JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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pubs.acs.org/JAFC

# Steam-Dried Ginseng Berry Fermented with *Lactobacillus plantarum* Controls the Increase of Blood Glucose and Body Weight in Type 2 Obese Diabetic *db/db* Mice

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**ABSTRACT:** This study examined whether steam-dried ginseng berries fermented with *Lactobacillus plantarum* (FSGB) could improve the indices of type 2 diabetes mellitus (T2DM) in obese *db/db* mice. FSGB was shown to have an effect on body weight and blood glucose/serum parameters when administered at a dose of 0.5 g/kg. In the intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (ITT), FSGB was clearly shown to improve insulin tolerance and glucose tolerance. Moreover, FSGB was shown to enhance immune activities by increasing the immune cell population, and glucose transpoter 1 (GLUT1) mRNA expression in L6 cells was up-regulated, suggesting that FSGB can increase glucose transport activity in target cells. These results indicate that steam- and dry-processed ginseng berries fermented with *L. plantarum* can be used to effectively control blood sugar metabolism via improving insulin and glucose tolerance and body weight gain in *db/db* mice.

**KEYWORDS:** *ginseng berries, Panax ginseng, steam-dried, Lactobacillus plantarum, blood glucose, diabetes*

## INTRODUCTION

In diabetes mellitus, which is caused by impaired insulin signaling and decreased insulin secretion, pancreatic $\beta$ cells play a crucial role in glucose homeostasis through insulin secretion, whereas pancreatic $\alpha$ cells are responsible for synthesizing and secreting the peptide hormone glucagon, which elevates glucose levels in the blood. It has become clear that both the number and volume of $\beta$ cell vary under different physiological and pathological conditions.[1] Pathologically, an excessive loss of $\beta$ cells can cause diabetes mellitus, and the dysfunction of $\beta$ cells has been shown to be a common feature of both type 1 (T1DM) and type 2 diabetes mellitus (T2DM).[2−4] It has been known that obesity and its associated low-grade inflammation may contribute to the development of T2DM.[5] Currently, the obesity epidemic has increased the incidence of T2DM, which has become worse due to the increase in child obesity. Moreover, excess weight has been established as a risk factor for T2DM, yet most obese individuals do not develop T2DM. Several previous studies have demonstrated that pro-inflammatory cytokines (tumor necrosis factor and interleukin-6), insulin resistance, and deranged fatty acid metabolism are involved in obesity and T2DM.[6] Because these interactions are complex, conventional pharmacological treatments for T2DM have a number of limitations, such as adverse effects and high rates of secondary failure. Thus, the development of alternative therapies with improved insulin sensitivity and weight reduction are needed for the treatment of T2DM.

Over the past few decades, various plant extracts have been clinically evaluated for the treatment of T2DM, including the root of *Panax ginseng*, also called Asian or Korean ginseng, which was shown to possess antihyperglycemic activity in vitro and in vivo in animal and clinical studies,[7−9] which mainly focused on T1DM. The root of ginseng is a commonly used herbal medicine; however, very little work has been done to evaluate the effect of the ginseng berry. Attele and others[10] reported that the *P. ginseng* berry extract contained high concentrations of ginsenoside Re and antihyperglycemic, and this extract displayed antiobesity effects in obese diabetic C57BL/6J *ob/ob* mice. Recently, we developed a fermented steam-dried ginseng berry extract (FSGB), using *Lactobacillus plantarum*, the so-called kimchi bacteria, which plays a crucial role in kimchi fermentation. In the present study, we examined the effect of FSGB on hyperglycemia and body weight in an obese T2DM animal model (C57BL/KsJ *db/db* mice).

## MATERIALS AND METHODS

**Sample Preparation and Fermentation of *P. ginseng* Berry Extracts.** Fresh berries of *P. ginseng* C.A. Meyer were obtained from Korea Genetic Pharm Co. Ltd. (Gyeonggi, Republic of Korea). All berries were gathered from at least 4-year-old plants, and whole ginseng berries were steamed at 100 °C for 2 h and dried for 24 h at 50 °C. These steam and dry procedures were repeated seven times, and the final products were prepared after sunlight-drying for 7 days. For high-performance liquid chromatography (HPLC) analysis, steam-dried ginseng berries were extracted in 80% methanol for 24 h. For treatment, 100 g of finely ground berries was autoclaved and boiled for 2 h in autoclaved deionized water (1.5 L). Before experimental use, *L. plantarum* (KCCM 11322, Korean Federation of Culture Collection, Seoul, Republic of Korea) was propagated twice in 50 mL of Difco lactobacillus MRS broth (Becton Dickinson, Franklin Lakes, NJ, USA) at 30 °C overnight. One liter of SGB containing *L. plantarum* ($10^7$

Received: February 2, 2012
Revised: April 23, 2012
Accepted: May 7, 2012
Published: May 7, 2012

**Table 1. Primer Sequences Used for Real-Time RT-PCR**

| gene | primer | amino acid sequence | product size (bp) | accession no. |
|---|---|---|---|---|
| IL-2 | 5′ | 5′-GCTCTACAGCGGAAGCACAG | 235 | NM_008366 |
| | 3′ | 5′-GTCAAATCCAGAACATGCCG | | |
| TNF-$\alpha$ | 5′ | 5′-AGCACAGAAAGCATGATCCG | 247 | NM_013693 |
| | 3′ | 5′-GTTTGCTACGACGTGGGCTA | | |
| GLUT1 | 5′ | 5′-AGCACAGAAAGCATGATCCG | 265 | M_13979 |
| | 3′ | 5′-GTTTGCTACGACGTGGGCTA | | |
| $\beta$-actin | 5′ | 5′-CTAGGCACCAGGGTGTGATG | 291 | NM_007393 |
| | 3′ | 5′-CTACGTACATGGCTGGGGTG | | |

CFU/mL) was fermented at 30 °C for 72 h. FSGB was then precipitated overnight, centrifuged at 10000*g* for 10 min at 4 °C, filtered through a 60 $\mu$m nylon net filter (Millipore, Billerica, MA, USA), lyophilized, and stored in desiccators at room temperature before use.

