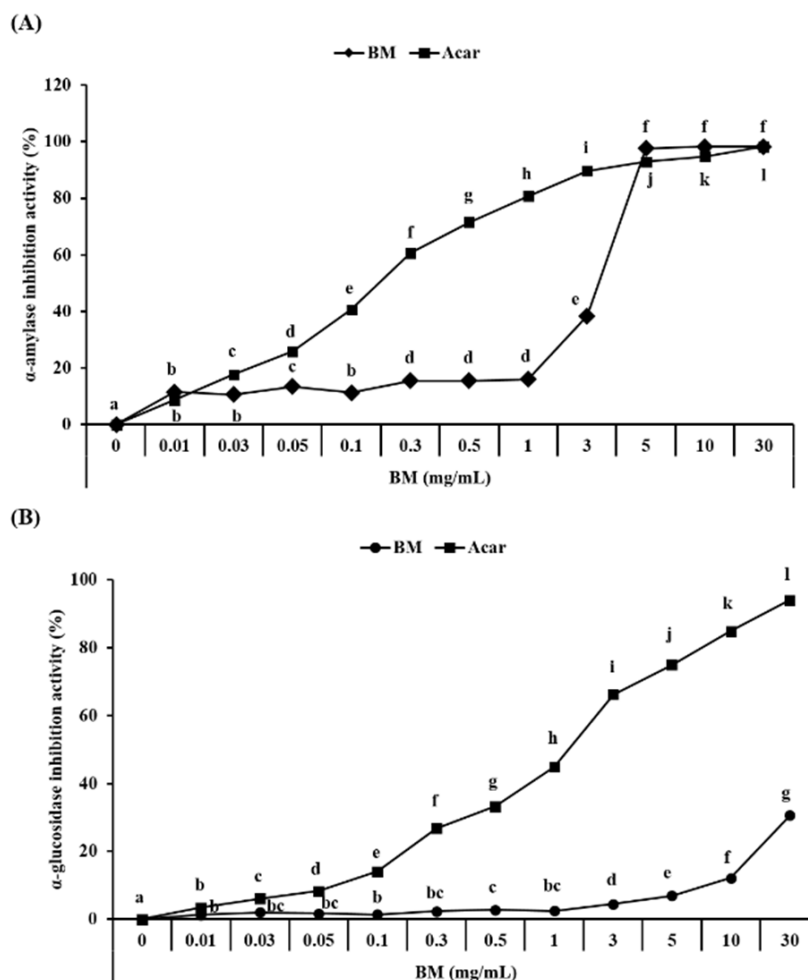


Figure 1. Inhibitory activity of BM on α -amylase and α -glucosidase. (A) α -amylase inhibition activity, (B) α -glucosidase inhibition activity. "a~l" indicate significant difference at $p < 0.05$. Data are expressed as mean \pm SEM

3. Results

3.1. BM Inhibits Glucose Absorption after a Meal

To evaluate the inhibitory effect of BM on postprandial glucose uptake *in vitro*, the activities of α -amylase and α -glucosidase were measured (Figure 1). α -amylase and α -glucosidase are representative carbohydrate enzymes and are used in many studies as indicators to measure blood glucose after a meal. Results indicated that the inhibitory effect on α -amylase activity did not change significantly up to a concentration of 1 mg/ml with BM treatment but showed a strong inhibitory activity from 3 mg/ml (Figure 1A). These results were similar to those obtained for the positive control cells. The inhibitory effect on α -glucosidase activity was weaker than that on α -amylase, and increased from 10 mg/ml (Figure 1B).

Next, OATT and OSTT were measured using SD rats for *in vivo* evaluation (Figure 2). OATT results showed

that the increased blood glucose in the control group was decreased in a concentration-dependent manner when administered BM orally (Figure 2A). The AUC result also showed that the blood glucose levels in the control group (184.12 ± 10.91) increased significantly compared to that of the NC group (152.69 ± 12.72) (Figure 2B). In the BM groups, the increased blood glucose was decreased in a concentration-dependent manner; however, this decrease was only slightly significant in the BM100 group (175.56 ± 10.77), and the difference was not significant in the BM30 (182.76 ± 12.63) and BM50 (177.55 ± 9.66) groups. The results of OSTT indicated that the blood sugar, which had been increased at 60 min, decreased in a concentration-dependent manner owing to BM administration (Figure 2C). The AUC result of OSTT showed a significant increase in blood glucose compared to that in the control group (270.09 ± 16.27) and a significant decrease in the BM50 (252.60 ± 18.61) and B100 (246.02 ± 20.14) groups. In the BM30 group (264.79 ± 18.00), a decrease was observed, but the difference was not significant (Figure 2D).

