



## Anti-hyperglycemic effects and mechanism of *Bidens pilosa* water extract

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### ABSTRACT

**Aim of study:** *Bidens pilosa* has traditionally been used as an anti-diabetic phytomedicine. However, its alleged benefits and mechanism remain elusive. This study aimed to evaluate the effect and action of *Bidens pilosa* water extract on type 2 diabetes.

**Materials and methods:** A daily dose of *Bidens pilosa* water extract or glimepiride, a positive control, was given orally to C57BL/KsJ-db/db mice once or for 28 days. Levels of blood glucose, serum insulin, and glycosylated hemoglobin A1C, glucose tolerance, and islet structure were used to evaluate its anti-diabetic effects in db/db mice. Rat pancreatic islets and streptozocin-treated mice were tested for insulin-releasing mechanism of *Bidens pilosa* water extract.

**Results:** A daily dose of *Bidens pilosa* water extract given once or for 28 days significantly decreased blood glucose levels and increased serum insulin levels in db/db mice. Besides, 28-day treatment with *Bidens pilosa* water extract significantly improved glucose tolerance, decreased HbA1C levels and protected islet structure in db/db mice. Mechanism study showed that *Bidens pilosa* water extract stimulated insulin secretion via pancreatic islets.

**Conclusions:** Our results suggest that *Bidens pilosa* water extract ameliorates type 2 diabetes in db/db mice via regulation of insulin secretion and islet protection.

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### 1. Introduction

Diabetes mellitus is a chronic metabolic disease which now afflicts 3% of the world population. Diabetes mellitus is classified into two types (type 1 and type 2) based on individual etiologies. Around 95% of diabetic patients are diagnosed with type 2 diabetes (Attele et al., 2002). A major feature of type 2 diabetes is insulin resistance and/or insulin deficiency which can cause hyperglycemia (Laakso, 2001). Therefore, a key strategy in treating patients with type 2 diabetes is maintenance of blood glucose level. Current oral anti-diabetic agents, which include insulin releasers, insulin

sensitizers and α-glucosidase inhibitors, have modest efficacy and limited of modes of action. In addition, current anti-diabetic drugs usually have adverse side effects, decreased efficacy over time, ineffectiveness against some long-term diabetic complications and low cost-effectiveness (Grover et al., 2002). Therefore, discovery and development of novel drugs for diabetes is still needed.

Plants are recognized as a wonderful source for medicines. It is estimated that 1200 species of plants are used as folk medicines for diabetes (Marles and Farnsworth, 1995). Most of them lack of scientific evidence for their alleged benefits. Among them, *Bidens pilosa* belongs to the Asteraceae family has been used to treat diabetes in different regions of the world (Marles and Farnsworth, 1995; Brandao et al., 1997; Pereira et al., 1999; Ubillas et al., 2000). One report showed that an aqueous ethanol fraction of *Bidens pilosa* had an anti-hyperglycemic effect in db/db mice (Ubillas et al., 2000). Moreover, a mixture of two acetylenic compounds showed a higher anti-hyperglycemic effect than of its component alone (Ubillas et al., 2000). However, the mechanism by which *Bidens pilosa* can treat type 2 diabetes is not yet clear.

In this study, we investigated the anti-hyperglycemic effects of *Bidens pilosa* water extract (BPWE) in db/db mice, a leptin receptor-deficient mouse model for type 2 diabetes study (Cefalu, 2006). We

**Abbreviations:** BPWE, *Bidens pilosa* water extract; BW, body weight; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; GLM, glimepiride; HbA1C, glycosylated hemoglobin A1C; OGTT, oral glucose tolerance test; PBS, phosphate-buffered saline; STZ, streptozocin; KRB buffer, Krebs–Ringer bicarbonate buffer.

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also studied the mode(s) of action of BPWE using pancreatic islet cells and streptozocin (STZ)-treated mice.