**Gas Chromatography−Mass Spectrometry (GC-MS).** For component analysis, the unprocessed raw ginseng berry (RGB) and steam-dried ginseng berry (SGB) powder (3 g) were extracted with 80% methanol (50 mL) under reflux at 80 °C for 2 h and three times. The samples were then filtered using Whatman no. 1 filter paper. The ginseng berry and steam-dried ginseng berry were analyzed using an Agilent Technologies 5975C GC-MS instrument equipped with a CTC CombiPAL autosampler system. Chromatographic separation of the ginseng berry and steam-dried ginseng berry were carried out using a helium carrier gas on an HP-5 column (250 $\mu$m × 0.25 $\mu$m × 30 m, Agilent Technologies, Santa Clara, CA, USA). A 10 $\mu$L volume of the sample was injected into a split injector that was operated in split mode using a 5:1 split ratio with split flow and column flow of 5 and 1 mL/min, respectively. The injector temperature was held at 250 °C and the transfer line at 250 °C. The GC oven was held at 50 °C for 5 min, ramped at 10 °C/min to 320 °C, and held for 5 min. The ion source temperature was 250 °C with an electron impact ionization energy of −70 V. Following a 4 min delay, data were collected from $m/z$ 35 to 250 using a detector voltage of 1059 V. The components were identified by comparing their relative retention time and mass spectra with Wiley7N library data of the GC-MS system.

**Animals.** Five-week-old male C57BL/KsJ *db/db* and C57BL/KsJ *db/m+* mice were purchased from Central Lab. Animal Inc. (Seoul, Republic of Korea). *db/m+* (hetero) mice were used as the control during the experiment. Mice were housed in an air-controlled semi-SPF room with a 12 h light/dark cycle at a temperature of 22 ± 1 °C and humidity of 50 ± 10%. The mice were fed standard rodent chow (Samyang Co., Gapyeong, Republic of Korea) and purified tap water ad libitum. The animals were acclimated to the laboratory environment for 1 week before the experiments were started. Each group was orally given either FSGB (0.05, 0.1, 0.5 g/kg) or saline for 35 days (5 weeks). Both 0.1 and 0.5 g/kg FSGB were shown to produce no adverse effects, whereas 2 g/kg FSGE induced an anergic status without any fatal signs or death within 24 h (data not shown). All experimental procedures were approved and carried out in accordance with the Institutional Animal Care and Use Committee of Laboratory Animal Research Center at Chungbuk National University, Republic of Korea.

**Cell Culture.** Mouse spleens were aseptically isolated from each group immediately after euthanasia, and single primary splenocytes were prepared by mechanical dissociation in cold phosphate-buffered saline (PBS) at pH 7.2. Erythrocytes were depleted using a red blood cell lysis buffer (eBioscience, San Diego, CA, USA) containing ammonium chloride, which lyses red blood cells and only minimally affects lymphocytes. Collected splenocytes were resuspended in Roswell Park Memorial Institute (RPMI) 1640 complete medium with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA). The cells were seeded at $1 \times 10^7$ cells/mL on 24-well plates and maintained at 37 °C in a humidified atmosphere containing 5% $CO_2$. Cell counts were carried out using a hemocytometer (Paul Marienfeld, Lauda-Königshofen, Germany). Rat myoblastoma cells derived from neonatal thigh skeletal muscle, L6 (Korean Cell Line Bank, Seoul, Republic of Korea), were grown in Dulbecco's modified Eagle's Medium (DMEM) (Hyclone) supplemented with 10% FBS, 100 U/mL penicillin, and 100 $\mu$g/mL streptomycin (Invitrogen, Carlsbad, CA, USA). Cultures were maintained under 5% $CO_2$ at 37 °C in tissue culture flasks. For all experiments, cells were grown to >90% confluency and subjected to no more than 20 cell passages.

**Measurement of Body Weight, Blood Glucose, and Serum Parameters.** Fasting blood glucose levels were measured three times at the indicated time points (days 0−35) after the animals were fasted for 4 h. Blood glucose levels were determined in blood samples obtained from the tail vein using ACCU-CHEK go (Roche Diagnostics, Mannheim, Germany). Animal body weight was recorded just before the blood glucose levels were measured. The assays used to determine serum glucose, triglyceride, total cholesterol, and high-density lipoprotein cholesterol (HDL cholesterol) levels were conducted on the last day of the study (day 35) with sera obtained from the abdominal vein using a CardioCheck PTS PANELS Lipid Panel Test Strip (Polymer Technology System, Indianapolis, IN, USA). Weights of the major organs (liver, kidney, and pancreas) were also measured on the last day of the study.

