

Anti-diabetic effects of mulberry (*Morus alba* L.) branches and oxyresveratrol in streptozotocin-induced diabetic mice

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Abstract Despite with accumulating evidences on the anti-diabetic effects of mulberry branch (MB), the major active component for the activity has not been known. Oral administration of MB ethanol (EtOH) extracts [0.5 or 1 g/kg body weight (BW)] once a day for 22 days to streptozotocin-induced diabetic ICR mouse significantly reduced fasting blood and plasma glucose level in a dose dependent manner compared to those of the diabetic control. Administration of oxyresveratrol [ORT, 0.6 g/kg BW], a major compound of MB EtOH extracts, to diabetic ICR mouse also significantly reduced fasting plasma glucose level. Further, ORT increased hepatic glucose transporter 2 transcription and glycogen content. Plasma insulin concentration and intestinal disaccharidase activity were not different between diabetic control and ORT groups. This suggests that ORT reduced plasma glucose by stimulating hepatic glucose uptake and glycogen storage. MB EtOH extracts and ORT could be potential adjunct therapies for diabetes management.

Keywords Diabetes · Mulberry branch · Oxyresveratrol · Streptozotocin · Mouse

Introduction

The prevalence of diabetes mellitus (DM) is steadily increasing worldwide. In 2013, 382 million was diagnosed with DM and this is expected to rise to 592 million in 2035

[1]. The pathophysiology of type 1 DM (T1DM) is characterized by absolute defect of insulin secretion caused by pancreatic injury and type 2 DM (T2DM) is characterized by insulin resistance. Chronic DM can develop into serious complications such as micro- and macro-vascular complications, notably retinopathy, neuropathy and nephropathy as well as atherosclerosis and hypertension in the latter [2]. Thus, the primary aim of diabetes management is effective control of blood glucose to achieve near normal levels. Patients with T1DM require insulin therapy but occasionally oral hypoglycemic agents are used as adjunct therapy. Currently, metformin (Met) is the only oral hypoglycemic agent approved by the Food and Drug Administration in the United States for juvenile T1DM [3]. However, the efficacy of combined treatment of Met and insulin is not clear. The management of T2DM commonly consists of use of biguanides such as metformin, sulfonylureas, α -glucosidase inhibitors, and thiazolidinediones, in addition to diet control and exercise [4]. However, the currently available drugs have been reported to exert several side effects such as hypoglycemia, loss of insulin secretion capacity, gastric upset, renal and hepatic impairment [4]. In addition, some drugs that are associated with severe hepatotoxicity or cardiovascular side effects have been prohibited for use. Therefore, in recent years, plant-derived phytochemicals with limited side effects have been extensively studied for the treatment of DM.

The fruits, leaves, branches, and root barks of mulberry (*Morus alba* L.) have long been used for the treatment of diabetes in traditional Korean and Chinese medicine [5]. Recent studies have identified its anti-hyperglycemic mechanism and major active components in the sericultural products [6–10]. In particular, mulberry branch (MB) was recently found to exhibit significant hypoglycemic activities in vitro and in vivo [8, 9]. Ye et al. [8] reported that the

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aqueous extracts of MB possess potent intestinal α -glucosidase inhibitory activity and significantly reduced fasting blood glucose level in alloxan-induced diabetic rats and mice [8]. The effect was comparable to that of acarbose, an anti-diabetic drug that acts as an α -glucosidase inhibitor. Our recent study also showed that MB exerts stronger α -glucosidase inhibitory effect than other parts of the mulberry tree [11]. However, the major active component for anti-diabetic activity of the MB extracts has not been known. Guo et al. [9] reported that polysaccharides isolated from MB reduced blood glucose level in streptozotocin (STZ)-induced diabetic mice. Inflammatory response and oxidative stress in pancreatic tissues were inhibited whereas body weight and insulin levels were restored after polysaccharide treatment. These effects were comparable to those of the metformin.

We recently isolated and identified that MB ethanol (EtOH) extracts contains oxyresveratrol (ORT, *trans*-2',3,4',5-tetrahydroxystilbene), resveratrol, and moracin [12]. Among these, oxyresveratrol 3',4-*O*- β -D-diglucoside (mulberroside A, MSA) and its aglycone (ORT) are the major functional constituents of MB and they are widely known for their anti-inflammatory [13], anti-melanogenic [14], and antioxidant activities [15]. However, the anti-diabetic action of ORT in STZ-induced diabetic mice has not been studied *in vivo*.

In this study, we therefore investigated the anti-diabetic effect of MB EtOH extracts and its major active component, ORT, in STZ-induced diabetic mice.

Materials and methods

Plant materials and chemicals

The branch of "Chongilppong" cultivar including *M. alba* L. were collected on early March in the Yeongcheon Province, Korea and identified by Gyoo-Byung Sung, National Institute of Agricultural Sciences, Jeonbuk, Korea. A voucher specimen has been deposited in the herbarium of the Institute of Natural Sciences of Catholic University of Daegu. All solvents for HPLC analysis were of Merck HPLC grade (Darmstadt, Germany). All other reagents used in this study were of analytical grade.

Preparation of ethanol extracts of MB and oxyresveratrol

Dried MB (2 kg) were cut into small pieces and refluxed twice in 60% EtOH (4 L) for 2 h. The EtOH solution was cooled, filtered, and evaporated under reduced pressure at 50 °C. The crude EtOH extract was redissolved in the same EtOH solution and left overnight at room temperature. The

upper layer was evaporated *in vacuo* to obtain EtOH extract of MB (yield: $7.4 \pm 0.3\%$). ORT and MSA were isolated from the ethanolic extract of mulberry (*M. alba* L.) twigs by a series of separation procedures, including solvent fractionation, and silica-gel, ODS-A, and Sephadex LH-20 column chromatographies as previously reported [12].