## 2. Materials and methods

### 2.1. Chemicals

Dimethyl sulfoxide (DMSO), STZ, glimepiride (GLM), hematoxylin, eosin Y diaminobenzidine tetrahydrochloride, and Hank's buffer were purchased from Sigma–Aldrich (St. Louis, MO, USA). GLM was dissolved in DMSO to make a stock solution of 25 mg/ml. Insulin and anti-insulin antibody (H-86) were purchased from Novo Nordisk (Princeton, NJ, USA) and Santa Cruz Biotechnology (Santa Cruz, CA, USA). RPMI 1640, Fetal bovine serum (FBS), collagenase XI, penicillin/streptomycin/glutamine, 2-mercaptoethanol and sodium pyruvate were purchased from Invitrogen (Carlsbad, CA, USA).

The water extract of *Bidens pilosa* L. var. *radiata* was prepared by shade-drying whole *Bidens pilosa* plants. The dried plants were then ground into homogenized powder. 100 g of powder was boiled in water (1:10, w/v) for 2 h twice. The water extract was filtered by the Advantec No. 2 filter paper (diameter 125 mm), concentrated with rotary evaporators, dried by lyophilization and then dissolved in water to make stock solutions. The yield of water extract was 19.4 g of BPWE out of 100 g of dried plants. Different batches of BPWE were chemically characterized by HPLC analysis. The *Bidens pilosa* plants were collected from the campus of Academia Sinica, Taiwan, on 18 and 31 August, and 3 September 2005 and authenticated by Dr. Ching-I Pen (Biodiversity Center, Academia Sinica, Taiwan). A voucher specimen (No. 120085) was deposited at the herbarium of Academia Sinica.

### 2.2. Cells and animals

Both single-dose experiments and long-term dose experiments were performed on female C57BL/KsJ-db/db mice purchased from Jackson Laboratory (Bar Harbor, ME, USA). Female C57BL mice and Wistar rats from National Laboratory Animal Center (Taipei, Taiwan) were used to study the anti-diabetic action of BPWE. All animals were maintained in the institutional animal facility and handled according to the guidelines of the Academia Sinica Institutional Animal Care and Utilization Committee. Light cycle (12 h light and 12 h dark), temperature ( $23 \pm 3^\circ\text{C}$ ) and humidity ( $70 \pm 10\%$ ) were controlled in the animal facility. Proper care and use of the animals were monitored. Hank's buffer containing collagenase XI (1.5 mg/ml) was injected into the pancreatic ducts of fasting 8-week-old Wistar rats. Pancreatic islets were collected after 30 min digestion and grown in a glucose-free RPMI 1640 medium supplemented with 10% FBS, penicillin (100 U/ml), streptomycin (100  $\mu\text{g}/\text{ml}$ ), 2-mercaptoethanol (50  $\mu\text{M}$ ), sodium pyruvate (1 mM), and glutamate (292  $\mu\text{g}/\text{ml}$ ) and subsequently used for insulin release assays.

### 2.3. Drug administration in db/db or C57BL mice

In the single-dose experiments, 6–8-week-old diabetic db/db mice were fasted for 16 h. After 2 h food intake, the mice were grouped and mice were tube-fed with vehicle (0.2 ml PBS), BPWE (10, 50 and 250 mg/kg body weight (BW)) or GLM (2.5 mg/kg BW). Postprandial blood glucose and insulin levels were monitored for 4 h. In the long-term dose experiments, 6–8-week-old db/db mice were grouped and tube-fed with PBS, BPWE (50 mg/kg) or GLM (2.5 mg/kg), once a day, for 4 weeks. Body weight, daily food intake and oral glucose tolerance were measured at the beginning of the experiment and at the end of the fourth week. Levels of postprandial blood glucose and serum insulin were determined once a week.

Glycosylated hemoglobin A1C (HbA1C) level was determined at the end of the experiment.

