New insights into the biological activities of *Chrysanthemum morifolium*: Natural flavonoids alleviate diabetes by targeting α -glucosidase and the PTP-1B signaling pathway

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ABSTRACT

As dual regulators, the PTP-1B signaling pathway and α -glucosidase slow glucose release and increase the degree of insulin sensitivity, representing a promising therapeutic target for type 2 diabetes. In this study, we systematic examined the *in vivo* and *in vitro* anti-diabetic activities of natural flavonoids **1–6** from *Chrysanthemum morifolium*. Flavonoids **1–6** increased glucose consumption-promoting activity and the phosphorylation of GSK-3 β and Akt, and decreased PTP-1B protein level along with slightly inhibitory activity of the PTP1B enzyme. Moreover, flavonoids **1–2** treatment induced insulin secretion in INS-1 cells. Besides, *in vivo* study revealed that flavonoids **2** and **5** demonstrated potent anti-hyperglycemic and anti-hyperlipidemic activity, and improved maltose and glucose tolerance. Although flavonoid **2** exhibited lower inhibitory activity against α -glucosidase *in vitro*, it could deglycosylated *in vivo* to diosmetin to function as an α -glucosidase inhibitor. Taken together, these results led to the identification of the natural flavonoids **1–6** from *C. morifolium* as dual regulators of α -glucosidase and the PTP-1B signaling pathway, suggesting their potential application as new oral anti-diabetic drugs or functional food ingredients.

Keywords: *Chrysanthemum morifolium*; Flavonoid; Diabetes; α -Glucosidase; PTP-1B signaling

1. Introduction

Chinese “Hangbaiju” comprises the flowering head of *Chrysanthemum morifolium*, which constitutes an important traditional Chinese medicine used for treatment purposes, e.g., “scattering cold”, “cleaning heat and toxins”, and “brightening eyes” [1, 2]. *C. morifolium* has been widely used for approximately 2000 years in China as an herbal tea, and is considered a functional food as it contains many types of biologically active components, such as flavonoids [3-5], triterpenoids [6], and volatile oils [7]. Among these, flavonoids are considered to represent the main bioactive components and have been found to exhibit

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antioxidant [8, 9], cardiovascular-protective [10, 11], and vasorelaxant activities [2, 10]. However, few studies have reported the application of *C. morifolium* as the anti-diabetic functional food and traditional Chinese medicine. The focus of our research is the identification of natural bioactive flavonoids to facilitate their application as functional foods and drug candidates. Our previous studies have identified several series of flavonoids as potent α -glucosidase inhibitors [21, 22]. In the present study, we identified two natural flavonoids **1–2** and their aglycone **3**, along with flavones **4–6** (Fig. 1B) from *C. morifolium* and evaluated their *in vitro* and *in vivo* anti-diabetic activities.

2. Materials and methods

2.1. Liquid chromatography-mass spectrometry (lc-ms) conditions

For LC-MS, we utilized a DGU-20A3R high-performance LC instrument coupled to a diode array detector and mass spectrometer (Shimadzu Corporation, LC-MS-IT-TOF, Kyoto, Japan). A 250 mm $\times$ 4.6 mm, 5- μ m, Symmetry C18 column (Agela Technologies Inc., Torrance, CA) with 20 mm $\times$ 3.9 mm i.d, 5- μ m, Sentry guard column was used at a flow rate of 1.0 mL/min. The column oven temperature was set at 25 °C. The mobile phase consisted of a combination of A (0.1% trifluoroacetic acid in water) and B (0.1% trifluoroacetic acid in acetonitrile). The gradient was varied linearly from 10% to 26% B (v/v) in 40 min, 65% B at 70 min, and finally to 100% B at 71 min and held at 100% B to 75 min. The diode array detection was set at 350, 310, 270 and 520 nm for real-time monitoring of the peak intensity and full spectra (190–650 nm) were continuously recorded for plant component identification. Mass spectra were simultaneously acquired using electrospray ionization in the positive and negative ionization modes at low and high fragmentation voltages (–3.5 and 4.5 kV) over the range of m/z 100–1,000.