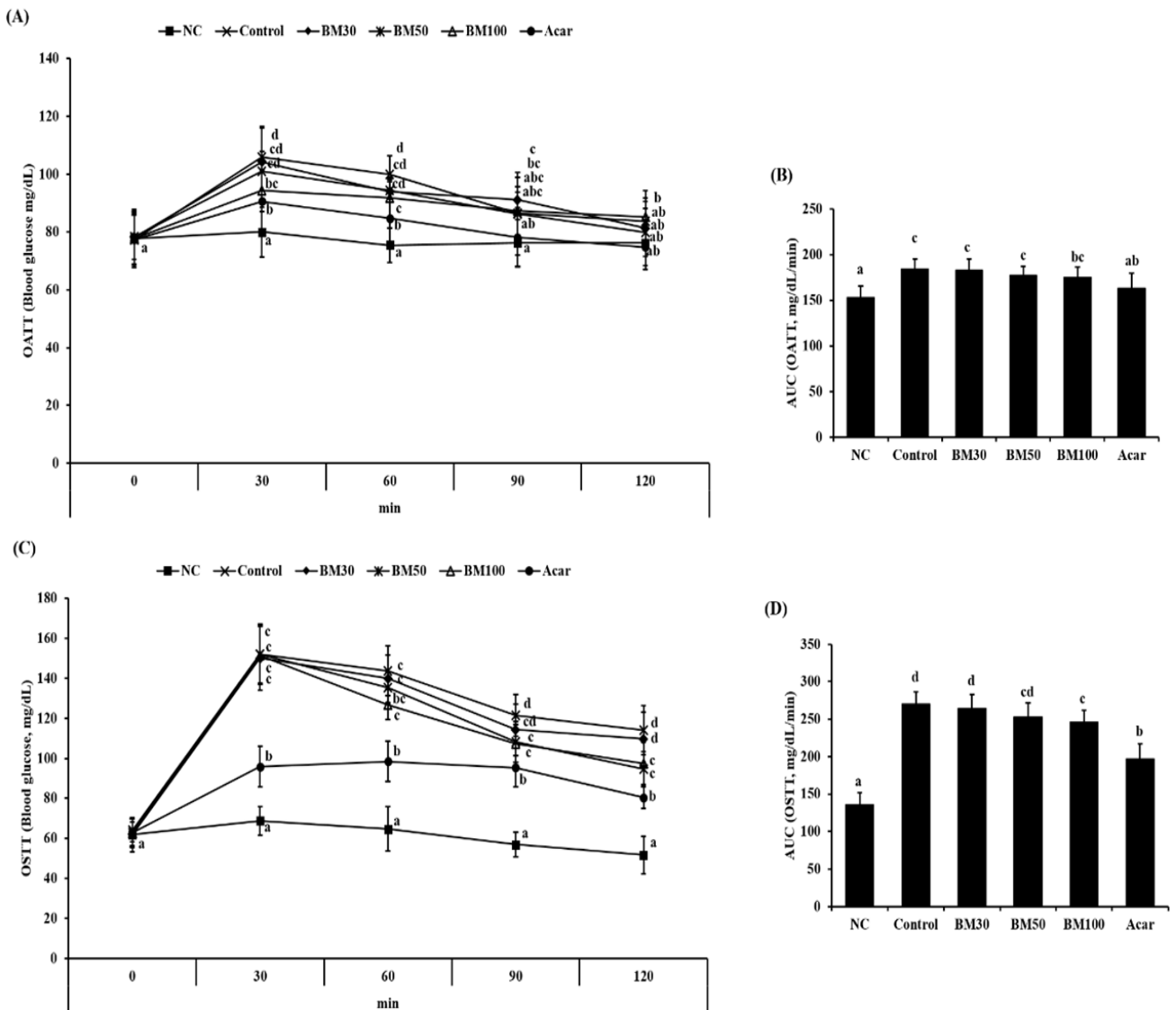


Figure 2. Effect of BM on OATT and OSTT in db/db diabetic mouse model. (A) OATT, (B) AUC of OATT, (C) OSTT, (D) AUC of OSTT. NC, normal control; Control, starch or sucrose only; BM30, BM (30 mg/kg) + starch or sucrose; BM50, BM (50 mg/kg) + starch or sucrose; BM100, BM (100 mg/kg) + starch or sucrose; Acar, acarbose (3mg/kg) + starch or sucrose. "a-d" indicate significant difference at $p < 0.05$. Data are expressed as mean \pm SEM

Table 1. Body weight changes and food intake in db/db mouse diabetic mouse model

Time variables/group	Initial	1 week	2 week	3 week
Food intake (g)				
db/+	-	3.03±0.22	2.82±0.10	3.02±0.37
db/db	-	5.04±0.05	4.99±0.06	5.05±0.89
BM10	-	4.94±0.13	5.22±0.11	5.22±0.32
BM30	-	4.85±0.63	5.13±0.15	5.66±0.30
BM50	-	4.61±0.22	5.24±0.02	5.45±0.44
Met	-	4.57±0.22	4.83±0.18	5.21±1.03
Weight (g)				
db/+	20.65±0.33 ^a	21.82±0.42 ^a	23.42±0.47 ^a	24.26±0.42 ^a
db/db	25.27±0.66 ^b	30.37±0.68 ^b	33.66±0.70 ^b	35.55±0.65 ^c
BM10	25.06±0.60 ^b	30.15±0.49 ^b	33.54±0.45 ^b	34.40±0.43 ^{bc}
BM30	25.13±0.73 ^b	29.38±0.63 ^b	32.52±0.52 ^b	34.28±0.31 ^{bc}
BM50	24.22±0.53 ^b	29.38±0.47 ^b	32.87±0.37 ^b	33.70±0.52 ^{bc}
Met	24.14±0.47 ^b	29.08±0.37 ^b	32.74±0.38 ^b	34.27±0.38 ^c

^{“a-c”} indicate significant difference at $p < 0.05$. Data are expressed as mean±SEM.

3.2. Effect of BM on General Symptoms and Changes in Feed Intake in the Diabetes Model

