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Anti-diabetic effect of black ginseng extract by augmentation of AMPK protein activity and upregulation of GLUT2 and GLUT4 expression in db/db mice

Ok-Hwa Kang^{1†}, Mi-Yae Shon^{2†}, Ryong Kong¹, Yun-Soo Seo¹, Tian Zhou¹, Do-Yeon Kim², Yeong-Soo Kim³ and Dong-Yeul Kwon^{1*}

Abstract

Background: Black ginseng (*Panax ginseng* C. A. Meyer), three to nine times-steamed and dried ginseng, has biological and pharmacological activities. In this study, the anti-diabetic effects of the black ginseng ethanol extract (GBG05-FF) in typical type 2 diabetic model db/db mice were investigated.

Methods: The effect of GBG05-FF in Type 2 diabetic mice was investigated by their blood analysis, biological mechanism analysis, and histological analysis.

Results: The mice group treated with GBG05-FF showed decreased fasting blood glucose and glucose tolerance compared to that of the nontreated GBG05-FF group. In the blood analysis, GBG05-FF decreased main plasma parameter such as HbA1c, triglyceride, and total-cholesterol levels related to diabetes and improved the expression of genes and protein related to glucose homeostasis and glucose uptake in the liver and muscle. The histological analysis result shows that GBG05-FF decreased lipid accumulation in the liver and damage in the muscle. Moreover, GBG05-FF increased the phosphorylation of the AMPK in the liver and upregulated the expression of GLUT2 in liver and GLUT4 in muscle. Therefore, the mechanisms of GBG05-FF may be related to suppressing gluconeogenesis by activating AMPK in the liver and affecting glucose uptake in surrounding tissues via the upregulation of GLUT2 and GLUT4 expression.

Conclusion: These findings provided a new insight into the anti-diabetic clinical applications of GBG05-FF and it might play an important role in the development of promising functional foods and drugs from the viewpoint of the chemical composition and biological activities.

Keywords: Black ginseng, Anti-diabetic, AMPK, Glut

Background

Black ginseng (*Panax ginseng* C. A. Meyer), three to nine times-steamed and dried ginseng undergoes Maillard browning reaction. The biological and pharmacological activities of steam-processed ginseng are greater than that of nonsteamed ginseng. During the steaming and drying process, the percentage of main ingredients including

saponin, ginsenosides, proteins, and phenolics is altered, because of newly produced active ingredients [1–4]. Black ginseng contains newly discovered ginsenosides and exhibits more potent biological activities than white and red ginseng [5]. Black ginseng possesses anticarcinogenesis, anti-diabetic, recovery from learning and memory damages, and antioxidative activities [6–9]. Previous studies were focused on verifying effectiveness of black ginseng on improvement of insulin resistibility or restoration of β -cell function [10]. In recent studies, we showed that supplementation with black ginseng extract improves diabetic parameters, thus it has a potential as a functional

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food for diabetes mellitus patients [11]. In this study, we focused on the regulation of hyperglycemia and hyperlipidemia caused by black ginseng extract.

Diabetes is a chronic metabolic disease that account for 3% of the world's mortality, because of its complications; especially, disease incidence in South Korea is the highest out of all of the OECD countries. It is a metabolic disease caused by either the destruction of pancreatic β -cells (type 1 diabetes) or unresponsiveness to insulin (type 2 diabetes) [12]. In particular, type 2 diabetes is an alarming public health issue, because it causes a broad range of chronic diseases and metabolic syndromes such as cardiovascular disease, hypertension, non-alcoholic fatty liver disease (NAFLD), and obesity.

The homeostasis of blood glucose is maintained as a result of the action of various vital organs. Blood glucose influx is mediated by glucose transporter (GLUT). When the hyperglycemia broke out, plasma glucose entered the cytoplasm of hepatocytes through GLUT2 [13]. In contrast, the glucose uptake in adipose tissue and muscle is mostly mediated by GLUT4 and plays an important role in glucose utilization by insulin stimulation [14]. Hepatic glucose uptake and glucose production are the key parameters in glucose homeostasis. Adenosine monophosphate-activated protein kinase (AMPK), a serine/threonine kinase, has a critical function in this process, because it can suppress gluconeogenesis in the liver and promote glucose uptake in peripheral tissues [15]. Previous studies have found that the activation of AMPK signal pathways increased the insulin- and contraction-stimulated GLUT4 translocation in muscles [16].