2.2. Materials and in vitro protocols

2.2.1. Materials

Dried *C. morifolium* flower was purchased from Tianfu Tea Co. Ltd. (Zhejiang, China). Baker's yeast α -glucosidase, p-nitrophenyl α -D-glucopyranoside (pNGP), 4-nitrophenyl phosphate disodium salt (pNPP), NaVO₄, acarbose, glibenclamide, and streptozocin (STZ) were purchased from Sigma-Aldrich (St. Louis, MO). Recombinant human PTP1B protein was purchased from Abcam (Cambridge, UK). The BCA Protein Assay Kit was obtained from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China). PTP-1B antibody, Akt (pan) (11E7) rabbit mAb, phospho-Akt (Ser473) (193H12) rabbit mAb, GSK-3 β (D5C5Z) XP rabbit mAb, phospho-GSK-3 β (Ser9) (D3A4) rabbit mAb, α / β -tubulin antibody, and goat anti-rabbit IgG(H+L)-HRP were purchased from Cell Signaling Technology (Danvers, MA, USA). HepG2 (human liver cancer), CHO-K1 (Chinese hamster ovary), and INS-1 (rat insulin-secreting beta) cells were acquired from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China).

2.2.2. Glucose consumption (GC) assay

The GC assay was performed as previously described [22]. Briefly, HepG2 cells were

cultured in Dulbecco's-modified Eagle's medium (DMEM) containing 25 mM D-glucose, 10% heat-inactivated fetal bovine serum (FBS), 10 U/mL penicillin, and 10 mg/mL streptomycin at 37 °C under a 5% CO₂ atmosphere. Cells were deprived of serum for 24 h. The cells were washed twice with phosphate buffered saline (PBS), and the medium was replaced by RPMI-1640 containing glucose (11.1 mM) supplemented with 0.2% bovine serum albumin. Then, metformin (0.5 mM) or test flavonoids were added to the medium at different concentrations. Dimethyl sulfoxide (DMSO) was used as the blank control. The glucose concentration in the medium was determined via the glucose oxidase method after 24 h treatment.

2.2.3. MTT method

The MTT assay was performed before the GC experiment. MTT (20 µL, 5 mg/mL dissolved in PBS) was added to each well. After 4 h, DMSO (100 µL) was added to dissolve the crystals, and the plate was placed on a shaker for 10 min. The OD value was measured using a microplate reader at a wavelength of 492 nm. The MTT results were used to normalize the GC results. GC owing to cell proliferation can be deducted by calculating the ratio of the GC and MTT (GC/MTT = mM/OD).

2.2.4. Western blotting

CHO-K1 cells were cultured in DMEM/F-12 Ham's medium supplemented with 10% FBS, penicillin (50 U/mL), and streptomycin (100 µg/mL) at 37 °C in a humidified atmosphere with 5% CO₂ [23]. Cells (5×10^4 /mL) were seeded in a 96-well cell plate for 24 h and then treated with compounds **1–6** (20 µM) or DMSO for 48 h. NaVO₄ was used as a positive control. Then, the cells were lysed with ice-cold RIPA buffer containing freshly added aprotinin and phenylmethanesulfonyl fluoride. The BCA Protein Assay Kit was used to determine protein concentration. Cell lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto a polyvinylidene fluoride membrane (Millipore, Billerica, MA). The membranes were blocked with 5% non-fat milk in 1× PBS containing 0.5% Tween 20 (PBST) for 1 h at room temperature and were then incubated with a primary antibody overnight at 4 °C. The following day, the membranes were washed three times with PBST and probed with a secondary antibody. Pierce™ ECL Western Blotting Substrate (Thermo Scientific, Waltham, MA) was used to detect the bands [24].

2.2.5. Glucose-stimulated insulin secretion and basal insulin secretion

INS-1 cells were cultured in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% FBS, streptomycin (100 µg/mL), and penicillin (50 U/mL) at 37 °C in a humidified atmosphere containing 5% CO₂. Cells (2×10^5 cells/mL) were seeded in a 48-well cell plate for 24 h. The cell culture medium was replaced by IMDM containing 40 mM glucose then cells were incubated with compounds or DMSO for 48 h. Glibenclamide was used as a positive control. Thereafter, the cell culture medium was carefully removed, the cells were washed with PBS, and 300 µL Krebs-Ringer's bicarbonate (KRB) buffer containing 2% FBS was added. After 30 min, the cells were stimulated with KRB buffer containing 5 or 30 mM (BIS or GSIS) glucose for 60 min at 37 °C. The cell medium was then collected for analysis of insulin secretion, which was determined using a rat insulin enzyme-linked immunosorbent

assay kit (Nanjingjiancheng, Nanjing, China), whereas 100 μ L RIPA Lysis buffer was added to the cells, which were saved for determination of the total protein content [25].