Quantification of MSA and ORT by HPLC

The EtOH extract (1.0 g) of MB was solubilized in EtOH (100 mL), followed by dilution and filtration by 0.45 μ m membrane filter (Whatman, Maidstone, England), and finally injected into an analytical HPLC. HPLC was performed on a Waters e2690/5 HPLC system (Milford, MA, USA) equipped with 2998 photodiode array detector at 320 nm and autosampler. HPLC analysis was carried out using a YMC-Pack Pro C₁₈ column (46 mm i.d. \times 250 mm, YMC Inc., USA) with a Guard-Pak C₁₈ precolumn insert. The separation was conducted using a linear gradient of two solvent systems; solvent A, 0.05% H₃PO₄ in H₂O; solvent B, CH₃CN at a flow rate of 0.8 mL/min. The gradient elution program was performed as follows: initial 5 min run of 10% B (v/v), followed by a 45 min linear gradient to 80% B, and holding for 5 min. The injection volume was 10 μ L. MSA and ORT were identified by a comparison of their retention time with those of the two standard compounds isolated previously [12]. Linear correlation coefficients were superior to 0.995 for two compounds. Levels of two compounds were determined by calibration curves of the two standards (MSA; $y = 1.5083x + 1.4372$, ORT; $y = 5.7642x - 1.5011$) and expressed as mg per 100 g of dried weight of MB. Recovery rates of two compounds were above 97%. The typical HPLC chromatograms of the two standard compounds and of the EtOH extract of MB are shown in Fig. 1.

Animals

Six-week-old male ICR mice were purchased from the Koatech bio (Busan, Korea). The animals were kept under controlled conditions (humidity of $50 \pm 10\%$, 12 h light/dark cycle, and at 25 ± 2 °C). The mice received food and water *ad libitum* during the experimental period. Their body weight was measured twice a week throughout the entire experimental period. Animal experiments were performed in compliance with the institutional guidelines and the protocols were approved by the Animal Care and Use Committee at Catholic University of Daegu (IACUC-2015-022, Geongsan, Korea).

Experimental procedure

For experiment I, mice were randomly divided into four groups: normal control (NC, $n = 10$), diabetes control