**Intraperitoneal Glucose Tolerance Test (IPGTT) and Insulin Tolerance Test (ITT).** The IPGTT and ITT assay were performed on day 35. Animals were fasted for 4 h, followed by an intraperitoneal (ip) administration of glucose (2 g/kg) and insulin (5 U/kg) (Sigma-Aldrich, St. Louis, MO, USA). Blood glucose levels were measured after 0 (baseline), 30, 60, 120, and 150 min.

**Measurement of Serum Insulin.** In the insulin analysis, fasting blood samples were collected via retro-orbital sinus puncture and transferred into serum separator tubes (Microtainer Tubes; Becton Dickinson). Serum was obtained by centrifugation (30 min at 4 °C) at 1200*g*, and serum insulin concentrations were determined using a mouse insulin ELISA kit (Alpco Diagnostics, Salem, NH, USA) and mouse insulin as a standard.

**Quantitative Real-Time Polymerase Chain Reaction.** Total RNA extracts from mouse splenocytes obtained from *db/m+*, untreated *db/db*, and FSGB-treated *db/db* mice were prepared at day 35 using the Trizol method (Invitrogen). In addition, glucose transporter 1 (GLUT1) mRNAs were analyzed from the L6 skeletal muscle cell line. cDNA was synthesized from RNA by reverse transcription of 1 $\mu$g of total RNA using the Improm-II reverse transcription system and oligo dT primers in a total volume of 20 $\mu$L (Promega, Madison, WI, USA). PCR amplification was performed using the primers described in Table 1 (Bioneer, Deajeon, Republic of Korea). Quantitative real-time PCR (qPCR) reactions were run on a Rotor-Gene 6000 (Corbett Research, Sydney, Australia) using SYBR Green PCR Master Mix (Qiagen, Valencia, CA, USA) in a reaction volume of 20 $\mu$L. Each real-time PCR master mix contained 10 $\mu$L of 2× enzyme Mastermix, 7 $\mu$L of RNase-free water, 1 $\mu$L of each primer (10 pmol each), and 1 $\mu$L of diluted template. PCR was performed with an initial preincubation step of 10 min at 95 °C, followed by 45