C57BL mice aged 6–8 weeks were treated with an intraperitoneal injection of STZ (200 mg/kg). Mice with postprandial blood glucose level over 500 mg/dl and serum insulin level below 0.18 ng/ml were grouped and tube-fed with PBS, BPWE (10, 50 and 250 mg/kg) or injected with insulin at 5 IU/kg BW. Blood glucose levels were measured for 4 h.

### 2.4. Measurement of glucose, insulin and HbA1C

Blood glucose concentration was measured using an Elite glucometer (Bayer, Pittsburgh, PA, USA). Insulin levels in serum samples and rat islet cell supernatants were determined using ELISA assays (Mercodia, Uppsala, Sweden). The percentage of HbA1C in blood samples was measured using a DCA 2000 analyzer (Bayer, Pittsburgh, PA, USA).

### 2.5. Insulin release

Pancreatic islets (10 islets/ml) from fasting Wistar rats were incubated with glucose-free KRB buffer (Miwa et al., 2000) containing PBS, GLM (10  $\mu\text{M}$ ) or BPWE (0.15, 0.75 and 1.5 mg/ml) for 30 min. The KRB buffer was then collected for ELISA assays.

### 2.6. Oral glucose tolerance test (OGTT)

On week 0, db/db mice were fasted for 12 h. An oral dose of vehicle (PBS) was given to all the mice at 0 h (half an hour before oral glucose administration). Afterward, a daily dose of vehicle (PBS), GLM, or BPWE was administered to the mice of each group for 4 weeks. On week 4, the mice were fasted for 12 h. An oral dose of vehicle (PBS), GLM (2.5 mg/kg) or BPWE (50 mg/kg) was given half an hour before oral glucose administration (1 g/kg BW) at 0 h. The blood glucose levels were monitored from 0.5 h before glucose administration to 3 h after glucose administration.

### 2.7. Immunohistochemical staining

Multiple parallel sections of the pancreata from db/db mice that had been administered BPWE for 4 weeks were flash-frozen. The sections were stained with hematoxylin and eosin or anti-insulin antibody, with development of diaminobenzidine tetrahydrochloride, followed by image analysis (Chang et al., 2007).

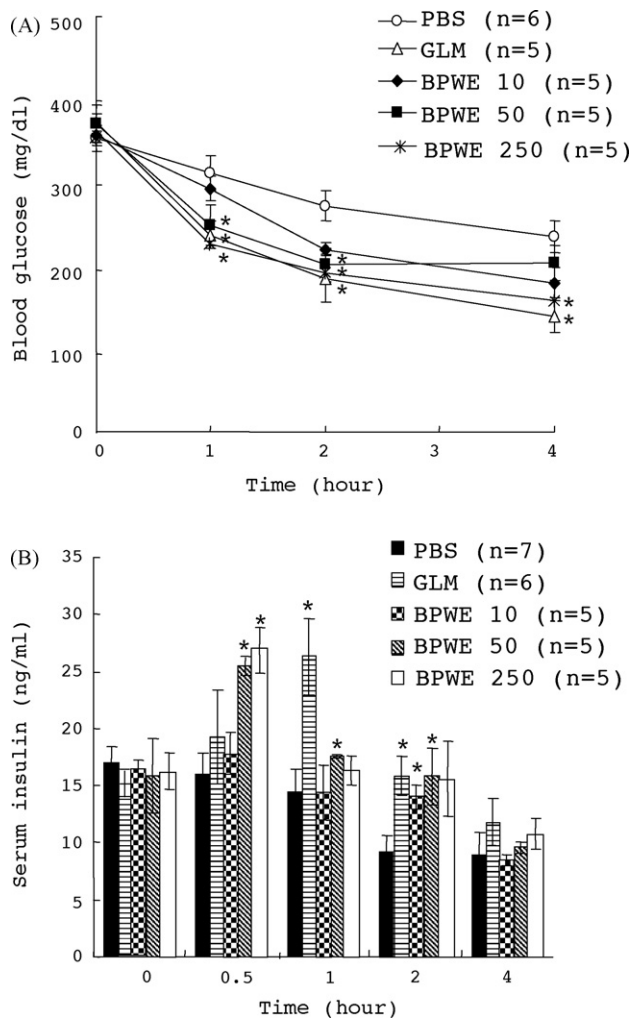
### 2.8. Statistical analysis

The results from three or more independent experiments were presented as mean  $\pm$  S.E. Differences between group averages were analyzed by ANOVA, followed by Fischer-LSD test. Differences of *p*-value less than 0.05 were considered statistically significant.