2.2.6. Inhibition studies of PTP-1B

pNPP was used as a substrate for the measurement of PTP1B activity. Compounds **1-6** were dissolved in DMSO. PTP-1B, pNPP, and NaVO₄ were dissolved in assay buffer (pH 6.0), which contains 150 mM NaCl, 50 mM 2-(N-morpholino) ethanesulfonic acid, 1 mM ethylenediaminetetraacetic acid, 1 mM dithiothreitol, and 0.05 % NP-40. Enzymatic reaction mixtures, composed of PTP-1B (20 μ g/mL, 5 μ L), test compounds (10 μ L), and buffer (75 μ L), were incubated at 30 °C for 5 min, then the reactions were initiated by adding pNPP (100 mM, 10 μ L) and incubated at 30 °C for 10 min. Enzymatic activity was detected by spectrophotometry at 405 nm [27].

2.2.7. Inhibition studies of α -glucosidase

Test compounds **1-6** and acarbose were dissolved in DMSO. α -Glucosidase and pNGP were dissolved in potassium phosphate buffer (0.05 M, pH 6.8). Enzymatic reaction mixtures, composed of α -glucosidase (4 mU, 20 μ L), pNGP (75 μ M, 30 μ L), test compounds (10 μ L), and potassium phosphate buffer (140 μ L), were incubated at 37 °C for 30 min. Enzymatic activity was detected by spectrophotometry at 405 nm.

2.3. Materials and in vivo protocols

2.3.1. Animals and diets

Four- to six-week-old male Kunming mice (18–22 g) were procured from The Institute for Laboratory Animal Science, Chinese Academy of Medical Sciences and Peking Union Medical College [Beijing, China, License SCXK (Jun) 2012-0004]. Animal procedures were approved by the Tianjin University of Science and Technology Institutional Animal Care Committee and performed in strict compliance with local and national ethical guidelines. The mice were kept in polypropylene cages (five in each cage) and housed under laboratory conditions (18–23 °C, 55–60% humidity, 12 h light/dark cycle). The mice were fed a standard pellet diet for 1 week after arrival and randomly divided into two groups: mice fed a standard pellet diet (normal mice), and mice fed a high-fat, high-fructose diet to induce type 2 diabetes. All mice had free access to food and water [21].

2.3.2. Induction of experimental type 2 diabetic mice and treatment

After ingesting the high-fat, high-fructose food for 3 weeks, the mice were subjected to a 12 h fast. Type 2 diabetes was induced by an intraperitoneal injection of STZ [100 mg/kg of body weight, dissolved in 0.05 M citrate buffer (pH 4.5)]. After another 2 weeks of the high-fat, high-fructose diet, fasting blood from the tail vein was used to determine blood GC. The mice were classified as having type 2 diabetes if blood glucose levels were > 11.0 mM. Type 2 diabetic mice were fed the high-fat, high-fructose diet throughout the study. Blood glucose and body weight were checked every week. Type 2 diabetic mice were divided into 6 groups with 10 mice per group. Each day, 10 mL of saline/kg, 50 mg of acarbose in saline/kg, 50 and 100 mg of diosmin in saline/kg, and 50 and 100 mg of apigenin in saline/kg were

orally administered to groups 1 (T2D model), 2 (acarbose), 3 (diosmin, 50 mg/kg), 4 (diosmin, 100 mg/kg), 5 (apigenin, 50 mg/kg), and 6 (apigenin, 100 mg/kg), respectively.

2.3.3. Determination of blood glucose levels

Blood glucose levels were determined as previously described [28]. After receiving treatment for 13 and 14 days and fasting overnight, all mice were orally administered glucose or maltose (2 g/kg), respectively, 60 min after treatment. Blood samples were taken from the tail vein after 0, 30, 60, and 120 min, and blood glucose was measured using a glucometer (Sinocare, Changsha, China). Areas under the curve (AUCs) were calculated using the trapezoidal rule.