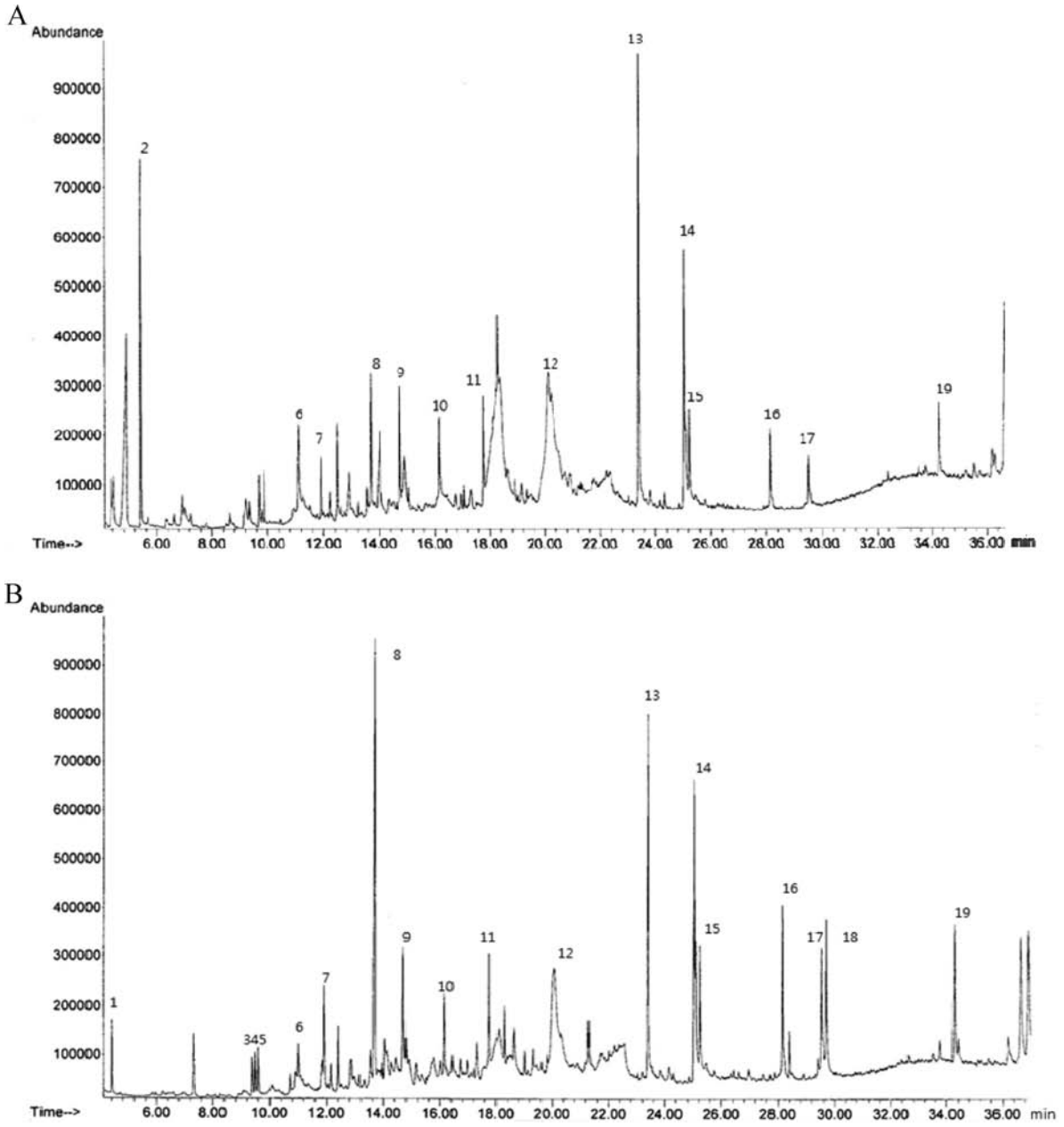


**Figure 1.** GC-MS chromatograms of RGB and SGB. The peaks between RGB (A) and SGB (B) were compared on the basis of the GC-MS library as listed in Table 2.

cycles of 95 °C for 15 s, annealing at 52 °C for 15 s, and extension at 72 °C for 10 s. Melting curve analysis was used to confirm formation of the expected PCR product, and products from all assays were subjected to 1.2% agarose gel electrophoresis to confirm that the products had the correct lengths. An inter-run calibrator was used, and a standard curve was created for each gene to determine PCR efficiencies. Relative sample expression levels were calculated using Rotor-Gene 6000 series software 1.7 and were expressed relative to $\beta$-actin and corrected for between-run variability. Data for the experimental samples were expressed as the percentage of the internal control gene.

**Hydroxyl Radical-Mediated Oxidation Assay.** Hydroxyl radical-mediated oxidation experiments were performed using a metal-catalyzed reaction as described previously,[11] with some modifications. The target protein, bovine serum albumin (BSA), was dissolved in a 150 mM phosphate buffer (pH 7.3) to a final concentration of 0.5 mg/mL. The BSA solution was incubated with and without 100 $\mu$M copper ($Cu^{2+}$) and 2.5 mM $H_2O_2$ in the presence and absence of the samples. The control antioxidant was 50 $\mu$M ascorbate, which was directly dissolved in PBS. The reactions were carried out in open tubes and placed in a shaking water bath that was maintained at 37 °C. After the reaction was complete, each mixture was separated on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and stained with 0.1% Coomassie blue solution.

**Histopathological Analysis.** The pancreas was fixed by inflation with 10% formalin. The tissues were then embedded in paraffin, cut into sections (5 $\mu$m), and stained with a hematoxylin–eosin (H&E) solution. All tissue samples were examined and imaged in a blinded fashion. Images were captured using an Olympus DP controller and manager at a magnification of ×200.

**Statistical Analysis.** The Kruskal–Wallis one-way analysis of variance with Dunnet's post hoc test, which was performed using SPSS software (v. 13), was used for statistical analyses to determine if the differences between the groups were statistically significant. Statistical significance was set a priori at $p < 0.05$.

Table 2. Comparison of the GC-MS Library of Ginseng Berry and Steam-Dried Ginseng Berry[a]

| | ginseng berry | | | steam-dried ginseng berry | | |
|---|---|---|---|---|---|---|
| peak | retention time (min) | compound | area (%) | retention time (min) | compound | area (%) |
| 1 | | | | 4.415 | acetic acid methyl ster | 0.45 |
| 2 | 4.887 | acetic acid | 3.36 | | | |
| 3 | | | | 9.387 | dihydro-2(3*H*)-furanone | 0.40 |
| 4 | | | | 9.493 | pyrrolidine | 0.41 |
| 5 | | | | 9.599 | 2-cyclopenten-1-one | 0.48 |
| 6 | 11.102 | carbonic acid allyl ethyl ester | 1.41 | 11.102 | carbonic acid allyl ethyl ester | 0.40 |
| 7 | 11.902 | phenylacetaldehyde | 0.40 | 11.902 | phenylacetaldehyde | 1.12 |
| 8 | 13.704 | 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) | 1.32 | 13.685 | 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) | 4.79 |
| 9 | 14.716 | 2,3-dihydrobenzofuran | 1.33 | 14.706 | 2,3-dihydrobenzofuran | 1.80 |
| 10 | 16.171 | 2-methoxy-4-vinylphenol | 1.41 | 16.171 | 2-methoxy-4-vinylphenol | 1.15 |
| 11 | 17.780 | 1,1-bis(1,3-phenylene)ethanone | 0.98 | 17.771 | 1,1-bis(1,3-phenylene)ethanone | 1.75 |
| 12 | 20.113 | D-(−)-quinic acid | 5.95 | 20.055 | D-(−)-quinic acid | 6.55 |
| 13 | 23.408 | palmitic acid | 4.15 | 23.399 | palmitic acid | 3.22 |
| 14 | 25.051 | linoleic acid | 2.83 | 25.061 | linoleic acid | 4.13 |
| 15 | 25.239 | stearic acid | 1.24 | 25.239 | stearic acid | 1.52 |
| 16 | 28.035 | glycerol monopalmitate | 0.83 | 28.150 | glycerol monopalmitate | 1.98 |
| 17 | 29.508 | glycerol monolinolate | 0.48 | 29.508 | glycerol monolinolate | 1.62 |
| 18 | | | | 29.682 | glycerol monostearate | 1.97 |
| 19 | 34.250 | sitosterol | 0.94 | 34.259 | sitosterol | 1.72 |

[a]The components were identified on the basis of the comparison of their relative retention time and mass spectra with Wiley 7N library data of the GC-MS system.