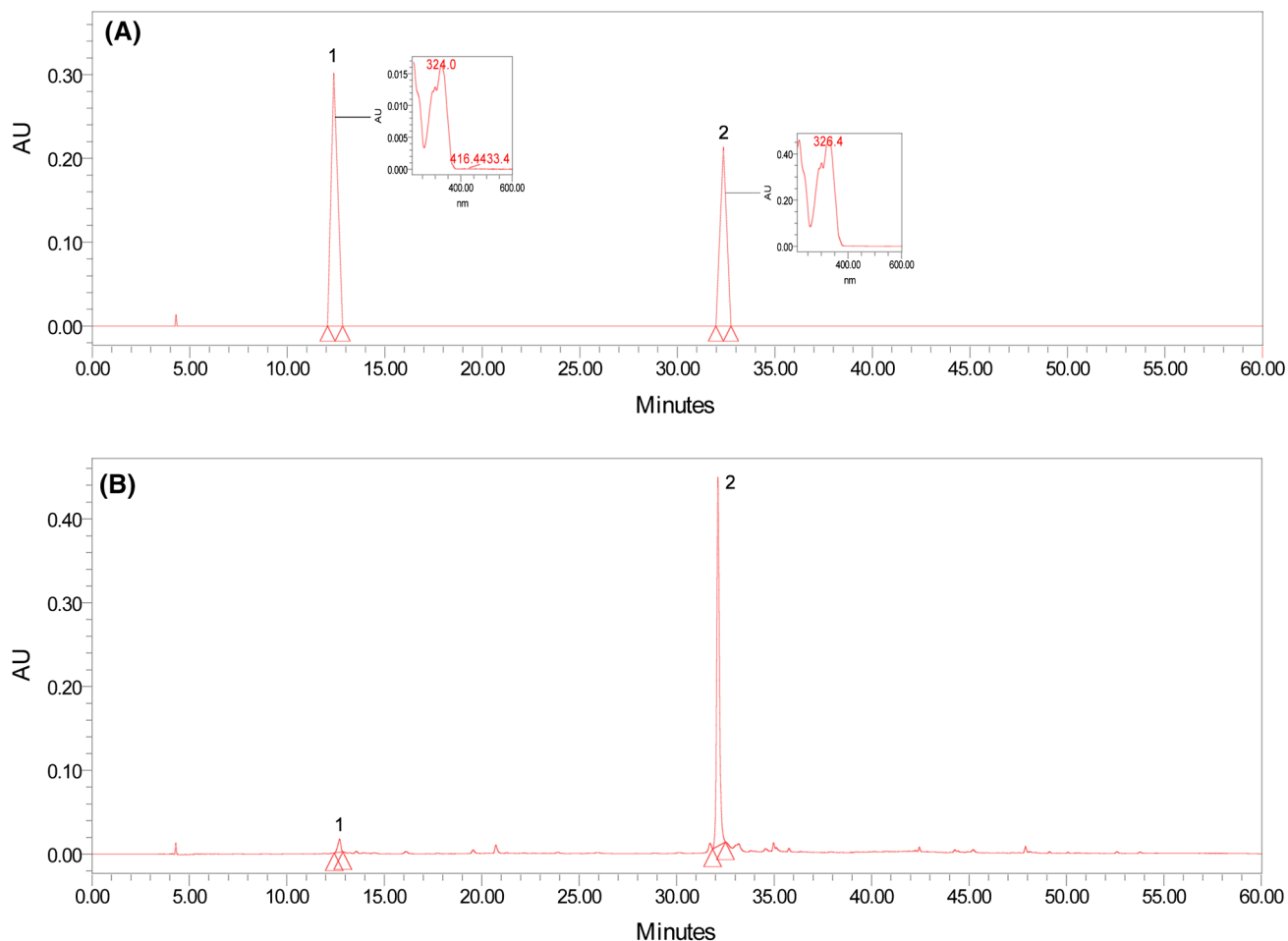


Fig. 1 HPLC chromatograms of two standards, mulberroside A (MSA) and oxyresveratrol (ORT) (A), and the EtOH extract of mulberry branch (MB) (B). 1: Mulberroside A (MSA), 2: oxyresveratrol (ORT)

(DC, $n = 10$), diabetes + MB EtOH extracts 0.5 g/kg BW (MBE0.5, $n = 10$), and diabetes + MB EtOH extracts 1 g/kg BW (MBE1, $n = 10$) (Fig. 2). For experiment II, mice were divided into four groups: NC ($n = 7$), DC ($n = 7$), DORT (diabetes + ORT, $n = 10$), and DMet (diabetes + metformin, $n = 10$). To induce diabetes, STZ (Sigma chemical, St. Louis, Missouri, USA) was dissolved in 0.1 M of citrate buffer (pH 4.5) and intraperitoneally-injected (40 mg/kg/day) to mice for five consecutive days. After we evaluate that blood glucose concentration from the tail vein is reached > 300 mg/dL by STZ injection, animals were orally administered with MB EtOH extracts (0.5 or 1 g/kg BW) once a day for 22 consecutive days in experiment I. In experiment II, mice were orally administered with ORT (0.6 g/kg BW) or Met (0.6 g/kg BW) for

22 consecutive days. Mice in NC and DC groups were administered with vehicle.

Tissue preparations

When the experiments I and II were completed, mice were fasted for 12 h, anaesthetized with CO_2 and sacrificed. Blood was taken from the inferior vena cava in a heparin-coated tube and centrifuged at 3000g for 15 min at 4 °C to separate the plasma. After the blood was collected, the liver was promptly removed, rinsed, weighed, frozen in liquid nitrogen, and stored at -70 °C until analysis. The small intestine (the section 10 cm away from the pylorus and just before the appendix) was immediately removed and gently flushed with ice-cold phosphate buffer saline (PBS). It was

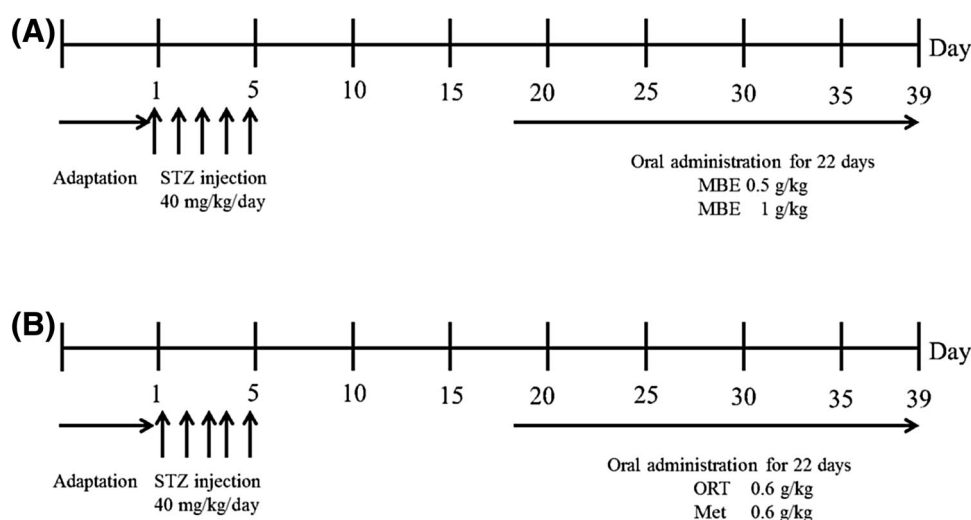


Fig. 2 Schematic representation of the experiments in T1DM animal model. **(A)** Experiment I; after 1 week of adaptation, male ICR mice were injected 5 times with STZ (40 mg/kg/day). Experimental supplement was orally administrated to mice in each group once a day for 22 days after the last injection of STZ. STZ, streptozotocin;

MBE, mulberry branch EtOH extract, **(B)** experiment II; After 1 week of adaptation, male ICR mice were injected 5 times with STZ (40 mg/kg/day). Experimental supplement was orally administrated to mice in each group once a day for 22 days after the last injection of STZ. STZ streptozotocin, ORT oxyresveratrol, Met metformin

divided into three parts (proximal, middle, and distal) and was used to prepare the crude enzyme solution for determination of disaccharidase activity.

Plasma and whole blood biomarkers

The concentration of fasting blood glucose was measured with whole blood obtained from the tail vein after 12 h of fasting using a glucose analyzer, blood glucose monitoring kit (Arkray, Japan). The plasma glucose and insulin levels were analyzed with commercially available kits: glucose (Asan, Seoul, Korea) and mouse ultrasensitive insulin ELISA Kit (Alpco, Salem, NH, USA).