Changes in feed intake are evaluated in the db/db mouse diabetes model as a common clinical symptom in diabetic mice (Table 1). Therefore, in this study, the intake of feed was evaluated for 3 weeks. Our results showed that the intake of feed and water was higher in the diabetic group than in the db/+ group, but there was no difference in the intake of the BM groups compared to that in the diabetic group. In addition, change in weight is one of the common clinical symptoms, and weight increased in the diabetes group compared to that in the normal group, and decreased in the BM groups compared to the diabetes

group. These results show that BM alleviates common clinical symptoms in diabetes.

3.3. Blood Glucose Reduction Effect of BM in the Diabetes Model

Regarding the measurement of weekly blood glucose, at week 1, the diabetes-induced group showed higher blood glucose levels than the normal group (Table 2). However, in the group administered BM, the blood glucose level decreased compared to that in the diabetes-induced group. At week 2, there was no change with BM administration; however, at week 3, blood glucose decreased in the BM groups compared to that in the diabetes group. In particular, there was a significant difference in the BM50 group.

Table 2. Blood glucose in db/db mouse diabetic mouse model

Time variables/group	Initial	1 week	2 week	3 week
db/+	82.75±8.34 ^a	72.25±3.56 ^a	94.00±5.50 ^a	103.75±3.19 ^a
db/db	193.38±9.40 ^b	194.88±15.04 ^b	218.13±20.40 ^b	316.00±23.65 ^b
BM10	189.75±9.95 ^b	200.13±12.88 ^{bc}	196.00±12.28 ^{bc}	278.88±13.00 ^{bc}
BM30	203.50±10.43 ^b	148.50±13.98 ^{bc}	244.50±27.79 ^{bc}	331.88±16.05 ^{bc}
BM50	190.38±7.19 ^b	190.00±8.91 ^{bc}	275.88±10.13 ^{bc}	271.50±21.98 ^{bc}
Met	201.25±10.55 ^b	167.00±19.09 ^c	229.38±26.38 ^c	238.75±21.91 ^c

^{“a-d”} indicate significant difference at $p < 0.05$. Data are expressed as mean±SEM.