## RESULTS AND DISCUSSION

Diabetes mellitus is a major and serious chronic metabolic disorder, and T2DM has a higher incidence (~95%) than T1DM.[12] T2DM is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency and has been shown to be closely associated with obesity, which is the primary risk factor of T2DM.[13,14] Thus, in the present study, we examined whether FSGB can be used to control body weight and glucose levels by enhancing insulin/glucose resistance, which are the main causes of T2DM in *db*/*db* mice.

We first analyzed the components contained in SGB to identify the potential active ingredients using GC-MS. In this analysis, a total of 19 peaks were detected and compared with the GC-MS library. RGB and SGB were shown to contain similar components except for some newly detected compounds in SGB, which included acetic acid methyl ester (0.45%), dehydro-2(3*H*)-furanone (0.40%), pyrrolidine (0.41%), and glycerol monostearate (1.97%). Figure 1 and Table 2 show that there were some differences in the components of RGB and SGB. Interestingly, 2,3-dihydro-3,5-dihydroxy-6-methyl-1,4*H*-pyran-4-one (DDMP) and sitosterol were highly increased by up to 3.6- and 1.8-fold, respectively, when compared to RGB. These components, which were typical in SGB, may be responsible for the antidiabetic effects of SGB by displaying free radical scavenging activity (DDMP),[15] lowering low-density lipoprotein (LDL) cholesterol (sitosterol),[16] and acting as a lipid emulsifier (GMS). The mechanisms involved in the chemical changes may be due to hydrolysis, dehydration, decarboxylation, and isomerization reaction during the steaming and drying processes.[17] In addition, ginsenosides are considered to be the major active ingredients in ginseng in regard to the treatment of diabetes mellitus,[10,18] which were also detected in SGB (Rg1, Re, Rf, Rb1, Rc, Rg2, Rb2, and Rd) (data not shown). On the basis of these findings, the antioxidant and LDL cholesterol lowering effects of SGB may also play a crucial role in preventing the development and progression of T2DM and its complications because increased oxidative stress has been shown to be closely associated with the development and progression of diabetes and its complications.[19,20] To determine whether SGB displayed protein-level antioxidant properties, degradation of BSA by $Cu^{2+}$ and $H_2O_2$ was monitored in the presence of 10−100 μg/mL RGB and SGB, as described under Materials and Methods. As shown in Figure 2, SGB more effectively protected against the degradation of BSA than the same amount of RGB, suggesting that SGB contained a higher amount of antioxidant components.

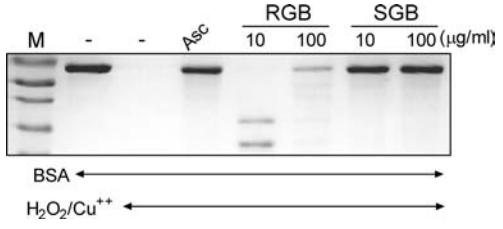


**Figure 2.** PAGE profiles of the BSA protein with $Cu^{2+}/H_2O_2$ in the presence of RGB and SGB. The gels show the protein obtained without treatment, with $Cu^{2+}/H_2O_2$, and at different concentrations of the extracts (10−100 μg/mL). Ascorbic acid (50 μM) was used as a positive control. The final steps included the incubation of all reactants, including BSA, for 2 h and electrophoresis in 10% SDS-PAGE.

In our previous study, we reported that fermentation of herbal extracts using *L. plantarum* successfully changed glucosides to aglycones, which are required for absorption through the human body.[21] Therefore, the final SGB samples were fermented with *L. plantarum* and used for the in vivo animal study. Panels A and B of Figure 3 shows that FSGB limited the increase in body weight, and Figure 3C shows that


