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# Increase in Insulin Secretion Induced by *Panax ginseng* Berry Extracts Contributes to the Amelioration of Hyperglycemia in Streptozotocin-induced Diabetic Mice

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*Panax ginseng* has long been used as a traditional herbal medicine. More recently, it has received attention for its anti-diabetic and anti-obesity effects in humans and in animal models of type 2 diabetes. In the present study, we tested the hypoglycemic effects of ginseng berry extract in beta-cell-deficient mice and investigated the mechanisms involved. Red (ripe) and green (unripe) berry extracts were prepared and administered orally (100 or 200 mg/kg body weight) to streptozotocin-induced diabetic mice daily for 10 wk. The body weight was measured daily, and the nonfasting blood glucose levels were measured after 5 and 10 wk after administration. Glucose tolerance tests were performed, and the serum insulin levels were measured. The proliferation of beta-cells was measured *in vitro*. The administration of red or green ginseng berry extract significantly reduced the blood glucose levels and improved the glucose tolerance in beta-cell deficient mice, with the higher doses resulting in better effects. Glucose-stimulated insulin secretion was significantly increased in berry extract-treated mice compared with streptozotocin-induced diabetic control mice. Treatment with ginseng berry extract increased beta-cell proliferation *in vitro*. Both red berry and green berry extracts improved glycemic control in streptozotocin-induced diabetic mice and increased insulin secretion, possibly due to increased beta-cell proliferation. These results suggest that ginseng berry extracts might have beneficial effects on beta-cell regeneration.

**Keywords:** *Panax ginsneg*, Red ginseng berry, Green ginseng berry, Diabetes, Beta-cell proliferation

## INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia. It is believed that absolute or relative insulin deficiency due to the inadequate beta-cell mass is the cause of hyperglycemia [1]. Type 1 diabetes results from the destruction of pancreatic beta-cells by beta-cell-specific autoimmune responses [2], and type

2 diabetes results from the progressive loss of beta-cell mass and function [3]. Therefore, strategies that preserve or restore beta-cell mass and function are logical therapeutic approaches for the treatment of diabetes.

Ginseng (*Panax ginseng*) has been used as an herbal medicine for thousands of years. Ginseng has pharmacological properties, including anti-cancer, anti-aging and anti-allergic effects [4-6], and is free from adverse effects, in contrast to chemical medicines [7]. Ginseng has also received increasing attention as a complementary and alternative medicine for the treatment of diabetes. Extracts of ginseng roots, berries and leaves have been reported to have hypoglycemic effects in animal models of type 1 and type 2 diabetes [8-10]. The anti-diabetic and anti-obesity effects of ginseng have been reported in animal experiments [11-16] and in clinical trials [17-19].

Previous studies have demonstrated that ginseng berry extract showed better anti-hyperglycemic activity than ginseng root extract when used at the same concentration [20], and berry extract has a higher content of total ginsenosides than root extract [9], with a distinct ginsenoside profile. It was reported that American ginseng (*P. quinquefolius*) berry extract reduced the blood glucose levels and body weight in *ob/ob* and *db/db* mice, models of type 2 diabetes [14,15]. However, the effects of ginseng berry extract on insulin-producing pancreatic beta-cells have not been specifically examined. In this study, we investigated the pharmacological effects of red (ripe) and green (unripe) ginseng berry extracts on beta-cells and insulin secretion using a streptozotocin (STZ)-induced diabetic mouse model, in which pancreatic beta-cells are damaged and insulin production is deficient.

## MATERIAL AND METHODS

### Preparation of ginseng berry extract

Fresh green berries (GBs, unripe) or red berries (RBs, ripe) of *P. ginseng* were freeze-dried. Approximately 500 g of berries was extracted with 10 L of 70% ethanol at 37°C for three days in a shaking incubator, and this extraction was repeated three times. After evaporating the solvent, the extracts were dissolved in distilled water (30 mL) and extracted three times with 30 mL of methylene chloride in a separatory funnel. The residual aqueous phase was extracted two more times with ethyl acetate, and the ethyl acetate extracts were then mixed with *n*-butanol. The butanol phase was collected and evaporated. The dried powder was used for HPLC analysis and subsequent experiments. For administration to mice, the berry extracts were dissolved in phosphate-buffered saline (PBS).