Disaccharidase activities

Intestinal disaccharidase activities were determined by measuring the amount of glucose released from lactose, sucrose, and maltose [16]. Briefly, individual intestinal parts were opened and flushed with ice-cold PBS, and the mucosa was collected by scraping with a glass slide and homogenized in 4 volumes of distilled water. After centrifugation at 7000g for 10 min at 4 °C, the homogenate supernatant was diluted onefold, eightfold, and tenfold to measure the activity of lactase, sucrase, and maltase, respectively. An equal volume of 0.1 M sodium maleate buffer (pH 6.0) containing 56 mM disaccharide solution was added to each diluents and incubated for 1 h at 37 °C. The mixture was heated in boiling water for 2 min and cooled, and a glucose oxidase reagent containing *o*-dianisidine as a chromogen was added. After 1 h of incubation

at 37 °C, the absorbance was measured at 420 nm. Disaccharidase activity was expressed as U/mg protein.

Measurement of hepatic glycogen content

The hepatic glycogen content was determined according to the method of Seifter and Dayton [17] with modification. Briefly, the liver tissue was homogenized in 6 volumes of 30% (w/v) KOH solution and dissolved at 100 °C for 30 min. Three volumes of 95% EtOH were added and O/N was incubated at 4 °C. Next day, it was centrifuged at 5000g for 15 min at 4 °C and the pellet was collected and dissolved in distilled water. The dissolved pellet was treated with an anthrone (Kanto, Osaka, Japan) reagent (0.02 g anthrone/L of 95% (v/v) H₂SO₄) and glycogen content was determined by measuring the absorbance at 620 nm.

RNA isolation and mRNA expression analysis

Total RNA was isolated from the liver. RNA was reversibly transcribed with reverse transcriptase (TaKaRa, Dalian, China) and oligo-(dT) primer (Macrogen, Seoul, Korea). The mRNA expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward: 5'-GAGGGGC-CATCCACAGTCTTC-3', reverse: 5'-CATCACCATCTCCAGGAGCG-3'), and glucose transporter 2 (GLUT2, forward: 5'-GCTGTCTCTGTGCTGCTTGT-3', reverse: 5'-CGTAACTCATCCAGG CGAAT-3') were determined by quantitative real time polymerase chain reaction (qRT-PCR). The reaction was carried out in 10 µL of SYBR

Green Master Mix (Invitrogen, Eugene, OR, USA) according to the manufacturer's instructions. Relative ratios were calculated based on the $2^{-\Delta\Delta C_t}$ method [18]. Relative quantification is based on the expression levels of GLUT2 (target gene) versus GAPDH (reference gene). Expression of GLUT2 mRNA was normalized by GAPDH and is presented as GLUT2/GAPDH ratio. PCR was monitored using the Mini Opticon Real Time PCR System (Bio-Rad, Philadelphia, PA).

Statistical analysis

All statistical analyses were performed using the SPSS program (ver. 19, IBM Korea, Seoul, Korea). The data were analyzed by one-way analysis of variance (ANOVA) and presented as mean \pm standard error mean (SEM). The differences were considered significant at $p < 0.05$.

Results and discussion

Levels of MSA and ORT in mulberry branches

Prior to selecting MB, the content of ORT and MSA in mulberry twigs (small and thin branches, < 1.0 cm in diameter) and stems (big and thick branches, 1.0 – 3.0 cm in diameter) were analyzed by HPLC. Levels of MSA of mulberry stems and twigs were 2.08 and 2.21%, respectively, while those of ORT of mulberry stems and twigs were 11.95 and 3.83%, respectively (Table 1). Thus, ORT level of mulberry stems was higher than that of mulberry twigs, whereas MSA level of mulberry twigs was higher than that of mulberry stems. The EtOH extracts of MB including mulberry twigs and stems were herein used as a sample.

MB decreased the fasting blood and plasma glucose in diabetic mice

Hypoglycemic effects of mulberry tree-derived products including mulberry fruits [6], leaves [7], and root barks [10] are well documented. However, limited studies have

reported the hypoglycemic activity of MB in diabetic animal models.

Ten days after the oral administration of MB, fasting blood glucose (FBG) was measured. FBG level in mice of the DC group (583.3 ± 10.57 mg/dL) was significantly higher than that in mice of the NC group (86.2 ± 6.84 mg/dL). However, FBG level was significantly lower in the MBE0.5 group (42%) and MBE1 group (80%) than in the DC group [Fig. 3(A)]. The concentrations of plasma glucose were measured after animals were euthanized. The plasma glucose concentrations of NC and DC mice were 87.0 ± 3.14 and 225.7 ± 9.71 mg/dL, respectively [Fig. 3(B)]. MBE0.5 and MBE1 groups showed significant decrease of plasma glucose level compared to the DC group in a dose dependent manner. Notably, MBE1