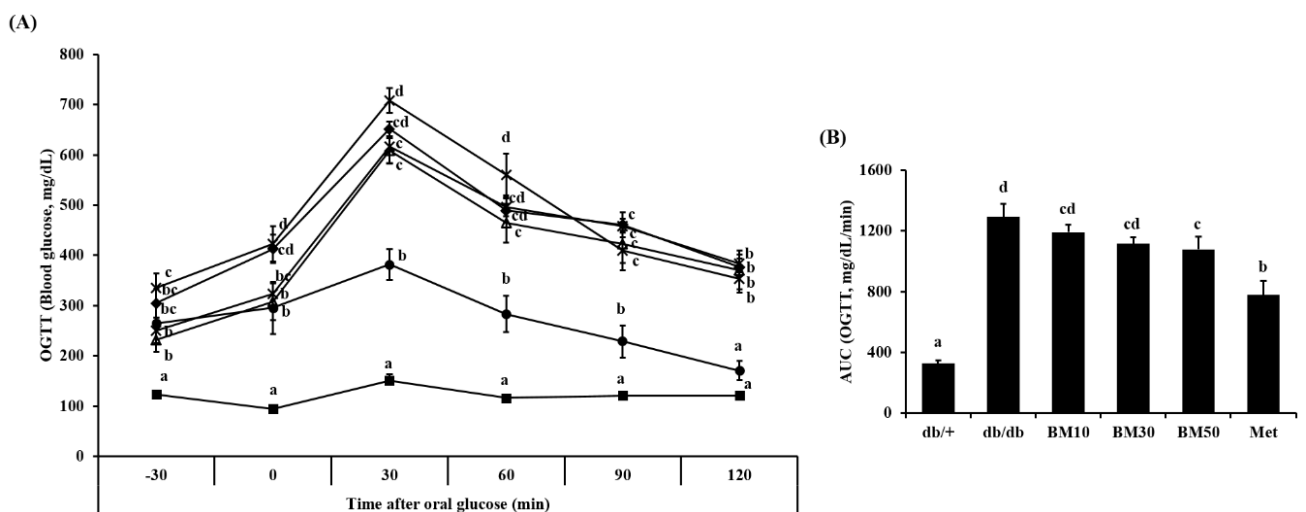


Figure 3. Effect of BM on oral glucose tolerance in db/db diabetic mouse model. (A) OGTT, (B) AUC of OGTT. db/+, non-diabetic mouse; db/db, diabetic mouse; BM10, BM (10 mg/kg) + diabetic mouse; BM30, BM (30 mg/kg) + diabetic mouse; BM50, BM (50 mg/kg) + diabetic mouse; Met, metformin (300mg/kg) + diabetic mouse. ^{“a-d”} indicate significant difference at $p < 0.05$. Data are expressed as mean±SEM

The OGTT results showed that compared to the db/+ group, blood glucose level was the highest in the db/db group 30 min after glucose administration (Figure 3A). However, blood glucose in the BM groups decreased in a concentration-dependent manner compared to that in the db/db group. The AUC results showed a significant increase in the db/db group compared to that in the db/+ group, and the concentration decreased significantly in the BM groups.

3.4. Effect of BM on Tissue Weight in the Diabetes Model

In this study, the weight of the liver, kidney, epididymis fat, and pancreas were measured to evaluate the effect of BM on diabetes-related tissue weight. Table 3 shows that liver weight was increased in the diabetes group compared to that in the db/+ group, and the kidney weight was lower in the diabetes group than in the db/+ group. However, there was no difference owing to the administration of BM. The weight of epididymis fat was significantly increased in the db/db group compared to that in the db/+ group, and decreased slightly in the BM groups, but the differences were not significant. The weight of the pancreas was significantly reduced to 0.13 g in the db/+ group and 0.11 g in the db/db group. A significant increase was observed in the BM10, BM30, and BM50 groups compared to the positive control. These results show that BM is related to insulin secretion.