dx.doi.org/10.1021/jf300460g | J. Agric. Food Chem. 2012, 60, 5438−5445

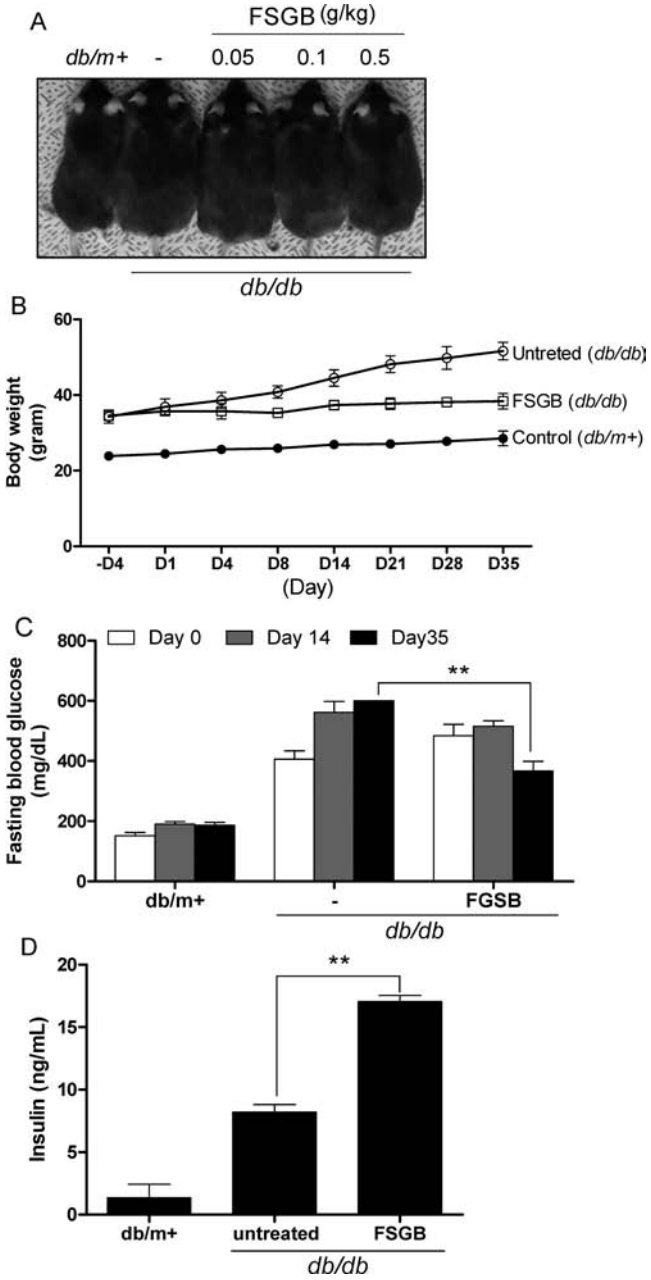


**Figure 3.** Body weight, blood glucose, and plasma insulin: (A) comparison of *db/m+*, *db/db*, and *db/db* + FSGB (0.05, 0.1, 0.5 g/kg); (B) changes of body weights in each group over the test period (35 days) (0.1 g/kg FSGB was administered during the study); (C) fasting blood glucose levels analyzed at days 0, 14, and 35; (D) plasma insulin levels measured at day 35. Results are expressed as the mean ± SD ($n$ = 5). **, $p < 0.01$.

the blood glucose levels were better controlled when compared to untreated *db/db* mice. Coincidently, administration of FSGB significantly elevated the plasma insulin levels (17.05 ± 0.49 ng/mL) when compared to the *db/m+* and untreated *db/db* mice (1.36 ± 1.06 and 8.21 ± 0.60 ng/mL, respectively) (Figure 3D). Generally, the plasma insulin levels of *db/db* mice increase rapidly during the first few weeks of life and then decrease to normal or less than normal levels around 10–weeks.[22] In the present study, plasma insulin in the FSGB group was significantly increased by approximately 2-fold when compared to the untreated *db/db* group. Although we cannot provide direct evidence of the effect of FSGB on insulin release in *db/db* mice, Rb2, which is abundant in ginseng berry, was recently reported to stimulate the expression of leptin mRNA in 3T3-L1 preadipocytes, in which plasma insulin levels showed a positive correlation with leptin levels in *db/db* mice.[23,24]

In addition, the levels of serum parameters (total cholesterol, high-density lipoprotein (HDL) cholesterol, and trigyceride) in mice treated with FSGB were similar to the levels in the *db/m+* control group (Table 3). Because T2DM and obesity are

**Table 3. Hematological Analysis**[a]

| | group ($n$ = 5) | total cholesterol (g/L) | HDL cholesterol (g/L) | triglyceride (g/L) |
|---|---|---|---|---|
| *db/m+* | untreated | <1.00 | 0.51 ± 0.04 | 0.72 ± 0.18 |
| *db/db* | untreated | 2.12 ± 0.17 | >1.00 | 1.74 ± 0.33 |
| | FSGB (0.1 g/kg) | <1.00 | 0.68 ± 0.25 | 1.39 ± 0.54 |

[a]Data represent the mean ± SD.

pathophysiologically associated, the successful modulation of serum parameters and body weight indicates that FSGB may be efficacious for the treatment of T2DM, in which *db/db* mice induce severe depletion of the insulin-producing $\beta$ cells of the pancreatic islets and a drastic decrease in body weight at the time of death.[22] In addition, a comparison of the major organs (liver, kidney, and pancreas) demonstrated that administration of FSGB was not harmful in T2DM-associated organs (liver and kidney) (Figure 4A–C). Interestingly, liver weight in FSGB-treated mice was significantly reduced when compared to the *db/m+* control group. This might be due to the restoration of a hypertrophic liver, which is secondary to fatty infiltration in *db/db* mice.[25] In addition, the weight of the pancreas for FSGB-fed mice was as low as that for the *db/m+* control group, even though it was not significantly reduced, which was probably due to the reduced islet hypertrophy in the FSGE mice.[26] Reduced islet hyperplasia was also observed in the H&E staining of the pancreas tissue (Figure 4D). IPGTT and ITT were investigated to better understand the anti-T2DM effect of FSGB. *db/db* mice used in this test had already reached the maximum glucose level, whereas the glucose levels in the FSGB-treated mice were significantly controlled at 35 days. Thus, both glucose baselines for IPGTT and ITT started at relatively high levels, and the results were compared between the untreated group, FSGB-treated group, and *db/m+* control group. As shown in Figure 5A, after 35 days of treatment with FSGB (0.1 g/kg), glucose tolerance was markedly improved when compared with the untreated *db/db* mice 120 min after glucose administration. Likewise, blood glucose levels in the FSGB group were effectively controlled 90 min after insulin administration, implying that insulin resistance was gradually improved (Figure 5B). These results strongly suggest that the effect of FSGB in T2DM may result from enhanced insulin and glucose resistance.