2.3.4. Determination of blood lipid levels

Blood lipid levels were determined as previously described [29]. At the end of the experimental period, blood samples were collected and the serum samples obtained by centrifugation (2000 g for 20 min) were stored at -20 °C for further analysis. Serum total cholesterol (TC) and triglycerides (TGs) were analyzed using commercial diagnostic kits (Jiancheng, Nanjing, China).

3. Results and discussion

3.1. Identification of *C. morifolium* flavonoids

In order to gain some anti-diabetic insight into the flavonoids of *C. morifolium*, the flavonoids were purified and identified purposely. Dried *C. morifolium* flower was finely powdered. The sample (60 g) was refluxed and extracted with ethanol-water (480 mL, 75:25, v/v) using a Soxhlet extractor 3 times for 1 h. The extract was combined and lyophilized to give crude powder (15.64 g), of which 15 mg was dissolved in methanol (1 mL) and filtered through a 0.22 µm nylon filter. Filtered sample (20 µL) was injected for analysis. Chromatograms of the *C. morifolium* extract are shown in Fig. 1A. The retention times (t_R), molecular ions ($[M + Na]^+/[M - H]^-$), and major fragment ions of the major peaks are listed in Table 1. Flavonoids in *C. morifolium* flower were identified based on a comparison of retention times and high-resolution mass spectra for authentic standards obtained using the same reported methods [1]. As shown in figure 1, two flavonoids **1-2** and flavones **3-6** in the aqueous ethanol extract of *C. morifolium* were identified as diosmetin 7-glucoside (**1**, peak 1), diosmin (**2**, peak 2), diosmetin (**3**, peak 3), luteolin (**4**, peak 4), apigenin (**5**, peak 5), and acacetin (**6**, peak 6). The assignment of nuclear magnetic resonance and mass spectrometry spectra is described in the supporting information.

(Figure 1)

3.2. Promotion of GC by flavonoids **1-6** in HepG2 cells

To assess the anti-diabetic activity of flavonoids **1-6** in *C. morifolium* flower, the GC in HepG2 cells was evaluated. The GC results were normalized using their MTT results. As shown in Fig. 2A, all flavonoids **1-6** significantly promote glucose-consumption at doses of 0.4–10 µM. Of these, flavone **4** was found to have the highest activity, being similar potent at dose of 4 µM with metformine at dose of 500 µM. Besides, Flavonoid **2** was slightly more

potent than **1**.

(Figure 2)

3.3. Enhanced insulin signaling by flavonoids **1–6**

Insulin signaling is dependent on activation of the insulin receptor, which phosphorylates and recruits different downstream signaling molecules. Thus, phosphorylation of the signaling molecules serves as an indicator of pathway activation. Insulin-stimulated phosphorylation of GSK-3 β (Ser9) was significantly increased by flavonoids **1–6** treatment in the cell cultures (Fig. 3A and B). Phosphorylation of Akt (Ser473) was also obviously enhanced (Fig. 3A and C). Moreover, the protein levels of PTP-1B were evidently decreased by **1–6** (Fig. 3A and D). To further determine the effect on PTP-1B, the inhibitory activity of hPTP-1B was performed. The results showed that flavonoids **1–6** exhibited weak inhibitory activity against hPTP-1B at 1, 5, and 20 μ M (Table 2). These results suggested that flavonoids **1–6** directly enhanced the insulin signaling activity and decreased the protein level of PTP-1B along with slightly inhibiting the activity of the PTP1B enzyme.

(Figure 3)

(Table 2)

3.4. Effects of flavonoids **1–2** on insulin secretion in high glucose-treated INS-1 cells

To further determine the effect of insulin signaling pathway by representative flavonoids **1–2**, we performed an insulin secretion test in the high glucose-treated INS-1 cellular model. Glibenclamide used as a positive control. As shown in Fig. 2B and C, after treatment with 30 and 5.5 mM glucose, insulin secretion of cells significantly decreased (normal vs. high glucose-treated model). Treatment with 0.1, 1, 10, and 100 μ M flavonoids **1** and **2** increased dose-dependently the level of insulin secretion, which previously decreased by glucose in both groups. These results suggested that flavonoids **1–2** treatment induced insulin secretion in INS-1 cells.