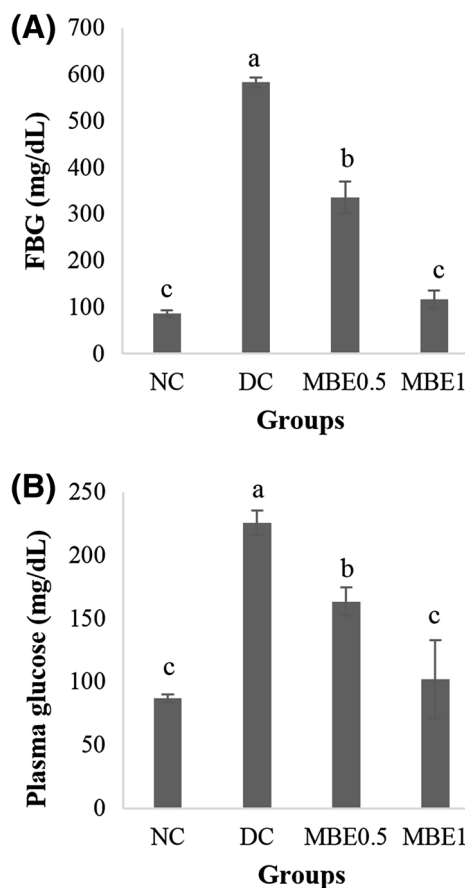


Fig. 3 Hypoglycemic effects of MB EtOH extracts. In STZ-induced diabetic ICR mouse, EtOH extracts of MB (0.5 or 1 g/kg BW) were orally administered for 22 days. After 10 days of oral administration, fasting blood glucose (FBG) (A) was measured from mouse tail vein. Concentration of plasma glucose (B) was measured after euthanization. Values are presented as the mean \pm SE. Means with different letters are significantly different at $p < 0.05$ using Duncan's multiple range test. NS, not significant; NC, normal control; DC, diabetes control; MBE0.5, mulberry branch EtOH extract 0.5 g/kg BW; MBE1, mulberry branch EtOH extract 1 g/kg BW

Table 1 Contents of ORT and MSA of mulberry branches including twigs and stems

Part of mulberry branch	Content (% , EtOH ext.)	
	MSA	ORT
Stem	2.08 \pm 0.23	11.95 \pm 1.12
Twig	2.21 \pm 0.29	3.83 \pm 0.31
Average	2.14 \pm 0.26	7.89 \pm 0.71

Data represent mean \pm SD of triplicate determinations

decreased the level of plasma glucose relative to that in the NC group.

STZ is a naturally occurring chemical with selective toxicity to insulin-producing beta cells in the pancreatic islets in mammals and has long been used to induce T1DM in experimental animals. Since its structure is highly similar to glucose, it has been thought that STZ is transported to cells through glucose transport protein, GLUT2, and it has been shown that STZ was not recognized by other glucose transporters [19]. This finding explains its specificity to beta cells because these cells express relatively high levels of GLUT2 [20].

It has been reported that normal and alloxan-induced diabetic mice and rats treated with water extracts of MB at doses of 1.25, 2.5, and 5 g/kg BW demonstrated blood glucose concentration reduction in a dose-dependent manner [8]. Guo et al. [9] reported that oral administration of polysaccharides (PS) isolated from MB at a dose of 0.6 g/kg BW significantly reduced fasting blood glucose level in STZ-diabetic mice. Moreover, the severely damaged structures and number of islets of pancreatic beta cells were effectively restored following PS administration. In this study, we identified that MB EtOH extracts also possesses potent hypoglycemic activity.

Notably, the levels of FBG and plasma glucose were quite different in this experiment. FBG levels of diabetic animals were about 2 times higher than plasma glucose level. Although we do not know an exact reason, we speculate that the temporary mouse health or metabolic state caused this difference. The reported levels of FBG varied in the range of 150–500 mg/dL in STZ-induced diabetic mice within the same species [21, 22]. Nevertheless, MBE administration significantly reduced both FBG and plasma glucose in a dose dependent manner.

ORT improved hyperglycemia in diabetic mice

In the previous HPLC analysis of MB EtOH extract (Fig. 1), we found the extracts are consisted of major ORT ($7.89 \pm 0.71\%$) and minor its glycoside, MSA ($2.14 \pm 0.26\%$) (Fig. 1). We therefore hypothesized that ORT is the primary bioactive compound accounts for the anti-diabetic action of MB.

To test our hypothesis, we administered ORT at 0.6 g/kg BW to the diabetic animals for 19 days and compared to the blood glucose lowering effect of Met (0.6 g/kg BW). The dose of Met used in reported mouse studies are varied in the range of 0.25–1 g/kg BW. We chose 0.6 g/kg BW of ORT in our study as Guo et al. [9] compared the anti-diabetic effect of polysaccharides isolated from mulberry branches to that of Met at 0.6 g/kg BW on STZ-diabetic mice. FBG level was significantly lower in the DORT and DMet groups than in the DC group [Fig. 4(A)]. The plasma

glucose levels of DORT group and DMet group were reduced to a similar level to that of NC group [Fig. 4(B)]. Similar to the result of MB, STZ injection significantly reduced plasma insulin level [Fig. 4(C)]. However, no significant difference was found between the diabetic groups. Again, these results suggest that the anti-diabetic effect of ORT was not dependent on insulin secretion.