## 3. Results

### 3.1. Single-dose effects of BPWE on blood glucose levels and insulin levels in db/db mice

The aqueous ethanol layer and two acetylenic compounds of *Bidens pilosa* were previously reported to have low anti-hyperglycemic effects in db/db mice (Ubillas et al., 2000). However, this result contradicts the fact that this plant is widely used as an anti-diabetic remedy across Africa, America and Asia. Therefore, we decided to reevaluate the potential of this plant to treat type 2 diabetes. We first evaluated the anti-hyperglycemic effect of BPWE in db/db mice. The water extract, rather than the aqueous ethanol fraction of this plant was chosen because of the use



**Fig. 1.** Single-dose effects of BPWE on the levels of postprandial blood glucose and serum insulin in db/db mice. Levels of blood glucose (A) and serum insulin (B) from db/db mice which have received a dose of PBS, GLM and BPWE were measured from 0 to 4 h after tube-feeding. The mouse number (n) is indicated. Values are expressed as mean ± S.E. \*p < 0.05.

of the water extract as a folk medicine in Taiwan. Diabetic db/db mice aged 6–8 weeks that were fed normally, had hyperglycemia, with postprandial blood glucose levels somewhere between 350 and 400 mg/dl (Fig. 1A). The levels of blood glucose in mice receiving a dose of vehicle decreased from 355 to 238 mg/dl in 4 h (Fig. 1A). In contrast, an oral dose of 2.5 mg/kg GLM effectively decreased blood glucose levels from 374 to 144 mg/dl in db/db mice (Fig. 1A). Similarly, single-dose of BPWE significantly lowered blood glucose levels in a dose-dependent fashion (1 h, Fig. 1A). We also compared serum insulin levels in db/db mice that received vehicle, GLM and BPWE. Consistent with their glucose-lowering effects, both GLM and BPWE significantly elevated the serum insulin levels

in db/db mice compared to vehicle alone (Fig. 1B). The increase in serum insulin levels by BPWE seemed to be dose-dependent (0.5 h, Fig. 1B) and BPWE and GLM seemed to have different kinetics in insulin secretion (Fig. 1B).

Overall the data in Fig. 1 showed that BPWE was able to lower blood glucose levels and increase serum insulin levels in db/db mice.

### 3.2. Long-term effects of BPWE on diabetes in db/db mice

To further evaluate the therapeutic effects of BPWE on diabetes, we examined the long-term anti-diabetic effects of BPWE in db/db mice. We found that BPWE at 50 mg/kg decreased blood glucose levels in fed db/db mice as effectively as GLM at 2.5 mg/kg (left panel, Fig. 2A). The blood glucose-lowering effects of BPWE might not be due to food uptake (Table 1). Consistently, BPWE at 50 mg/kg has similar serum insulin-increasing effects as GLM (right panel, Fig. 2A). Next, we assessed the effect of BPWE on glucose tolerance. Six-week-old diabetic db/db females showed similar glucose tolerance before treatment (left column, Fig. 2B). After 4-week administration, GLM improved glucose tolerance in db/db mice compared to control vehicle (right panel, Fig. 2B). The db/db mice treated with BPWE at 50 mg/kg also showed an improvement in glucose tolerance. Our data showed that BPWE was slightly more effective than GLM at improving glucose tolerance (right panel, Fig. 2B). Next, we examined the effects of BPWE on the percentage of HbA1C, a well-known indicator of long-term glycemic control in db/db mice following different treatments. In the blood from diabetic db/db mice aged 10–12 weeks, the percentage of HbA1C was 7.4% (Fig. 2C). In contrast, the percentage of HbA1C was 7.0% and 6.5% in the blood of age-matched mice following treatment with 2.5 mg/kg GLM and 50 mg/kg BPWE, respectively (Fig. 2C). These data suggest that BPWE (0.9% decrease of HbA1C is relatively better at controlling glycemia than GLM (0.4% decrease of HbA1C in db/db mice (Fig. 2C). Diabetic db/db mice usually develop severe atrophy of pancreatic islets. Therefore, we assessed the protective effect of BPWE on islet destruction in db/db mice. Pancreatic islets of GLM-treated or untreated control mice were destroyed (Fig. 2D). In contrast, the treatment of pancreatic islets of mice with BPWE increased islet preservation (Fig. 2D).