3.5. α -Glucosidase inhibition of *C. morifolium* flavonoids

To discover the targets of anti-diabetic, α -glucosidase inhibitory activity of flavonoids **1–6** was investigated. Acarbose, a clinical α -glucosidase inhibitor, was selected as a reference compound. As shown in Table 3, flavone **5** (IC₅₀ = 9.04 μ M) was found to have the highest activity, being even more active than acarbose (IC₅₀ = 236.5 μ M). Moreover, no apparent inhibitory activity against α -glucosidase was observed for flavonoids **1** and **2**, with IC₅₀ values >100. However, diosmetin, the aglycone (**3**) of flavonoids **1** and **2**, exhibited comparable α -glucosidase inhibitory activity with acarbose. As it has been reported that deglycosylation of flavonoids constitutes a major metabolic pathway for these compounds [30, 31]. We considered that although flavonoids **1** and **2** exhibit no α -glucosidase inhibitory activity *in vitro*, they might be deglycosylated to diosmetin *in vivo*, which is positive for anti-diabetic activity.

3.6. Flavonoids ameliorates hyperglycemia in vivo

To further evaluate the *in vivo* anti-diabetic activity and validate our hypotheses of deglycosylation, the hypoglycemic activity of representative flavonoid **2** and **5** was

determined using STZ-induced diabetic mice. Compared with normal mice (health group), diabetic mice (T2D model group) exhibited hyperglycemia with blood glucose levels of approximately 23 mM (Fig. 4A). After 4-week treatment with flavonoids **2** and **5**, blood glucose levels significantly decreased compared with those of the vehicle group (Fig. 4A and B).

(Figure 4)

3.7. Flavonoids improves maltose and glucose tolerance *in vivo*

To assess glucose homeostasis and insulin sensitivity in diabetic mice treated with flavonoid **2** and **5**, we evaluated the amelioration of glucose tolerance. Flavonoids **2** and **5** administration resulted in a significant improvement in glucose tolerance (Fig. 4C and D). To understand the basis of flavonoid **2** and **5** activity with regard to α -glucosidase suppression, we further investigated the influence of flavonoid **2** and **5** on blood glucose levels using an *in vivo* maltose tolerance test. Blood glucose levels after oral administration of maltose indirectly reflect the activity of α -glucosidase, which can hydrolyze maltose into glucose. As shown in Fig. 4E, after treatment with flavonoid **2** and **5** for 2 h, postprandial blood glucose level obviously improved compared with that of the diabetic model group. AUCs of flavonoid **2** were reduced by 11.2% and 13.5% at a dosage of 50 and 100 mg/kg, respectively (Fig. 4F). Thus, despite the lack of *in vitro* inhibitory activity against α -glucosidase, flavonoid **2** appeared capable of significant decreasing fasting blood glucose levels and improving maltose and glucose tolerance *in vivo*. Consequently, flavonoid **2** exhibited comparable *in vivo* anti-diabetic activity with acarbose. The results strongly supported our conjecture that flavonoid **2** might be deglycosylated *in vivo* to diosmetin to function as an α -glucosidase inhibitor.

3.8. Flavonoids reduces serum levels of TG and TC

After 4 weeks of treatment with flavonoid **2** and **5**, a significant decrease in serum levels of TG and TC was observed (Table 4). TG levels were decreased by 33.8% (50 mg/kg) and 27.3% (100 mg/kg) for flavonoid **2**, whereas TC levels were decreased by 8.3% (100 mg/kg) compared to those of diabetic model mice. Especially, TC levels were decreased by 22.1% (50 mg/kg) and 15.7% (100 mg/kg) for flavonoid **5** compared to those of diabetic model mice. However, acarbose at a dosage of 50 mg/kg exhibited no anti- hyperlipidemia activity.