To further verify the potential of using FSGB to treat T2DM, the effects of FSGB ex vivo and in vitro were evaluated. Several previous studies have reported that glucose transport activity increased several-fold in response to chronic insulin treatment due to an increase in GLUT1 mRNA and protein levels.[27,28] These findings imply that an increase in GLUT1 mRNA can elevate glucose uptake. Because T2DM is associated with an increased risk of acquiring infectious diseases and developing sepsis due, in part, to immune dysfunction,[29] enhancing

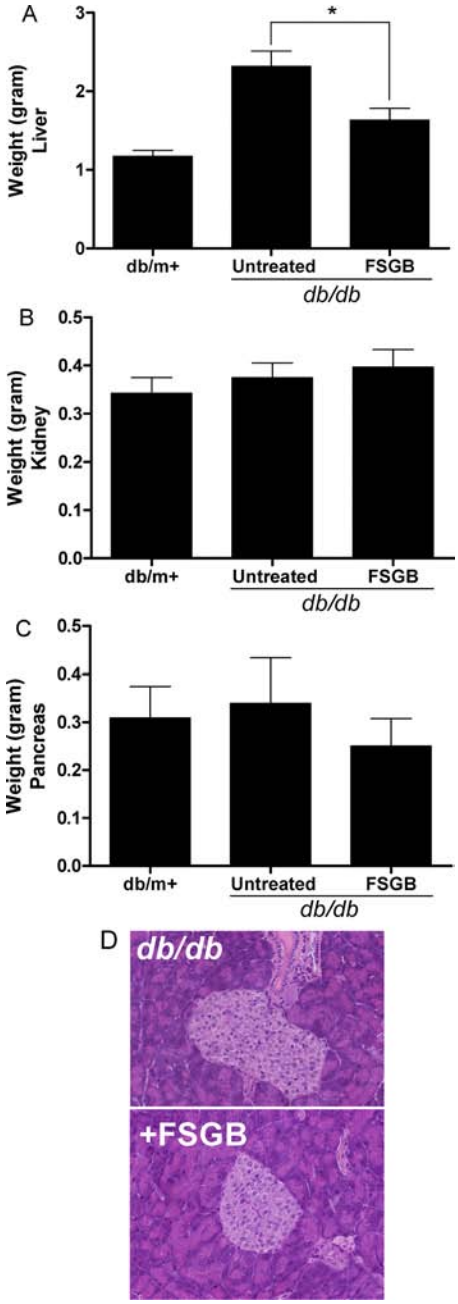


**Figure 4.** Weight of major organs and H&E staining of the pancreas: changes in (A) liver, (B) kidney, and (C) pancreas weight compared between groups. (D) H&E staining of the pancreas of untreated *db*/*db* and FSGB-treated *db*/*db* mice after 35 days. Results are expressed as the mean ± SD ($n$ = 5). *, $p < 0.05$.

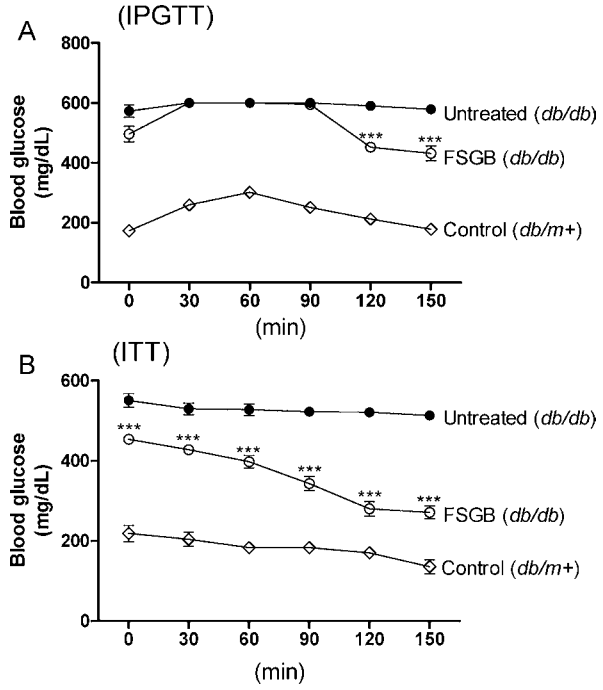


**Figure 5.** IPGTT and ITT at the indicated time points (0−150 min) after 35 days of treatment with or without FSGB: (A) IPGTT and (B) ITT analyzed on day 35 after the mice were fasted for 4 h, followed by an intraperitoneal administration of glucose (2 g/kg) and insulin (5 U/kg), respectively. Blood glucose was measured from baseline (0) to 150 min. Results are expressed as the mean ± SD ($n$ = 3). ***, $p < 0.001$.