### 3.3. Mechanism of anti-diabetic action of BPWE

To better understand the mechanism of BPWE as an insulin releaser, we tested its effect in rat pancreatic islets. We treated these islets with PBS, GLM or BPWE at different concentrations in KRBB buffer. As expected, GLM stimulated insulin release from rat pancreatic islets (Fig. 3A). Similarly, BPWE enhanced insulin secretion in a dose-dependent fashion (Fig. 3A). To further examine the *in vivo* response where BPWE reduced hyperglycemia via insulin production from pancreatic islets, we evaluated the anti-hyperglycemic effects of BPWE in STZ-treated C57BL mice whose pancreatic islets were already depleted. As expected, glimepiride, an insulin releaser, could not reduce hyperglycemia in these mice (Fig. 3B). BPWE was

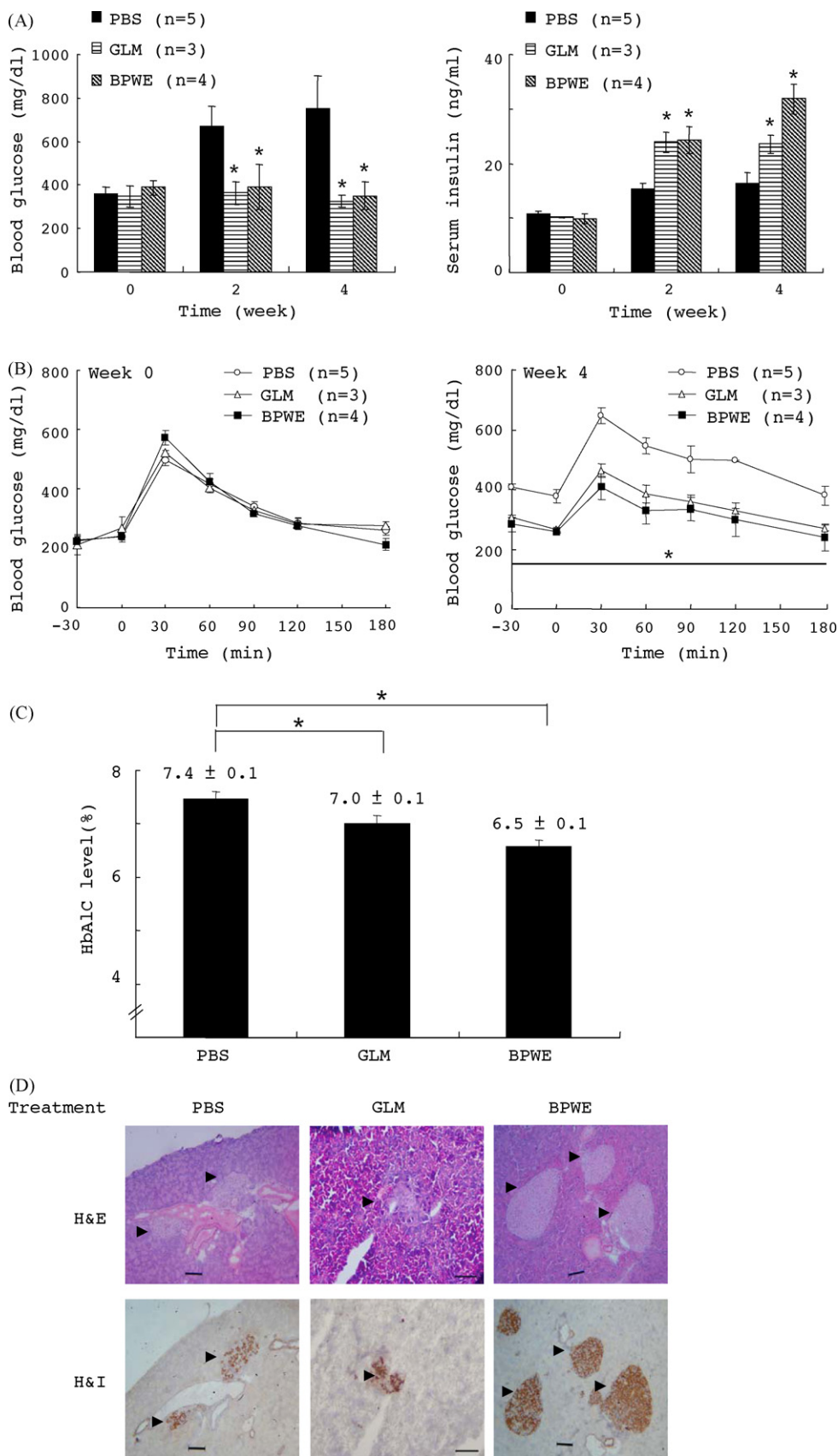
**Table 1**

Effect of BPWE on body weight and food intake in db/db mice. 6–8-Week-old db/db mice were given free access to food. The mice were daily tube-fed with PBS, glimepiride (GLM, 2.5 mg/kg) and BPWE (50 mg/kg) for 4 weeks. Body weight and food intake were measured.

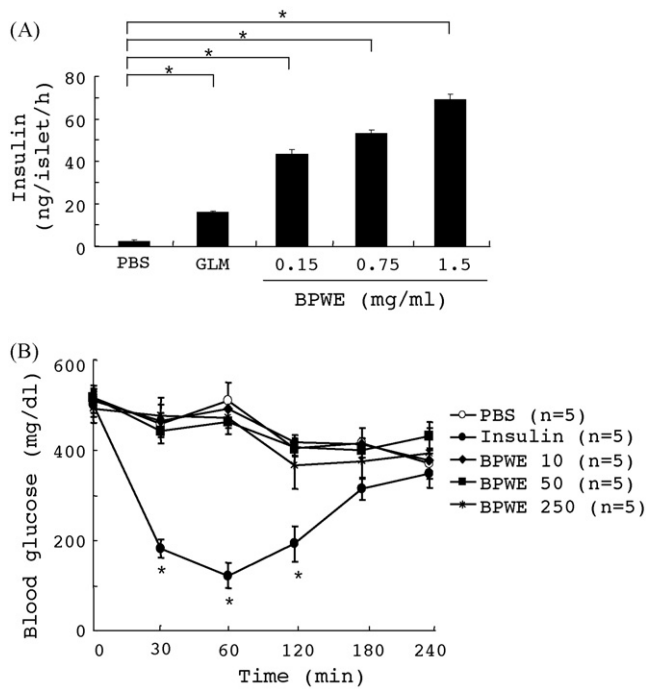
Treatment	Pre-treatment (week 0)			Post-treatment (week 4)		
	PBS	GLM 2.5	BPWE 50	PBS	GLM 2.5	BPWE 50
Mouse number	5	3	4	5	3	4
Body weight (g/mouse)	27.2 ± 0.7	25.9 ± 0.9	26.8 ± 0.7	37.7 ± 1.2	36.2 ± 1.0	32.5 ± 0.3*
Food intake (g/(day mouse))	3.0 ± 0.1	3.0 ± 0.1	3.1 ± 0.2	6.2 ± 0.4	5.4 ± 0.3	5.0 ± 0.5

The data are expressed as mean ± S.E.