3.5. Effect of BM on Diabetes-related Factors in the diabetes Model

In this study, hematologic analysis was performed to confirm the effect of BM on diabetes-related factors. Fructosamine, TC, HDL-C, and triglyceride levels were increased in the db/db group compared to those in the db/+ group, and in particular, fructosamine, TC, and HDL-C showed significant differences (Table 4). In the BM groups, fructosamine was decreased in a concentration-dependent manner compared to that in the db/db group. Among them, the BM50 group showed a significant difference. TC levels decreased with the administration of BM, but there was no significant difference in the B10 group and a slightly significant difference in the B30 and

B50 groups. There was a slight decrease in HDL-C in the BM30 and BM50 groups, but the difference was not significant, and regarding the triglyceride level, there was no difference owing to BM administration. The db/db group showed a significant decrease in the LDL-C level compared to that in the db/+ group, but there was no difference owing to the administration of BM.

3.6. Effect of BM on Insulin Secretion in the Diabetes Model

To confirm the effect of BM on insulin secretion, the levels of insulin in the serum, homeostatic model assessment insulin resistance (HOMA-IR) and HOMA of beta cell function (HOMA- β), were measured (Figure 4). The db/db group (20.55 \pm 2.17) showed a remarkable increase in insulin concentration as compared to that in the db/+ group (2.31 \pm 0.10) (Figure 4A). In contrast, BM10 was 19.47 \pm 1.77, BM30 was 18.85 \pm 1.80, and BM50 was 14.84 \pm 2.08, which showed a decrease in comparison to the db/db group. In particular, a significant decrease was observed in the B50 group. The measurement of HOMA-IR showed that the db/db group (4.53 \pm 0.57) had a remarkable increase in insulin resistance compared to that in the db/+ group (0.15 \pm 0.01), and a concentration-dependent decrease was confirmed with the administration of BM (Figure 4B). In particular, the B30 (3.99 \pm 0.39) and B50 (2.23 \pm 0.43) groups showed a significant decrease. Next, the measurement of the insulin secretion ability of β cells, showed that the db/db group (20.55 \pm 2.17) had a significant increase compared to that of the db/+ group (4.21 \pm 0.74) (Figure 4C). In contrast, the BM groups showed a concentration-dependent decrease, but the difference was not significant.

To confirm the effect on insulin secretion from a histological aspect, immunohistochemical analysis was performed on the pancreatic islets of Langerhans (Figure 5). The results of confirmed that the insulin concentration was high in the db/+ group, whereas it was decreased in the db/db group. However, it was shown that the concentration of insulin, which was decreased by the administration of BM, increased in a concentration-dependent manner. These results show that the administration of BM is related to pancreatic β cells.

Table 3. Tissue weight in db/db mouse diabetic mouse model

Time variables/group	Liver (g)	Kidney (g)	Epididymis fat (g)	Pancreas (g)
db/+	1.02 \pm 0.04	0.49 \pm 0.09	0.38 \pm 0.05	0.13 \pm 0.01 ^c
db/db	1.98 \pm 0.09	0.31 \pm 0.01	1.84 \pm 0.07	0.11 \pm 0.01 ^a
BM10	1.90 \pm 0.05	0.28 \pm 0.02	1.98 \pm 0.12 ^c	0.11 \pm 0.02 ^b
BM30	2.01 \pm 0.05	0.29 \pm 0.01	1.83 \pm 0.01	0.12 \pm 0.01 ^{ab}
BM50	1.91 \pm 0.04	0.30 \pm 0.01	1.85 \pm 0.01	0.12 \pm 0.01 ^b
Met	2.00 \pm 0.06	0.03 \pm 0.01	1.80 \pm 0.01	2.00 \pm 0.01 ^{ab}

^{a-c} indicate significant difference at $p < 0.05$. Data are expressed as mean \pm SEM.