### HPLC analysis

Ginsenosides were analyzed as described previously [21] using an HPLC system (NS-4000; Futecs Co., Daejeon, Korea) equipped with a SofTA 300s evaporative light scattering detector (SofTA Co., Westminster, CO, USA) and a ProntoSIL fractionation column (250×4.66 mm) (Bischoff, Berlin, Germany). The flow rate was set to 0.8 mL/min. The identification and quantification of ginsenosides were carried out by comparing the retention times and the peak areas, respectively, with those of ginsenoside standards or by the direct addition of the ginsenoside standard into the sample (spike test). Sample aliquots were filtered through a 0.45 μm polytetrafluoroethylene filter prior to injection. All samples were run in triplicate. The ginsenoside standards ($Rb_1$, $Rb_2$, $Rb_3$, Rc, Rd, Re, Rf, $Rg_1$, $Rg_2$, and $Rh_1$) were purchased from Canfo Chemical, Chengdu, China.

### Animals

C57BL/6 mice were purchased from the Korea Research Institute of Bioscience and Biotechnology (Daejeon, Korea). Mice were maintained under specific pathogen-free conditions in a temperature-controlled room (23±1°C) with a 12-h light/dark cycle and *ad libitum* access to food and water at the Animal Care Center, Lee Gil Ya Cancer and Diabetes Institute, Gachon University of Medicine and Science. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Lee Gil Ya Cancer and Diabetes Institute.

### Induction of diabetes and treatment with ginseng berry extract

At 8 wk of age, male mice were intraperitoneally injected with STZ (80 mg/kg body weight) in sterile citrate buffer, pH 4.0, for two consecutive days. Beginning 2 d after STZ injection, the glucose levels in the tail vein blood were monitored with a glucometer (One Touch Ultra; LifeScan Inc., Milpitas, CA, USA). Mice with blood glucose levels over 250 mg/dL were used for the subsequent experiments. Five days after the STZ treatment, animals were randomly divided into six groups (*n*=7 or 8 in each group): normal control mice, PBS-treated STZ-induced diabetic mice, and STZ-induced diabetic mice given 100 or 200 mg/kg RB or GB extract. Berry extracts were given by oral intubation daily for 10 wk. After 5 and 10 wk of treatment, the glucose levels and body weights were measured following the removal of food for 3 h, and food consumption was measured weekly.

## Intraperitoneal glucose tolerance tests

After 4 and 8 wk of extract treatment, mice were not fed for 14 h, and then a glucose solution (2 g/kg body weight in PBS) was injected intraperitoneally. The blood glucose levels were measured at 0, 30, 60, 90, and 120 min after glucose injection.

## Measurement of the serum insulin levels

After 10 wk of treatment, mice were not fed for 14 h, and then a glucose solution (2 g/kg body weight in PBS) was injected intraperitoneally. Blood samples were drawn into heparinized capillary tubes from the periorbital veins at 30 min after glucose injection. The blood was centrifuged (3,000 rpm, 15 min, 4°C), and the serum insulin levels were determined using a mouse insulin ultrasensitive EIA kit (Alpco Diagnostics, Salem, NH, USA).

## Cell culture and proliferation assays

INS-1 rat insulinoma cells were maintained in RPMI-1640 (Gibco BRL, Grand Island, NY, USA) supplemented with 11 mM glucose, 10% fetal bovine serum (Gibco BRL), 100 U/mL penicillin, 100 μg/mL streptomycin, 50 μM beta-mercaptoethanol and 1 mM sodium pyruvate at 37°C in a humidified 5% $CO_2$/95% air atmosphere. INS-1 cells were seeded in 96-well plates at a density of $5 \times 10^3$ cells/100 μL/well and incubated for 24 h. The cells were then incubated in culture medium containing ginseng berry extract (5, 10, and 20 μg/mL). Following 24 h of incubation, the cell viability was determined using a Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's protocol.