immune activity may be one potential therapeutic strategy for the treatment of complications associated with T2DM. In regard to this, we investigated the effects of FSGB on immune cells obtained from the spleen of mice used in this study. The mRNA levels of interleukin-2 (IL-2) and tumor necrosis factor-$\alpha$ (TNF-$\alpha$), which are known growth factors for T and B cells, respectively, were analyzed using qPCR. Figure 6A clearly demonstrates that the expressions of IL-2 and TNF-$\alpha$ mRNAs were significantly increased in FSGB-treated mouse splenocytes when compared to the *db*/*m*+ control group. In addition, the number of immune cells (mouse splenocytes) was markedly increased in the FSGB group, which corresponded to the qPCR results (Table 4). These results indicate that FSGB can effectively enhance immune activity as well as normalize the suppressed proliferative response of immune cells due to elevated serum insulin in T2DM.[30] Moreover, FSGB was shown to dose-dependently up-regulate the GLUT1 mRNA levels in a skeletal muscle cell line (L6), which suggests that FSGB may play a role in increasing glucose transport activity (Figure 6B). Because excess body fat in obese individuals appears to play a role in insulin resistance, which can cause T2DM along with heart disease, high blood pressure, stroke, and unhealthy cholesterol levels, combined therapeutic methods for the treatment of T2DM need to include diabetic dyslipidemia.[31] In light of this, the well-modulated serum parameters (total cholesterol, HDL cholesterol, and trigyceride) in the *db*/*db* mouse, which is a model for diabetic dyslipidemia, indicate that FSGB may prevent T2DM in overweight and obese individuals.[32] In the histological analysis, the islet architecture in *db*/*db* mice supplemented with FSGB was preserved, whereas control *db*/*db* mice exhibited a loss and degeneration of the islet boundary.

Xie and others[33] reported that a *P. ginseng* berry organic extract contained more ginsenosides, including Rb2 and Rd, than the extracts from *P. ginseng* root. In the present study, the patterns of ginsenosides were not changed between RGB and SGB, but both extracts contained Rb2 and Rd, which were typical in ginseng berry (data not shown). More convincing evidence for the anti-T2DM effect of FSGB was provided in a previous study, where the steaming of ginseng at high temperature was shown to potentially produce significant changes in the major ginsenosides, which may enhance biological activity.[34] In addition, changing glucosides to


dx.doi.org/10.1021/jf300460g | J. Agric. Food Chem. 2012, 60, 5438−5445

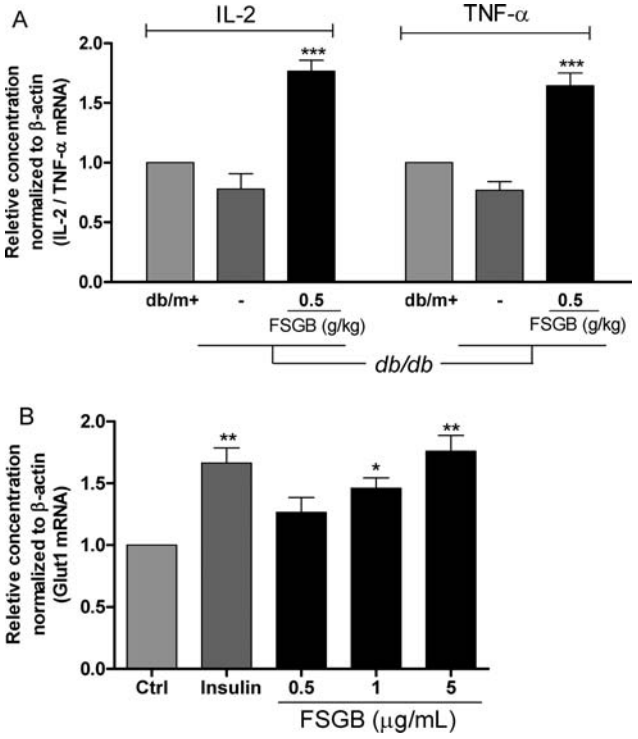


**Figure 6.** Quantitative analysis of mRNA expression. (A) To analyze IL-2 and TNF-$\alpha$ mRNA, splenocytes were prepared from euthanized mice at day 35, and mRNAs were isolated from each group (*db/m+*, untreated and FSGB-treated *db/db* mice) as described under Materials and Methods. (B) In vitro mRNA expression of GLUT1 mRNA with or without FSGB (0.5, 1, and 5 $\mu$g/mL) was investigated in the L6 skeletal muscle cell line. Insulin was used as a positive control. Experiments were performed in triplicate, and the results are expressed as the mean ± SD. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

**Table 4. Splenocyte Counts**[a]

| | group ($n$ = 5) | cell counts (cells/mL) |
|---|---|---|
| *db/m+* | untreated | $(4.25 \pm 0.51) \times 10^6$ |
| *db/db* | untreated | $(3.67 \pm 0.24) \times 10^6$ |
| | FSGB (0.1 g/kg) | $(5.13 \pm 0.38) \times 10^6$ |

[a]Data represent the mean ± SD.

aglycones was thought to synergistically enhance the anti-T2DM effect of FSGB in *db/db* mice.[35]

In conclusion, the present study demonstrated that administration of a steam-dried ginseng berry extract fermented with *L. plantarum* significantly improved the pathologic indices of T2DM by modulating insulin sensitivity and lowering plasma glucose levels in *db/db* mice. In addition, FSGB might have antiobese and antidyslipidemia activities, which would be of clinical importance to improving the management of T2DM. More in-depth studies are needed to determine the mechanisms and structural identity of FSGB. However, based on the results of this study, FSGB may be a potent functional supplemental material for the treatment of T2DM.

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### Author Contributions

‖Equally contributed to this paper.



### Funding

This study was supported by a grant of the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare and Family Affairs, Republic of Korea (A091121).

### Notes

The authors declare no competing financial interest.


## ACKNOWLEDGMENTS

We sincerely thank President Myung-Ae Park and Korea Genetic Pharm Co., Ltd. for their kind cooperation.

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