Table 4. Blood analysis in db/db mouse diabetic mouse model

Time variables/group	Fructosamine (μ M/L)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TC (mg/dL)
db/+	204.56 \pm 4.85 ^a	98.67 \pm 5.73	86.88 \pm 7.01 ^a	17.24 \pm 2.63	115.67 \pm 9.93 ^a
db/db	346.89 \pm 23.02 ^c	237.33 \pm 6.89	115.81 \pm 3.27 ^{bc}	13.07 \pm 0.48	137.22 \pm 4.49 ^b
BM10	333.56 \pm 10.83 ^{bc}	225.56 \pm 10.95	117.27 \pm 5.03 ^c	11.19 \pm 0.95	135.11 \pm 6.43 ^b
BM30	329.33 \pm 12.43 ^{bc}	260.78 \pm 12.90	115.03 \pm 2.78 ^{bc}	11.64 \pm 0.95	135.11 \pm 3.36 ^{ab}
BM50	304.67 \pm 7.61 ^b	229.67 \pm 14.17	109.77 \pm 3.34 ^{bc}	9.48 \pm 0.80	129.56 \pm 4.12 ^{ab}
Met	329.78 \pm 13.27 ^{bc}	242.89 \pm 12.54	101.63 \pm 4.10 ^b	12.20 \pm 0.80	123.33 \pm 4.34 ^{ab}

^{a-c} indicate significant difference at $p < 0.05$. Data are expressed as mean \pm SEM.

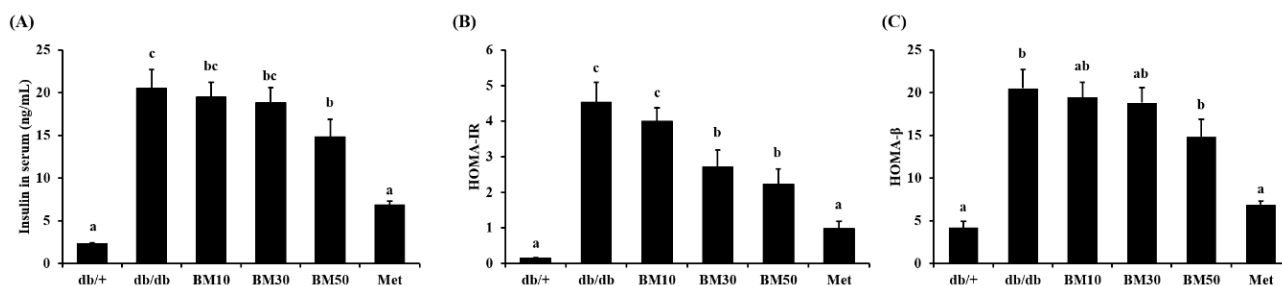


Figure 4. Effect of BM on insulin resistance in db/db diabetic mouse model. (A) Insulin concentration in serum, (B) HOMA-IR, (C) HOMA- β . db/+, non-diabetic mouse; db/db, diabetic mouse; BM10, BM (10 mg/kg) + diabetic mouse; BM30, BM (30 mg/kg) + diabetic mouse; BM50, BM (50 mg/kg) + diabetic mouse; Met, metformin (300mg/kg) + diabetic mouse. "a-c" indicate significant difference at $p < 0.05$. Data are expressed as mean \pm SEM