## Quantitative real-time polymerase chain reaction

Total RNA was isolated from the cells, and cDNA was synthesized using a PrimeScript 1st strand cDNA synthesis kit (Takara, Kyoto, Japan). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the Power SYBR green Master-mix (Takara) and Applied Biosystems Prism 7900HT sequence detection system. Polymerase chain reaction (PCR) was carried out and stopped at 40 cycles (2 min at 50°C, 10 min at 95°C, and 40 cycles of 10 s at 95°C and 1 min at 60°C). The primer sequences used were mouse cyclin D2: sense 5'-CCATGAATTA CCTGGACCGTTT-3' and antisense 5'-GCGCCCAGGAGCTGAAG-3'; mouse IRS2: sense 5'-CGCTCGACTGCTGTCTGAAC-3' and antisense 5'-GGTGTAGAGGGCGATCAGGTACT-3'; mouse PDX-1: sense 5'-CAAAGCTCACGCGTG-GAAA-3' and antisense 5'- CCTCTTATTCTCCTCCG-

GTTCTG-3'; and mouse cyclophilin: sense 5'-CCATGA ATTA CCTGGACCGTTT-3' and antisense 5'-GC-GCCCAGGAGCTGAAG-3'. The qRT-PCR results were evaluated using the comparative Ct method (User Bulletin No. 2; PerkinElmer Life Sciences, Waltham, MA, USA) with cyclophilin as the invariant control gene.

## Statistical analysis

Data are presented as the mean±SE. The significance of the differences was analyzed with an unpaired Student's *t*-test and ANOVA followed by a posteriori test. The values of statistical significance were set at $p<0.05$ and $p<0.01$.

# RESULTS

## Identification of ginsenosides in red and green ginseng berry extracts

The ginsenoside composition was determined by HPLC analysis (Fig. 1). The ginsenoside Re was the most abundant saponin in RB and GB, with the Re content of RB being higher than that of GB. However, the $Rb_1$, Rc, Rd, and $Rb_2$ contents of GB were higher than those of RB. The total ginsenoside concentration of GB was higher than that of RB.

## Recovery of body weight in streptozotocin-induced diabetic mice treated with red berry or green berry

To determine whether treatment with RB or GB affects body weight in STZ-induced diabetic mice, we measured the body weights of RB- or GB-treated mice. The body weights of STZ-induced diabetic mice were significantly lower than those of the normal age- and sex-matched control mice at 5 wk (10% lower) and 10 wk (15% lower) after the start of the experiment. In contrast, the body weights of the STZ-induced diabetic mice treated with 200 mg/kg GB for 5 wk were significantly higher (7% higher) than those of the STZ-induced diabetic group. In addition, the body weights of mice treated with 200 mg/kg GB or RB for 10 wk were significantly increased (9% higher) compared with the body weights of STZ-induced diabetic mice (Fig. 2A). The amounts of food consumed per week over the 10-week period were not significantly different among groups (Fig. 2B).

## Anti-hyperglycemic effects of red berry and green berry in streptozotocin-induced diabetic mice

We then investigated whether RB or GB treatment could lower the blood glucose level in STZ-induced
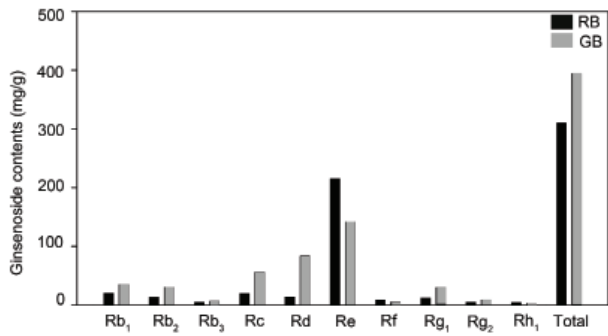
**Fig. 1.** Ginsenoside content of *Panax ginseng* red berry (RB) and green berry (GB) extracts as determined by HPLC.