(Table 4)

4. Conclusion

The PTP-1B signaling pathway plays an important in the regulation of insulin signaling and the development of type 2 diabetes and obesity. α -Glucosidase catalyzes the final step of carbohydrate digestion and releases glucose. As dual regulators, the PTP-1B signaling pathway and α -glucosidase slow the release of glucose and increase the degree of insulin sensitivity, resulting in alleviation of disorders of glucose and lipid metabolism and insulin resistance, thereby representing a promising potential therapeutic strategy for the treatment of type 2 diabetes. To identify natural compounds that may influence this process, we performed *in vitro* cell-based and enzyme-based experiments to determine the effect on proteins related to the PTP-1B signaling pathway and α -glucosidase. The results showed that flavonoids **1–6**

significantly promote glucose consumption (Fig. 2A). The phosphorylation of GSK-3 β and Akt was increased and the protein level of PTP1B was decreased by flavonoid **1–6** treatment (Fig. 3). However, flavonoids **1–6** showed only weak inhibitory activity against PTP-1B at 1, 5, and 20 μ M (Table 2). Moreover, flavonoids **1–2** were capable of increasing insulin secretion in INS-1 cells (Fig. 2B and C).

Given that the *in vitro* potencies of many PTP-1B regulators or α -glucosidase inhibitors are not consistent with their *in vivo* activities, a series of *in vivo* anti-diabetic activities were evaluated in STZ-induced type 2 diabetic mice. Moreover, as the oral availability of PTP-1B represents a challenge for the development of PTP-1B inhibitors [24], we examined the effects of 4-week oral administration of flavonoid **2** and **5** in diabetic mice. Flavonoid **2** administration resulted in a significant reduction in fasting blood glucose levels (Fig. 4A and B) combined with a significant improvement in glucose tolerance (Fig. 4C and D). Furthermore, flavonoid **2** and **5** ameliorated maltose tolerance comparing with that in the diabetic model group (Fig. 4E and F), which indirectly reflected the *in vivo* activity of α -glucosidase.

Dyslipidemia, characterized by hypertriglyceridemia including TG and TC levels, constitutes a major risk factor contributing to the burden of macrovascular disease in type 2 diabetes. In the present study, flavonoid **2** and **5** showed potent lipid lowering capability in the diabetic mice, eliciting reduced serum TC and TG levels (Table 4). It is considered that the alleviation of insulin resistance may have contributed to the lower TC and TG levels.

In conclusion, natural flavonoids **1–6** from *C. morifolium* were identified as α -glucosidase and PTP-1B signaling pathway dual regulators, which could serve as a new starting point for the discovery of novel oral anti-diabetic agents and potential functional food ingredients.

Conflict of interest statement

The authors declare no conflicts of interest.

Ethics statement

I have read and adhere to the Publishing Ethics.

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Supporting information

Supplementary data related to this article can be found at <http://>. These data include NMR and mass spectra signals of compounds **1–6** described in this article.

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Figure legends

Fig. 1. Chromatograms of the aqueous ethanol extract and structures of natural flavonoids **1–6** of *C. morifolium*. (A) Chromatograms (350 nm) of the aqueous ethanol extract. Chromatograms of the *C. morifolium* extract (a), diosmetin 7-glucoside (b), diosmin (c), diosmetin (d), luteolin (e), apigenin (f), and acacetin (g). (B) Structures of identified flavonoids **1–6** from *C. morifolium*.

Fig. 2. Effects of flavonoids **1–6** on promote glucose consumption (GC) and flavonoids **1–2** on insulin secretion. (A) Flavonoids **1–6** promote GC in HepG2 cells. Metformin (Met) was used as a reference compound. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs control. Effects of flavonoids **1–2** on insulin secretion in high glucose-treated INS-1 cells. INS-1 cells were preincubated with 40 mM glucose and compounds or DMSO for 48 h. Cells were incubated with 5.5 mM (B) and 30 mM (C) glucose for 60 min, respectively. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ vs model. Data represent the average of three independent experiments, each performed in duplicate.

Fig. 3. Effects of flavonoid **1–6** and NaVO₄ on insulin signaling. (A) Akt, GSK3 β , and PTP-1B phosphorylation status was determined via western blotting. Relative ratio of p-Akt (B), p-GSK3 β (C), and PTP-1B (D). Data are expressed as the means $\pm$ SD (n = 3). ** $P < 0.01$ and *** $P < 0.001$ vs control.