In this study, the anti-diabetic effects of black ginseng extract (GBG05-FF) in typical type 2 diabetic model db/db mice were investigated. GLUT2 expression in the liver, GLUT4 expression in the skeletal muscle, AMPK protein levels in the liver and white fat was detected to validate the beneficial effects of GBG05-FF as an anti-diabetic agent and to clarify the mechanism of action.

Methods

Materials

3,3-Diaminobenzidine tetrahydrochloride (DAB) was purchased from Sigma-Aldrich (St Louis, MO, USA). Guinea pig antihuman insulin and rabbit antiglycagon were obtained from Millipore Corporation (Billerica, MA, USA). Glycated hemoglobin (HbA1c), triglyceride (TG) cholesterol (CHO), and oral glucose tolerance test (OGTT) were purchased from Bio-Rad variant II turbo (Bio-Rad Laboratories, Hercules, CA, USA). Glucose 6-phosphatase (G6pase), phosphoenolpyruvate carboxykinase (PEPCK), liver glycogen phosphorylase (LGP), carnitine palmitoyltransferase 1 α (CPT1 α), citrate synthase (CS), glucokinase (GK), mitochondrial medium-chain acyl-CoA dehydrogenase (MCAD), Glucose transporter

2 (GLUT2) and glucose transporter 4 (GLUT4), and β -actin oligonucleotide primers were purchased from Bioneer Corp. (Daejeon, Korea). Anti- β -actin, GLUT2, and GLUT4 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-pThr172-5' AMP-activated protein kinase (AMPK) and antiAMPK antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Standard ginsenosides were purchased from Ambo institute (Daejeon, Republic of Korea).

Preparation of GBG05-FF

GBG05-FF was made by the business of master-work for geumsan ginseng through International ginseng and Herb Research Institute, it was studied [11]. Briefly, 5-Year old Korean white ginseng (*Panax ginseng* C.A. Meyer) was purchased from a local ginseng center (Geumsan, Korea). The black ginseng was prepared from white ginseng by repeated steaming at 95 °C for 6 h and drying at 60 °C. The dried black ginseng samples were extracted twice with 10 volumes of 70% ethanol at 80 °C for 8 h, using a Soxhlet extractor with a heating mantle. The extracts were subsequently filtered and lyophilized. The extraction yields of black ginseng was 25%. Black ginseng extract was designated as GBG05-FF and used for the research.

Analytical methods

GBG05-FF sample was extracted with an equal volume of n-butanol. The n-butanol fraction was then evaporated to dryness, and 50% methanol was added. HPLC system (Santa Clara, CA, USA) equipped with a Poroshell 120 EC-C18 column (3.0 mm \times 50 mm, 2.7 μ m, Agilent) was used at a detection wavelength of 203 nm. Water (A) and acetonitrile (B) were kept at 35 °C and used as the mobile phase at 0.8 ml/min with following gradient: 0–2 min, 10% B; 2–4 min, 11% B; 4–9 min, 13% B; 9–19 min, 21% B; 19–26 min, 28% B; 26–28 min, 28% B; 28–32 min, 32% B; 32–34 min, 32% B; 34–40 min, 45% B; 40–42 min, 45% B; 42–50 min, 55% B; 50–55 min, 55% B; 55–55.01 min, 10% B; 55.01–62 min, 10% B. To investigate the stability of the GBG05-FF water decoction, the same extraction step was repeated to obtain GBG05-FF and then the berberine hydrochloride content in GBG05-FF was detected by HPLC analysis at a detection wavelength of 203 nm with a C18 column (Fig. 1).

Experimental mouse model of diabetes

All the animal experiments were performed in accordance with the guidelines established by the Animal Ethics Committee of Wonkwang University, and this experiment was approved by the committee (Approval No. WKU15–101). To evaluate the effect of GBG05-FF