diabetic mice. STZ-induced diabetic mice showed significantly higher nonfasting blood glucose levels than normal control mice (192.8±20.3 *vs*. 531.3±60.5 mg/dL, $p<0.01$). However, treatment of the diabetic mice with 200 mg/kg of RB significantly lowered the blood glucose levels after 5 wk of treatment, with a further reduction after 10 wk, compared with the levels in untreated STZ-induced diabetic mice. Treatment with 200 mg/kg of GB for 5 wk did not result in hypoglycemic effects but significantly lowered the blood glucose levels after 10

wk of treatment. Treatment with 100 mg/kg of RB or GB extract was ineffective (Fig. 3).

### Improvement of glucose tolerance in green berry- and red berry-treated mice

To determine whether exogenous glucose is properly cleared in diabetic mice treated with 200 mg/kg RB or GB, we performed intraperitoneal glucose tolerance tests at 4 and 8 wk of treatment. The blood glucose concentrations in RB-treated mice were significantly lower than those in the vehicle-treated control diabetic mice at each time point after glucose injection (Fig. 4A) after 4 wk of treatment, and blood glucose clearance further improved after 8 wk of treatment (Fig. 4B). Although glucose clearance was improved in GB-treated mice, the effect was less than that observed in RB-treated mice (Fig. 4A, B).

### Increase in glucose-stimulated insulin secretion in red berry- and green berry-treated mice

To determine whether the improved glucose levels in RB- and GB-treated STZ-induced diabetic mice were a result of increased insulin secretion, we measured the
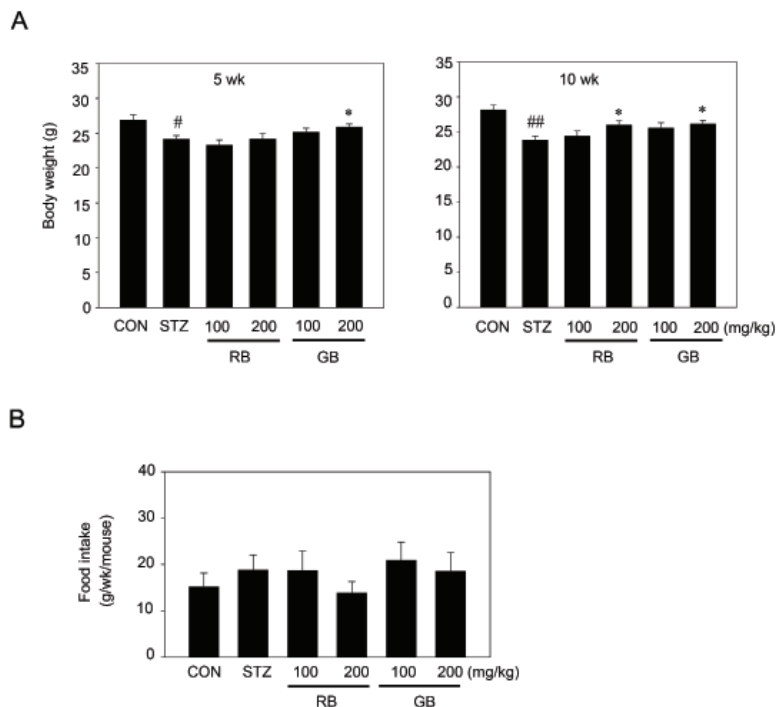