Fig. 4. Effects on blood glucose levels following administration of flavonoid **2** and **5** in diabetic mice. Healthy mice were treated with vehicle, and type 2 diabetes (T2D) mice were treated with vehicle, flavonoids **2**, **5**, and acarbose for 4 weeks. Fasting blood glucose concentrations (A) and corresponding AUC (B). Effects on maltose and glucose tolerance after administration of flavonoid **2** and **5** in diabetic mice. (C) Oral glucose tolerance test (OGTT), (D) AUC in OGTT, (E) Oral maltose tolerance test (OMTT), and (F) AUC in OMTT. Data are expressed as the means $\pm$ SD (n = 10). * $P < 0.05$ and ** $P < 0.01$ vs the T2D model.

Table 1Chemical components identified from the extract of *C. morifolium* flower.

Peak No.	t_R (min)	$[M+Na]^+/[M-H]^-$ (m/z)	Predicted values (m/z)	fragments (m/z)	Identification
1	35.83	485.1047/461.1079	485.1054/461.1089	217.0383, 418.7810, 475.3239 /130.9476, 285.0398	Diosmetin 7-glucoside
2	34.65	/607.1607	/607.1668	/130.9468, 255.2341, 285.0399	Diosmin
3	53.63	323.0518/	323.0526/	217.0446, 324.0552, 419.2826/	Diosmetin
4	47.61	309.0356/	309.0370/	310.0385, 418.7813/	Luteolin
5	52.55	293.0406/	293.0420/	217.0385, 301.1409, 475.8242/	Apigenin
6	61.10	307.0559/	307.0577/	217.0368, 418.7820/	Acacetin

Table 2Human PTP-1B inhibition of flavonoids **1–6**.

No.	Name	Inhibition (%) ^a		
		20 μ M	5 μ M	1 μ M
1	Diosmetin 7-glucoside	29.79 $\pm$ 1.03	23.36 $\pm$ 0.52	27.74 $\pm$ 2.10
2	Diosmin	30.53 $\pm$ 3.63	32.82 $\pm$ 2.84	36.26 $\pm$ 1.62
3	Diosmetin	28.27 $\pm$ 3.71	27.10 $\pm$ 2.89	22.81 $\pm$ 2.07
4	Luteolin	39.92 $\pm$ 1.62	40.75 $\pm$ 2.84	19.80 $\pm$ 2.18
5	Apigenin	39.17 $\pm$ 0.87	20.23 $\pm$ 0.10	29.77 $\pm$ 1.48
6	Acacetin	32.05 $\pm$ 2.85	36.26 $\pm$ 2.70	38.17 $\pm$ 1.62
	Sodium orthovanadate ^b	56.58 $\pm$ 4.86		

^a Results represent the average of three independent experiments, each performed in duplicate. ^b Reference compound.

Table 3 α -Glucosidase inhibition of flavonoids **1–6**.

No.	Name	IC ₅₀ (μ M) ^a
1	Diosmetin 7-glucoside	> 100
2	Diosmin	> 100
3	Diosmetin	46.45
4	Luteolin	16.65
5	Apigenin	9.04
6	Acacetin	> 100
	Acarbose ^b	51.30

^a Results represent the average of three independent experiments, each performed in duplicate. ^b Reference compound.

Table 4

TC and TG levels of mouse groups.

Group	Dosage (mg/kg)	TG (mM) ^a	TC (mM) ^a
Healthy		1.55 $\pm$ 0.24	5.23 $\pm$ 0.17
T2D model		3.08 $\pm$ 0.84	9.75 $\pm$ 0.11

Acarbose	50	3.02 ± 0.79	9.61 ± 0.27
2	50	$2.04 \pm 0.36^*$	9.18 ± 0.20
2	100	$2.24 \pm 0.38^*$	$8.94 \pm 0.18^*$
5	50	3.13 ± 0.35	$7.60 \pm 0.64^{**}$
5	100	3.06 ± 0.26	$8.22 \pm 0.38^{**}$

^aData are expressed as the means $\pm$ SEM (n = 10). ^{**} $P < 0.01$, ^{*} $P < 0.05$ vs the type 2 diabetes (T2D) model.

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