\* p < 0.05 is considered to be statistically significant when compared with control group.



**Fig. 2.** Anti-diabetic effects of BPWE in db/db mice during a 4-week treatment. (A) Blood glucose and serum insulin levels from db/db mice, which were daily tube-fed with PBS, GLM and BPWE, were monitored 0, 2 and 4 weeks after tube-feeding. (B) Oral glucose tolerance tests were conducted on the same mice at week 0 and week 4 (A). (C) After 4 weeks of treatment, the percentage of HbA1C was measured in blood samples from the db/db mice (A). (D) The pancreata of the mice were then stained with hematoxylin plus eosin Y (H&E) or hematoxylin plus anti-insulin antibody (H&I). The scale bars are 100 μm. All the data are expressed as mean ± S.E. The mouse number (n) is indicated. \**p* < 0.05.



**Fig. 3.** Insulin-releasing effects of BPWE on pancreatic islet cells *in vitro* and STZ-treated mice *in vivo*. (A) Insulin secretion of rat pancreatic islet cells by PBS, GLM and BPWE were measured. (B) The reduction of blood glucose by PBS, GLM and BPWE in STZ-treated C57BL mice (*n* indicates the mouse number). Values are expressed as mean  $\pm$  S.E. \**p* < 0.05.

also unable to reduce hyperglycemia (Fig. 3B). In contrast, insulin injected improved hyperglycemia. These data suggest that BPWE controls blood glucose via its regulation of insulin release from pancreatic islets.

#### 4. Discussion and conclusion

Our results show that BPWE can effectively treat diabetes in db/db mice. We demonstrate for the first time that BPWE ameliorates type 2 diabetes through enhancement of insulin secretion and possibly islet protection.

BPWE can augment serum insulin levels and, in turn, reduce blood glucose levels (Figs. 1, 2A and 3B). These data are consistent with our observation that serum insulin levels reached the plateau 0.5 h after oral uptake in db/db mice, preceding the peak of the decrease in blood glucose levels (Fig. 1). Type 2 diabetes always accompanies pancreatic islet destruction. However, most of the anti-diabetic drugs currently available can not prevent pancreatic atrophy in patients and animal models with type 2 diabetes. In contrast, BPWE but not GLM could protect pancreatic islets in db/db mice (Fig. 2D). This mechanism needs to be further investigated. Protection of  $\beta$  cells will be useful for prevention of type 2 diabetes in obese patients and newly diagnosed type 2 diabetic patients. However, it might not be effective for chronic type 2

diabetic patients with delayed diagnosis and in type 1 diabetic patients.

There is a discrepancy in the anti-hyperglycemic efficacy of *Bidens pilosa* in our study and other studies (Ubillas et al., 2000). This may be due to differences in the methods of preparing the extract of this plant. The boiling water extraction method used here and the aqueous ethanol extraction method used in Ubillas and colleagues' study, may result in different compositions or contents of active phytochemicals. This discrepancy may also be that the *Bidens pilosa* used in our study and the *Bidens pilosa* used in other studies have different compositions or contents of active constituents due to different environmental factors and therefore, have loss in anti-hyperglycemic efficacy. For instance, cytopiloyne, the most potent *Bidens pilosa* polyacetylene for type 1 diabetes, was isolated by Chiang et al. (2007) but was not detected in the same species of plant used by Ubillas et al.

This study provides scientific evidence for the ethnobotanical use and action of *Bidens pilosa*, which helps research and development of *Bidens pilosa* for type 2 diabetes. Further studies need to be conducted to elucidate the bioactive compounds and molecular mechanisms.

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