**Fig. 2.** Effect of ginseng berry extracts on body weight and food intake. Streptozotocin (STZ)-induced diabetic mice were orally administered red berry (RB) or green berry (GB) extract (100 or 200 mg/kg body weight) daily for 10 wk. (A) Body weights were measured at 5 and 10 wk of treatment. (B) Food intake was measured weekly. Values are the average weight of food consumed/mouse (per group of 6-8 mice)/wk (throughout the 10 wk). Values are mean±SE. CON, untreated, normal mice; STZ, vehicle-treated STZ-induced diabetic mice. #$p<0.05$, ##$p<0.01$ compared with control group. *$p<0.05$ compared with STZ-induced diabetic group.
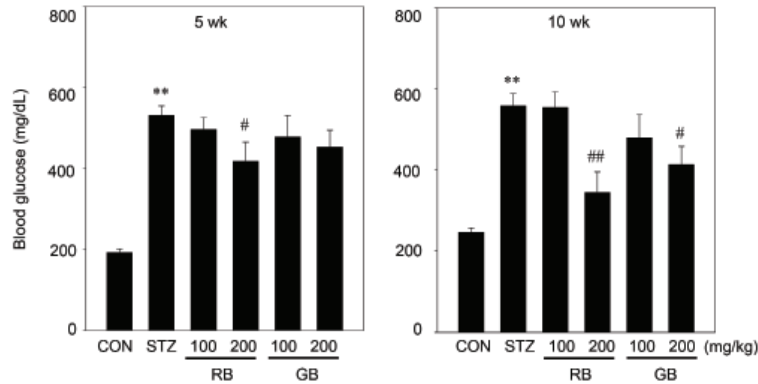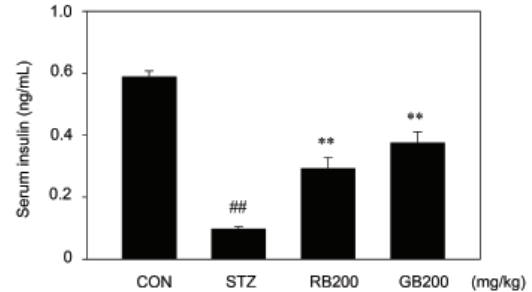
**Fig. 3.** Effect of ginseng berry extracts on blood glucose levels. Streptozotocin (STZ)-induced diabetic mice were orally administered red berry (RB) or green berry (GB) extract (100 or 200 mg/kg body weight) daily for 10 wk. Blood glucose levels were measured in fed mice ($n$=6-8) at 5 and 10 wk of treatment. Values are mean±SE. CON, untreated, normal mice; STZ, vehicle-treated STZ-induced diabetic mice. **$p < 0.01$ compared with control group. #$p < 0.05$, ##$p < 0.01$ compared with STZ-induced diabetic group.
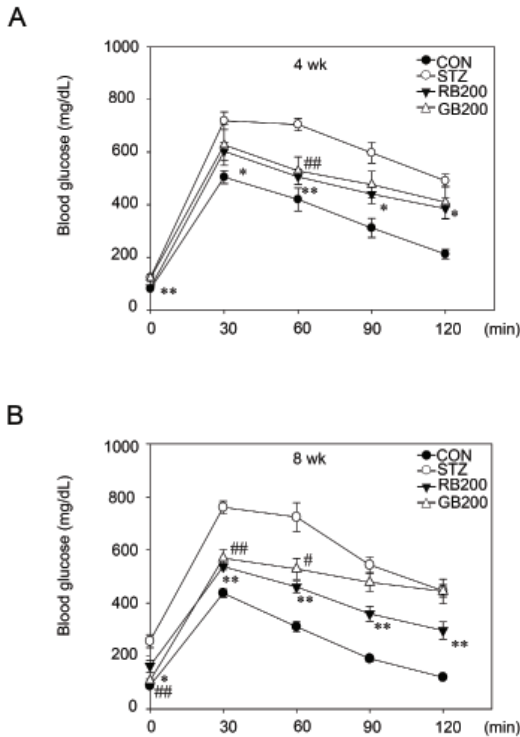