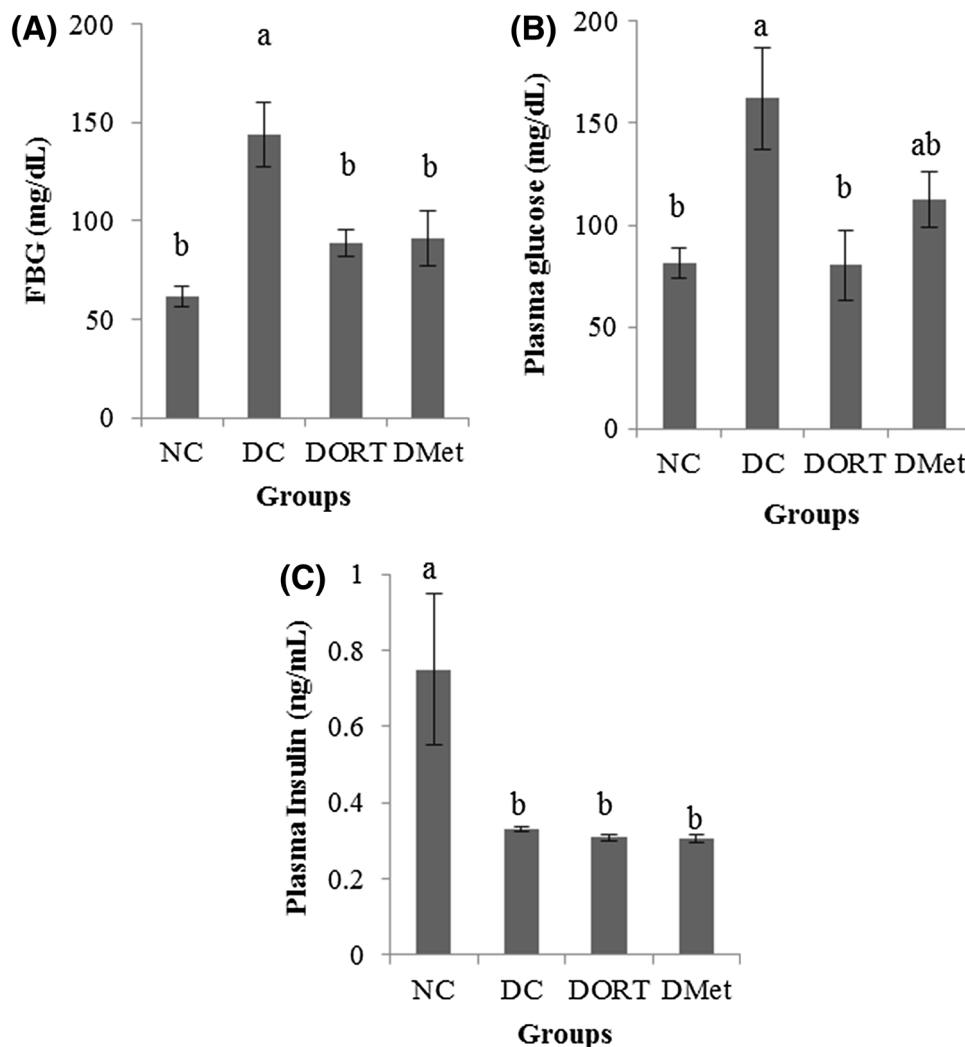
ORT, a hydroxylated form of resveratrol, is a natural hydroxystilbene found at high concentration in mulberry trees. Despite its good solubility in aqueous solution and high bioavailability (approximately 50%) [23], the biological activities of ORT have not been fully explored. ORT is known to inhibit tyrosinase [13], cyclooxygenase [24], and rat liver mitochondrial ATPase activities [25]. Further, ORT has been reported as a potent antioxidant and free radical scavenger in microglial cells [14]. Further, its anti-inflammatory [12] and anti-immune activities [26] have been demonstrated in vitro and in vivo. Mouihate et al. [26] reported that ORT-induced dampening of neuroimmune response was largely attributed to its inhibitory effect on tumor necrosis factor- α production. It has been reported that MSA from mulberry root barks exerted significant hypoglycemic effects on alloxan-induced diabetic mice [10]. MSA (50 mg/kg) markedly reduced plasma glucose level and the reduction was comparable to that of gliclazide. Taken together, although MSA was found to exhibit hypoglycemic activity in T2DM, the hypoglycemic effect of ORT has not yet been demonstrated in T1DM. To our knowledge, this is the first study to report the anti-diabetic activity of ORT and its ability as a potent blood glucose-lowering agent in STZ-induced T1DM diabetic animal model.

Intestinal disaccharidase activity of ORT in diabetic mice

To investigate the mechanism of the anti-diabetic activity of ORT, the disaccharidase activity in the small intestine was first evaluated. Since disaccharidases were predominantly active at the proximal part of the small intestine and monosaccharides are absorbed at the proximal jejunum, the enzyme activities were measured at the proximal region. As shown in Fig. 5, the activities of lactase, sucrase, and maltase were higher in all diabetic mice than those in NC mice. However, no significant difference was found among the experimental groups.

Carbohydrates are generally digested into oligosaccharides and disaccharides by digestive enzymes secreted into the small intestine. Disaccharides are then converted into monosaccharides by disaccharidase located on the brush border membranes in the small intestinal mucosa. Intestinal disaccharidases contain sucrase, maltase, and lactase. Sucrase and maltase are known as α -glucosidase since they

Fig. 4 Effect of ORT on the level of plasma glucose and insulin. In STZ-induced diabetic ICR mouse, ORT (0.6 g/kg BW) and Met (0.6 g/kg BW) were orally administered for 22 days. After 10 days of oral administration, fasting blood glucose (FBG) (A) was measured from mouse tail vein. Concentration of plasma glucose (B) and insulin (C) was measured after euthanization. Values are presented as the mean \pm SE. Means with different letters are significantly different at $p < 0.05$ using Duncan's multiple range test. NS not significant, NC normal control, DC diabetes control, DORT diabetes + oxyresveratrol; DMet diabetes + metformin



can catalyze the liberation of α -glucose from the non-reducing end of the substrate. The increase in disaccharidase activity would directly cause postprandial hyperglycemia, which is considered to be a major risk factor for diabetes patients [27]. In fact, significant increase in disaccharidase activities has been observed in the small intestine of experimental animals [28] and diabetic patients. Therefore, limiting and/or delaying glucose absorption after meals is known to be beneficial in controlling plasma glucose concentration and diabetic complications [4].