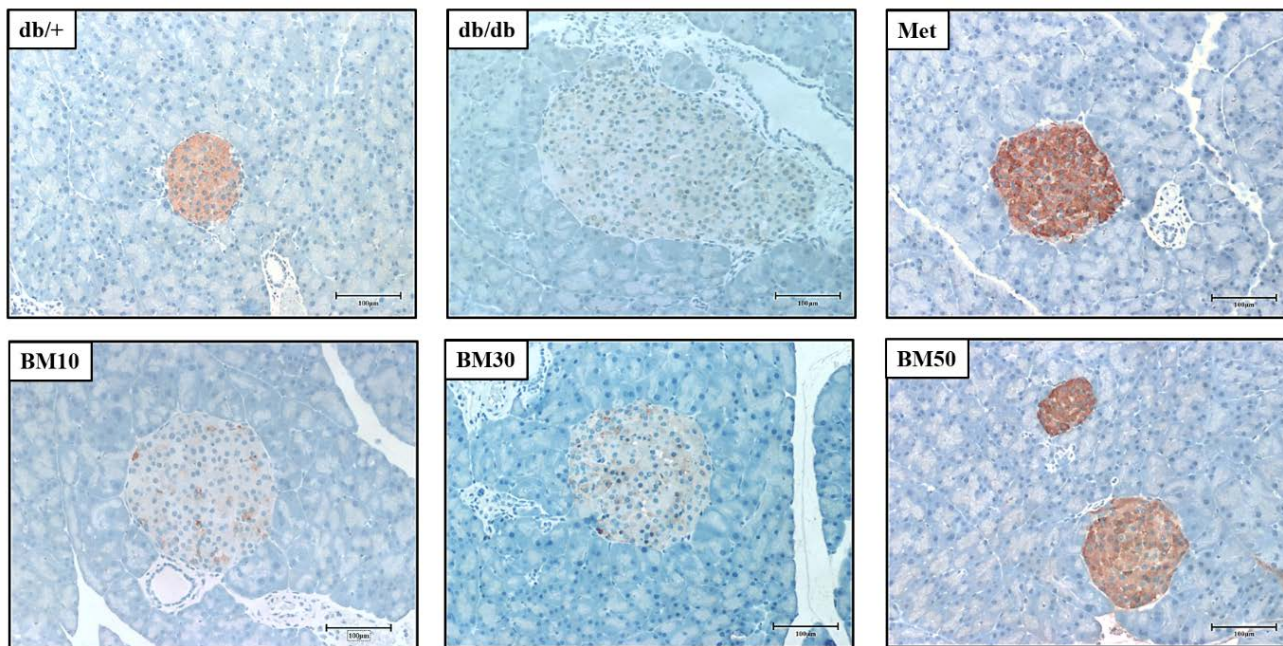


Figure 5. Effect of BM insulin in db/db diabetic mouse model of islets of Langerhans. db/+, non-diabetic mouse; db/db, diabetic mouse; BM10, BM (10 mg/kg) + diabetic mouse; BM30, BM (30 mg/kg) + diabetic mouse; BM50, BM (50 mg/kg) + diabetic mouse; Met, metformin (300mg/kg) + diabetic mouse. Scale bars represent 100 μ m

4. Discussion

Diabetes is a chronic degenerative disease and is classified as the third most dangerous disease worldwide [13]. In this study, we aimed to demonstrate the antidiabetic effect of BM. BM showed inhibitory activity against α -amylase and α -glucosidase and alleviated common symptoms of diabetes. In addition, it showed a reducing effect on blood glucose and diabetic factors in blood, and on tissue weight and insulin secretion.

Diabetes is caused by hyperglycemia and abnormal insulin production and secretion [14]. In general, α -amylase and α -glucosidase inhibitors are used to improve the hyperglycemic symptoms of diabetes [15,16]. α -amylase acts as a catalyst to decompose polysaccharides into disaccharides through hydrolysis of α -1,4-glycosidic linkages, and α -glucosidase acts as a catalyst to decompose disaccharides into monosaccharides [17,18,19]. In this experiment, to assess the inhibition of α -amylase and α -glucosidase activities, OATT and OSTT were measured to confirm the blood glucose improvement effect of BM. Our results indicated that the inhibition of α -amylase activity was strong at 3 mg/ml of BM, and the inhibition of α -glucosidase activity was observed from a

concentration of 10 mg/ml BM. However, inhibition of α -glucosidase activity was shown to lower than that of α -amylase. OATT results confirmed that blood glucose decreased with BM administration in comparison to that in the db/db group at 30 min after starch administration, and OSTT results showed that BM administration decreased blood glucose at 60 min after sucrose administration. However, the AUC of both OATT and OSTT decreased, but only that of OSTT in the BM50 group showed a significant difference. The results of the diabetes model showed a decrease in blood glucose owing to the administration of BM, and in particular, there was a significant difference in the BM50 group. In the OGTT, the increased blood glucose was decreased by the administration of BM 30 min after glucose administration, and the AUC also showed a significant decrease in the groups receiving BM. These results suggest that BM is effective in regulating blood sugar and is associated with inhibitory activity against α -amylase and α -glucosidase.