**Fig. 4.** Effect of ginseng berry extracts on glucose tolerance. Streptozotocin (STZ)-induced diabetic mice were orally administered red berry (RB) or green berry (GB) extract (100 or 200 mg/kg body weight) daily for 10 wk. Intraperitoneal glucose tolerance tests were performed at (A) 4 wk and (B) 8 wk of treatment (200 mg/kg body weight, $n$=5-7). Values are mean±SE. CON, untreated, normal mice; STZ, vehicle-treated STZ-induced diabetic mice. #$p < 0.05$, ##$p < 0.01$ GB-treated group compared with STZ-diabetic group. *$p < 0.05$, **$p < 0.01$ RB-treated group compared with STZ diabetic group.
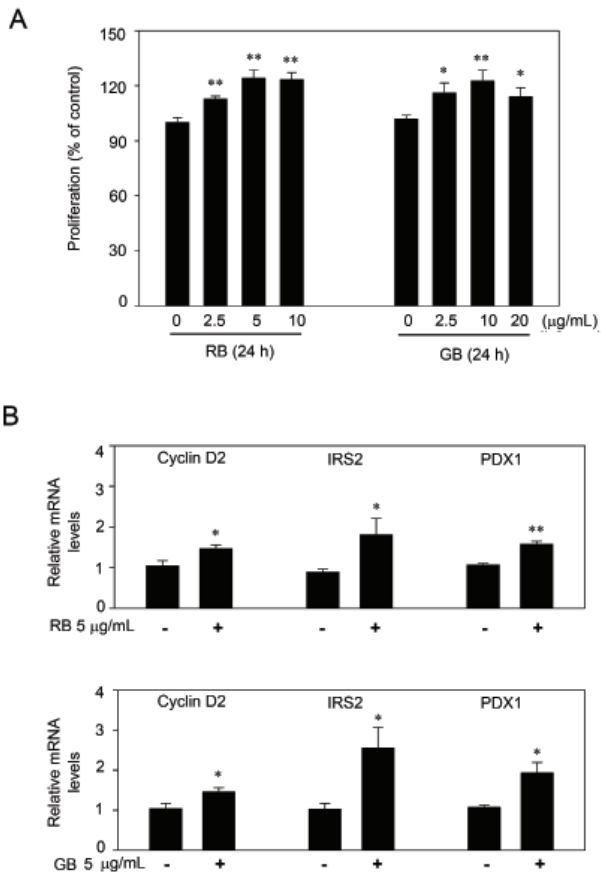


**Fig. 5.** Effect of ginseng berry extracts on serum insulin levels. Streptozotocin (STZ)-induced diabetic mice were orally administered red berry (RB) or green berry (GB) extract (200 mg/kg body weight) daily for 10 wk. After 10 wk of treatment, glucose (2 g/kg, i.p.) was injected, and blood was collected at 30 min later for serum insulin measurements. CON, untreated, normal mice; STZ, vehicle-treated STZ-induced diabetic mice. ##$p < 0.01$ compared with control group. **$p < 0.01$ compared with STZ-induced diabetic group.

serum insulin levels 30 min after intraperitoneal glucose injection. As shown in Fig. 5, the serum insulin levels in mice treated with RB or GB for 10 wk were significantly higher than those in vehicle-treated diabetic mice. These results indicate that increased insulin secretion contributes to lowering the blood glucose in diabetic mice treated with RB or GB.