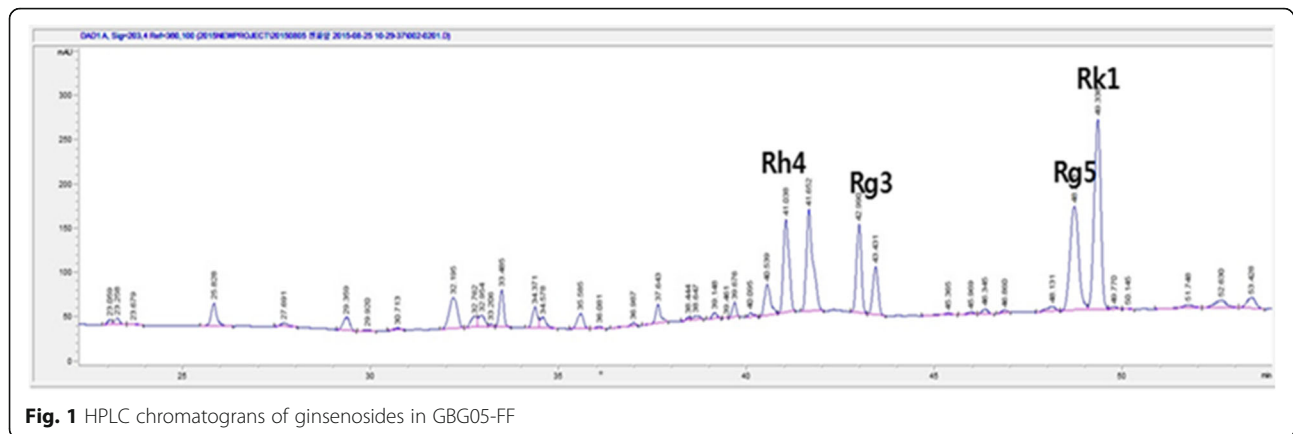


Fig. 1 HPLC chromatograms of ginsenosides in GBG05-FF

on fasting blood glucose levels, 30 C57BKS (db/db) mice (20–25 g, 4 weeks, male, BKS.Cg - +Lepr^{db}/+Lepr^{db}) and 10 C57BKS mice (20–25 g, four weeks, male, BKS.Cg-m+/+Lepr^{db}) were used. All the animals were purchased from SAMTACO (Osan, Korea). All the experiments in this study were conducted for four weeks. The mice were given free access to water and kept at a constant room temperature under a 12/12-hour light/dark cycle. All the mice were acclimatized for an adaptation period of seven days before the experiment, fed standard pellet chow, and given fresh water ad libitum. A total of 40 mice were randomly divided into four groups (ten mice per group): Group I (C57BKS group, Normal), Group II (C57BKS -db/db group, Control), Group III (C57BKS -db/db mice were fed a dietary supplement of 300 mg/kg of GBG05-FF, GBG05-FF 300), and Group IV (C57BKS-db/db mice fed a dietary supplement of 900 mg/kg of GBG05-FF, GBG05-FF 900). The weights were checked every week. Water and feed were always available. After four weeks, all the mice were sacrificed to collect blood and remove the liver and muscle.

Measurement of fasting blood glucose level

Fasting blood glucose levels were checked from blood samples taken from the tail vein of each mouse every week. To determine fasting blood glucose levels, mice were deprived of their diet for 12 h, but allowed free access to drinking water. Blood glucose levels were checked by using Accu-Check (Roche Diagnostics Korea co., Ltd., Seoul, Korea).

Measurement of oral glucose tolerance test (OGTT)

After GBG05-FF administering for four weeks, the OGTT were performed. Briefly, a normal control group, diabetic control group, and GBG05-FF-fed groups (GBG05-FF 300 and GBG05-FF 900) were deprived of their diet, but allowed access to drinking water. After fasting for 16 h, blood was drawn from the mouse tail

veins and glucose levels were determined by using Accu-Check of all the mice. Immediately after the blood check, glucose (0.2 g/100 g body weight) was orally administered to all mice. Blood glucose levels were checked at the indicated time intervals (0, 30, 60, 90, and 120 min).

Glycated hemoglobin, TG, and total cholesterol assays in the serum

After administering GBG05-FF for four weeks, the mice were sacrificed and the blood collected was used to evaluate HbA1c, TG, and total CHO levels. HbA1c levels were measured by a Bio-Rad variant II turbo (Bio-Rad Laboratories, Hercules, CA, USA). Plasma TG and total CHO levels were measured using an Automated chemical analyzer (Roche Diagnostics, Mannheim, Germany).

Histopathological analysis of liver and muscle

The liver and muscle tissues were fixed in 4% formalin over 24 h. The fixed tissues were processed for paraffin embedding. The sections were counterstained with hematoxylin and eosin. To observe collagen content, picrosirius red staining was performed by using a picrosirius red staining kit (Polysciences, Inc. Warrington). The sections of muscle were deparaffinized and rinsed with distilled water. The washed sections were soaked in picrosirius red solution A for 2 min. The sections were rinsed again with distilled water, soaked in picrosirius red solution B for 60 min and picrosirius red solution C for 2 min, immersed in 70% alcohol for 45 s, dehydrated, cleared, and mounted. Image acquisition was performed using an optical microscope (Nikon Eclipse 80i; Tokyo, Japan) with a magnifying power of 100 and 200.