Diabetes is classified as insulin-dependent type 1 diabetes and insulin-independent type 2 diabetes [13]. Type 1 diabetes is caused by a decrease in insulin secretion owing to the destruction of β cells, and type 2 diabetes is caused by impaired glucose metabolism owing

to insulin resistance and insulin exhibit symptoms such as obesity, insulin resistance, and hyperglycemia because of a mutation in the leptin receptor [21]. In this experiment, body weight, diet, and drinking water were measured to demonstrate the effect of BM on clinical symptoms, a common symptom of diabetes. The amount of food consumed increased in the db/db group compared to that in the db/+ group, but there was no difference owing to the administration of BM. Considering change in body weight, the increased body weight in the db/db group was shown to decrease with the administration of BM. This shows that BM relieves the common symptoms of diabetes.

In diabetes, various diabetes-related factors are increased in blood [22]. To diagnose diabetes, fructosamine is used as a marker of mean blood glucose [23]. Cholesterol causes various cardiovascular diseases and complications, and it is an important factor in the occurrence of diabetes [24]. Blood cholesterol levels have been shown to increase in diabetic patients [25]. Therefore, the control of cholesterol is important for the prevention of diabetes. In this study, hematological analysis was performed to demonstrate the effect of BM on increased blood diabetes-related factors in diabetes. The measurement of fructosamine indicated that the increased levels in the db/db group decreased in a concentration-dependent manner, and the blood cholesterol concentration was also decreased compared to that in the db/db group. It was confirmed that amount of LDL-C decreased in the db/db group compared to that in the db/+ group, but there was no difference owing to the administration of BM. This shows that BM regulates factors in the blood that develop in diabetes.

As diabetes progresses in patients with diabetes, changes in organ weight begin to appear [26]. In the case of the liver, insulin secretion is lowered owing to a decrease in immune function, resulting in impaired glucose metabolism and accumulation in liver lipids [27]. In the case of the pancreas, it is caused by destruction of β cells and a decrease in insulin [28]. In this study, organ weights were measured to confirm the effect of BM on tissue weight in a diabetes induction model. The weight of the liver was higher in the db/db group than that in the db/+ group, but there was no difference with the administration of BM. Considering pancreatic weight, it was confirmed that the weight of the pancreas decreased in the BM groups compared to that in the db/db group. This showed a greater increase than that in the positive db/db group. In diabetes, the function of β cells gradually decreases as hyperglycemia progresses, and the number of β cells decreases owing to degranulation of β cells and insulin resistance worsens [26,29]. The results of this study confirmed that insulin concentration was significantly increased in the db/db group and decreased in the BM-administered groups. Insulin resistance was increased in the db/db group and decreased in a concentration-dependent manner owing to the administration of BM. The same pattern was observed for the results of insulin secretion. Histological results showed that the insulin concentration in the db/db group decreased in the pancreatic islets of Langerhans compared to that in the db/+ group and increased in the BM-administered groups. These results indicate that BM reduces insulin resistance through the regulation of β cells in the pancreas.

In summary, this study was conducted to investigate the antidiabetic effects of BM. Common clinical symptoms in diabetes were alleviated with BM, which showed a glucose inhibitory activity through the control of α -amylase and α -glucosidase, and reduction in factors related to diabetes in the blood. Regarding weight of organs involved in diabetes, BM administration resulted in a decrease in liver weight and an increase in pancreatic weight. Regarding insulin resistance, BM decreased the increased insulin resistance and secretion ability and increased the insulin concentration in the islets of Langerhans. These results suggest that BM has an antidiabetic effect, which is likely owing to the regulation of glycolytic enzymes and pancreatic β -cells. Therefore, BM can be used as a health functional food to prevent diabetes.

Conflict of Interests

There is no conflict of interest.

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Availability of Data and Materials

The data that support the findings of this study are included in this published article or are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

The present study was approved and supervised by the the Institutional Animal Care and Use Committee of INVIVO Co., Ltd. All animal experiments carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Significant efforts were made in order to minimize both the number of animals used and their suffering.

Contributions of Authors

Conceived and designed the experiments: DSH, HJY. Performed the experiment: DYS, YMP. Analyzed the data: YMP, HYL and NRS. Contributed reagents/ materials/ analysis tool: BSK, MSK, HYL. Wrote the paper: NRS.

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