### Increase in beta-cell proliferation in red berry- or green berry-treated INS-1 cells

To determine whether the decreased blood glucose levels and increased insulin secretion in RB- and GB-treated mice might be a result of the increased proliferation of pancreatic beta-cells, we investigated whether RB and GB have proliferative effects on a beta-cell line (INS-1 cells) *in vitro*. We found that incubation of INS-1 cells with RB or GB for 24 h resulted in a significant increase in the number of cells relative to the number of untreated control cells (Fig. 6A). As cyclin D2, PDX1 and IRS2 are known to be involved in beta-cell proliferation [22-24], we determined whether RB and GB can induce the expression of cyclin D2, PDX1 and IRS2 mRNA in INS-1 cells. The treatment of INS-1 cells with RB or GB (5

**Fig. 6.** Effects of ginseng berry extracts on beta-cell proliferation. (A) INS-1 cells were treated with red berry (RB) or green berry (GB) extract at the indicated concentrations for 24 h, and cell proliferation was measured by Cell Counting Kit-8. (B) Cyclin D2, IRS2, and PDX1 mRNA levels were measured in INS-1 cells treated with RB and GB (5 μg/mL) for 4 h (n=4-5). Fold increase was determined as a ratio of mRNA levels of untreated cells. *$p<0.05$, **$p<0.01$ compared with control cells.

μg/mL) increased the levels of cyclin D2, PDX1, and IRS2 mRNA expression (Fig. 6B).

## DISCUSSION

The anti-diabetic and anti-obesity effects of ginseng have been reported in animal experiments [8,13-15] and clinical trials [17,25,26]. Although both ginseng roots and ginseng berries possess anti-diabetic effects [20], ginseng berries contain significantly more ginsenosides than the roots do [15,27]. In addition, the berries seem to have more potent anti-hyperglycemic activity in *ob/ob* mice [28]. Furthermore, only ginseng berries showed marked anti-obesity effects in *ob/ob* mice [20]. However, the effects of ginseng berry extract on beta-cell function have not been studied previously. In the present study, we investigated the anti-hyperglycemic effects of RB and

GB extracts in beta-cell deficient diabetic mice and investigated the effect of berry extracts on insulin secretion and beta-cell proliferation.

We observed a significant anti-hyperglycemic effect for both RB and GB extracts in STZ-induced diabetic mice, as evidenced by decreases in the blood glucose levels after 5 and 10 wk of treatment (200 mg/kg body weight) and by the improvement of the glucose tolerance. We also observed that ginseng berry extract treatment significantly ameliorated the body weight loss caused by the STZ-treatment without altering food intake, most likely as a consequence of ameliorated diabetic symptoms.

According to previous reports, the mechanisms of action of ginseng's anti-diabetic activity could be multifaceted, attributable to the modulation of gastrointestinal absorption, the regulation of insulin secretion and/or sensitivity, or a combination of these factors [8,13,14,18]. Our results show that the improvement in the blood glucose levels in ginseng berry extract-treated STZ-induced diabetic mice is at least in part associated with a significant increase in insulin secretion: there was a greater than 3-4 fold increase in insulin secretion in berry extract-treated mice. We observed that ginseng berry extract increased beta-cell proliferation in INS-1 cells, suggesting that an increase in beta-cell mass is one possible mechanism explaining the anti-diabetic effects of ginseng berry extract. It is widely accepted that type 1 diabetes and type 2 diabetes result from beta-cell deficiency: type 1 results from autoimmune destruction of beta-cells [2], and type 2 results from the progressive loss of beta-cell function and mass [29]. The identification of agents that stimulate beta-cell proliferation, such as the active component of ginseng berry extract, may be an important therapeutic strategy for both forms of diabetes.

The active components of ginseng are ginsenosides, a group of steroidal saponins [7,30]. To date, over 30 ginsenosides have been identified in the roots, leaves, stems, flower buds and berries of ginseng. However, the "total ginsenoside" content is usually based on HPLC analysis of 6 to 8 major ginsenosides: $Rg_1$, Re, Rf, $Rb_1$, Rc, $Rg_2$, $Rb_2$, and Rd [10,31,32]. Different parts of the plant contain distinct ginsenoside profiles, which may be responsible for their different pharmacological activities [14]. Our HPLC analysis indicated that both green and red ginseng berry extract contained a very high concentration of the ginsenoside Re relative to the concentrations of other ginsenosides. Whether the ginsenoside Re is the primary component responsible for the beta-cell proliferative effect remains to be tested in the future.

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