Quantitative reverse transcription polymerase chain reaction (RT-PCR) analysis

Total RNA was isolated from the muscles and liver in mice using an RNeasy Mini kit (iNtRon, Seongnam,

Korea), and complementary deoxyribonucleic acid (cDNA) was synthesized using a the QuantiTect Reverse Transcription kit (Qiagen, Seoul, Korea) according to the manufacturer's instructions. Real-time PCR was performed in triplicate using power SYBR[®] Green PCR master mix (iNtRon, Seongnam, Korea) in a StepOnePlus Real-Time RT-PCR System. The expression levels of the target genes relative to that of the endogenous reference gene β -actin were calculated using the delta cycle threshold method using StepOne software v2.3 (Applied Biosystems, Foster City, CA, USA). The primer sequences are listed in Table 1.

Western blot analysis

Protein expression was assessed by Western blot analysis according to the standard procedures. Each liver and muscle were homogenized in RIPA lysis buffer (ATTO Corp., Tokyo, Japan) on ice. The homogenates were centrifuged (13,000 rpm, 10 min, 4 °C), and the protein concentrations in the supernatant were determined using the Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Equal amounts of protein (20 μ g) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene membrane (Millipore, Bedford, MA, USA). The membrane was blocked for >1 h with 5% skim milk in Tris-buffered saline buffer (150 mM NaCl and 20 mM Tris-HCl, pH 7.4) with 0.05% Tween 20. The membrane was incubated with primary antibodies for 18 h, washed with tris-buffered saline containing Tween 20, and incubated with anti-mouse or anti-rabbit immunoglobulin G horseradish peroxidase-conjugated secondary antibodies combined with primary antibody of target protein. The proteins were supplemented with the ECL prime Western blotting detection reagents (GE Healthcare, Parsippany, NJ, USA),

Table 1 Primers for quantitative real-time polymerase chain reaction analysis

	Forward	Reverse
G6Pase	ATGACTTTGGGATCCAGTCG	TGGAACCAGATGGGAAAGAG
PEPCK	CTGGCACCTCAGTGAAGACA	TCGATGCCCTCCAGTAAAC
LGP	CCAGAGTGCTCTACCCAAT	CCACAAAGTACTCCTGTTTCAGC
GS	GACACTGAGCAGGGCTTTT	GGGCCTGGGATACTTAAAGC
CPT1 α	ACCCTGAGGCATCTATTGACAG	ATGACATACTCCCACAGATGGC
MCAD	GGCAAATGCCTGTGATTCTT	CCATTGCGATCTTGAAACCT
GK	TCCCTGTAAGGCACGAAGACAT	ATTGCCACCACATCCATCTCA
CS	TGCCACACAAGCCATTTG	CTGACACGTCTTGCCAACTT
SIRT4	CGCTGCTCAAGATCCCTAAG	GCGACACAGCTACTCCATCA
SIRT6	GGTACTGTGGATGAGGTGAT	GGCTCAGCCTTGAGTGCTAC
GLUT2	CTGCTCTTCTGTCCAGAAAGC	TGGTGACATCCTCAGTTCTC
GLUT4	TCGTCATTGGCATTCTGGTTG	AGCTCGTTTACTAAGAGCAC

and ImageQuant LAS 4000 Mini Biomolecular Imager (GE Healthcare, Parsippany, NJ, USA) was used to evaluate the bands, which were quantified by Image j.

Statistical analysis

All the experimental results were compared by one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS, ver. 22.0, SPSS Inc. Chicago, IL, USA). The data are expressed as means \pm SEM. Group means were considered to be significantly different at $p < 0.05$, as determined by the technique of protective least significant difference.

Results

HPLC analysis of GBG05-FF

The contents of minor ginsenoside Rh4, Rk1 and Rg5 in BGE (GBG05-FF) were 1.93, 6.90, and 11.74 mg/g, respectively. GBG05-FF contained ginsenosides such as ginsenoside Rg5, Rk1, and Rh4. These unique ginsenosides are nonexistent in white ginseng. The major ginsenosides including Rb1, Rb2, Rc, and Rd. in white ginseng can be transformed to the minor ginsenoside Rg3. This ginsenoside may also be converted to Rg5 and Rk1 by heating (Table 2).