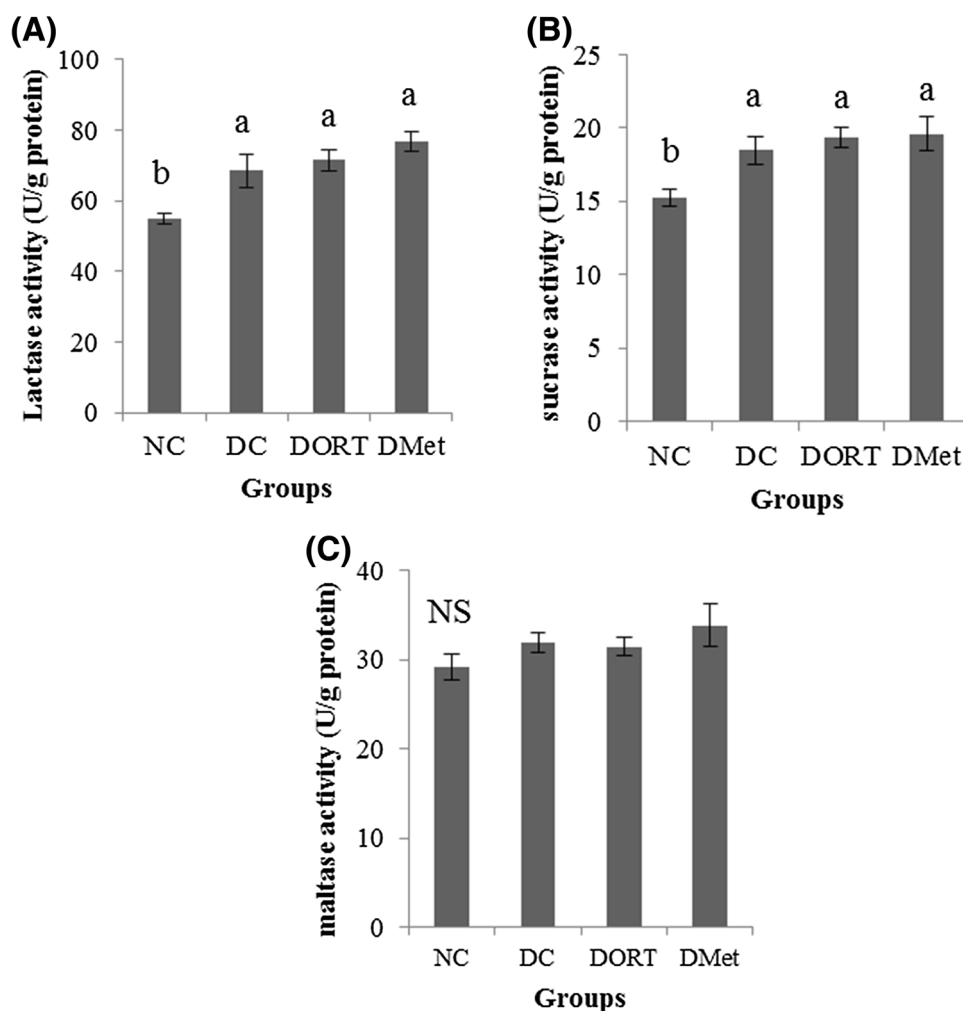
The aqueous extracts of MB have been reported as a potent α -glucosidase inhibitor in the small intestine of rats and mice after screening hundreds of traditional Chinese medicines [8]. The hypoglycemic effect of high dose MB (2.5 and 5 g/kg body wt.) was comparable to that of acarbose, an α -glucosidase inhibitor. Although increased disaccharidase activity was promoted by diabetes induction, it was found that ORT and Met administration did not affect enzyme activities.

ORT increased GLUT2 transcription and glycogen contents in the liver

Since intestinal disaccharidase activity and level of plasma insulin did not differ significantly among the diabetic groups, hepatic GLUT2 mRNA level was measured to investigate whether ORT reduced blood glucose level by stimulating hepatic glucose uptake. GLUT2 mRNA level in the liver was significantly lower in DC mice than in NC mice [Fig. 6(A)]. However, GLUT2 expression was significantly higher in the DORT and DMet groups than in the DC group.

In diabetes, hepatic glycogen is metabolized to glucose, which is released into the blood and contributes to hyperglycemia. As shown in Fig. 6(B), the level of hepatic glycogen in DC mice was significantly lower than that in NC mice. Consistent with the findings of GLUT2 expression, hepatic glycogen contents in DORT and DMet groups also increased to a similar level to that of the NC group.

Fig. 5 Effect of ORT on intestinal disaccharidase activity. Lactase (A), sucrase (B), and maltase (C) activity were measured at the proximal region of the small intestine. Values are presented as the mean \pm SE. Means with different letters are significantly different at $p < 0.05$ using Duncan's multiple range test. *NS* not significant, *NC* normal control, *DC* diabetes control, *DORT* diabetes + oxyresveratrol, *DMet* diabetes + metformin



Taken together, these suggest that ORT may be responsible for the anti-diabetic action of MB by stimulating hepatic glucose uptake and glycogen storage.