Effect of GBG05-FF on fasting glucose levels and glucose intolerance in type-2 diabetes model

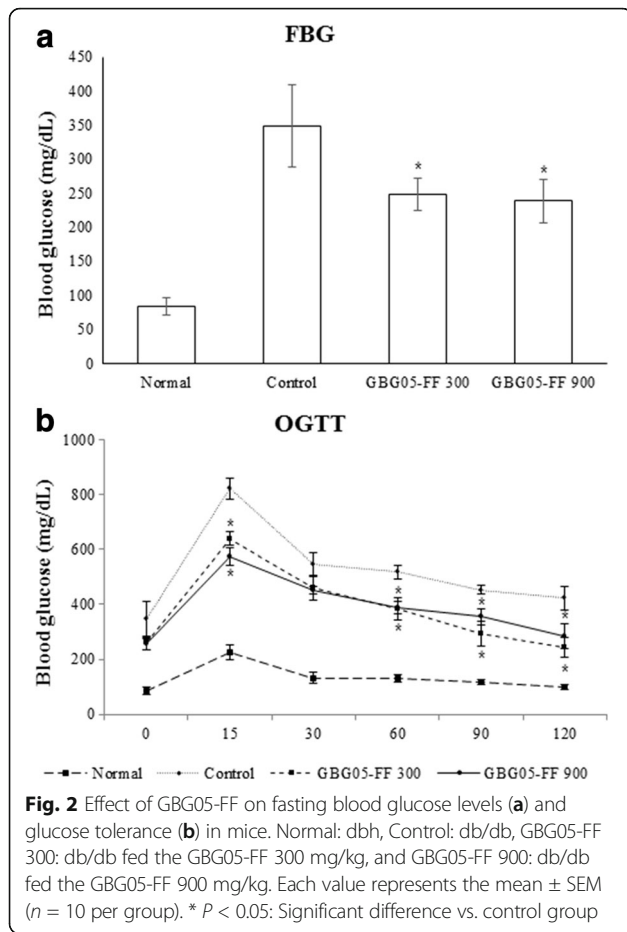
To confirm the effect of GBG05-FF on the development of diabetes, fasting blood glucose levels and glucose tolerance were investigated in Type 2 diabetic db/db mice. Before GBG05-FF was administered to mice, the zero-time fasting blood glucose levels were not significantly different among the groups. During four weeks of GBG05-FF administering, GBG05-FF suppressed the increase in the fasting blood glucose level in Type 2 diabetic db/db mice (Fig. 2). Moreover, after GBG05-FF administration for four weeks, OGTT was performed. The result of glucose tolerance in the mice group administered with GBG05-FF showed decrease in glucose level compared to that of the control group (Fig. 2). However, the result showed no dose dependent effect both on fasting glucose levels and glucose tolerance.

Effect of GBG05-FF on plasma parameters related to diabetes

To verify the effect of GBG05-FF in plasma parameters, blood samples were collected from mice after experiment during four weeks. The blood analysis results showed that