The liver plays an important role in glucose homeostasis by regulating glucose uptake and glucose storage. In contrast to muscle and fat, where glucose transport is stimulated by insulin, liver glucose transport is not sensitive to insulin [29]. GLUT2, a facilitated-diffusion glucose transporter, is expressed in a tissue-specific (liver, pancreatic β -cells, kidney tubular epithelial cells, and intestinal mucosa cells) manner and is the main isoform of glucose transporters in the liver [30]. Several studies have demonstrated that liver GLUT2 expression is regulated by glucose concentration. It has been shown that GLUT2 expression reduced during starvation but increased to normal levels after food intake [31]. However, in diabetic animals, liver GLUT2 expression appears to be contradictory. It has been reported that GLUT2 gene expression is upregulated in diabetic rats [30]. In cultured rat hepatocytes, the levels of GLUT2 mRNA and protein were significantly increased by glucose stimulation [32]. However, Akarte et al. [33]

showed that GLUT2 expression in the liver of STZ-induced diabetic rats was lower than that in normal rats. Immunostained hepatocytes of STZ-induced hyperglycemic animals demonstrated that hyperglycemic rats possess markedly fewer GLUT2 positive hepatocytes than control rats do. In the present study, GLUT2 mRNA expression was significantly lower in the DC group than in the NC group.

Glycogen synthesis in the liver is impaired during diabetes [33] hence, decreased glycogen content is commonly found in the liver of diabetic animals [34]. Similarly, our study showed that glycogen levels in liver tissues were significantly lower in the DC group than in the NC group. This decrease is largely attributed to reduced glycogen synthase activity either by insulin depletion or by insulin resistance. When insulin binds to an insulin receptor on the plasma membrane, Akt signaling pathway is activated, subsequently active Akt phosphorylates and inactivates glycogen synthase kinase 3 (GSK3). Inactive GSK3 is unable to phosphorylate glycogen synthase. Thereafter, unphosphorylated active glycogen synthase initiate

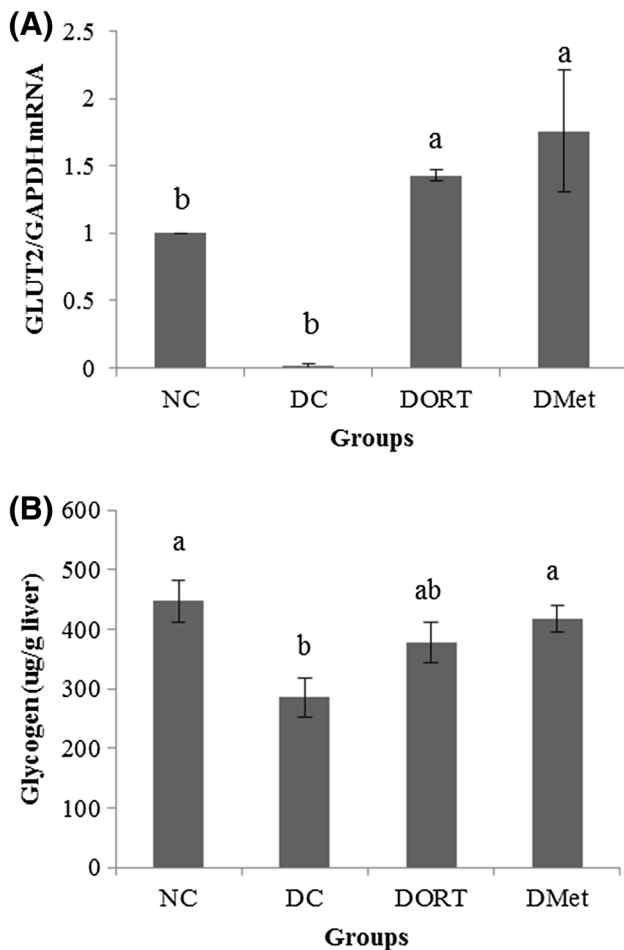


Fig. 6 Effect of ORT on GLUT2 expression and glycogen contents in the liver of diabetic mouse. In STZ-induced diabetic ICR mouse, ORT (0.6 g/kg BW) and Met (0.6 g/kg BW) were orally administered for 22 days. After euthanization, expression of glucose transporter 2 (GLUT2) mRNA in the liver (A) was measured by qRT-PCR. Concentration of liver glycogen (B) was measured. Values are presented as the mean \pm SE. Means with different letters are significantly different at $p < 0.05$ using Duncan's multiple range test. NS not significant, NC normal control, DC diabetes control, DORT diabetes + oxyresveratrol, DMet diabetes + metformin

glycogen synthesis from glucose-6-phosphate. Any defect in the glycogen synthesis pathway leads to diabetic complications [35]. Taken together, ORT showed hypoglycemic effects by increasing liver GLUT2 expression and glycogen content. Therefore, we propose that facilitated hepatic glucose uptake and glycogen synthesis is a mechanism of the anti-diabetic effect of ORT.

There are several limitations in this study. First, since the anti-diabetic effect of MB and ORT was investigated in two independent experiments, the hypoglycemic activity of MB and ORT could not be compared. Second, only single oral doses of ORT were evaluated. It will be interesting to investigate whether the anti-diabetic activity of ORT exhibit a dose-response relationship. Third, STZ-induced

diabetic animal model was employed to evaluate T1DM; therefore, it would be interesting to include insulin as a positive control. Nevertheless, to the best of our knowledge, this study is the first to report the anti-diabetic and hypoglycemic effects of MB and ORT in diabetic animals (T1DM). Considering that insulin injection is the only therapy available for controlling blood glucose concentration in T1DM patients, the insulin independent blood glucose lowering effect of ORT suggests that MB extract and its bioactive compound ORT could be developed as healthy functional food for T1DM patients.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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