Table 2 Ginsenoside contents in GBG05-FF

Ginsenosides (mg/g)		
Rh4	Rg5	Rk1
1.93 \pm 0.18	11.74 \pm 0.27	6.9 \pm 0.08



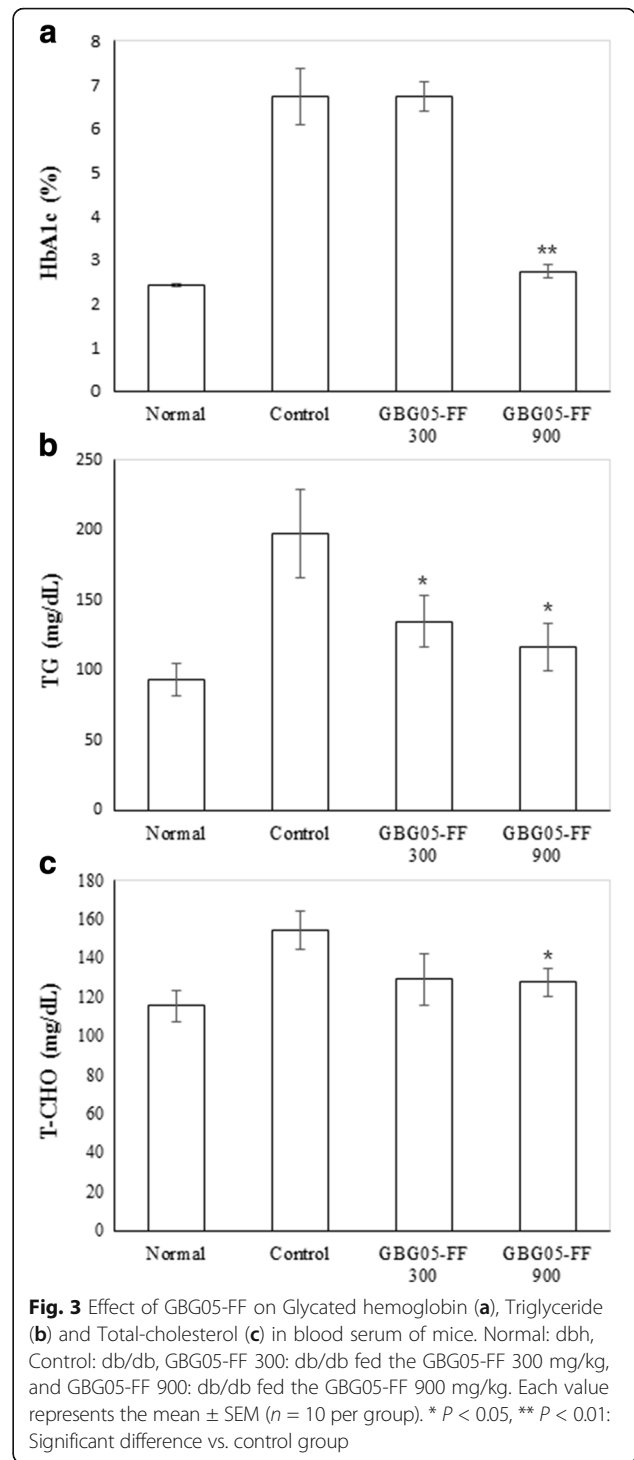
the TG and total CHO decreased in a dose-dependent manner, but HbA1c levels decreased only for the group administered to GBG05-FF 900 mg/kg with 900 mg/kg GBG05-FF (Fig. 3).

Effect of GBG05-FF on the expressions of genes related to glucose homeostasis in muscle and liver of type 2 diabetic db/db mice

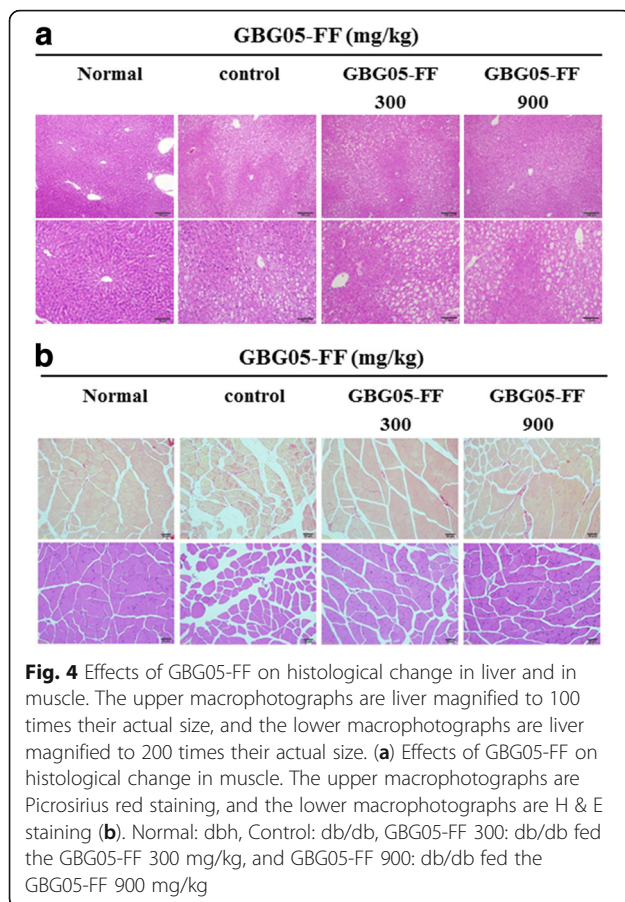
To prove the effect of GBG05-FF in glucose homeostasis mechanism of diabetic mice, quantitative real-time PCR analysis was performed. The results showed that GBG05-FF suppressed the expression of G6Pase, PEPCK, and LGP in the liver tissue, whereas GBG05-FF increased the expression of GS in the liver tissue and the expression of CPT1 α , CS, GK, and MCAD in the muscle tissue.

Histological analysis on effect of GBG05-FF in muscle and liver tissue

To investigate the morphological changes of the liver and muscle, histological analysis was performed. The liver of db/db group not treated with GBG05-FF showed lipid accumulation. However, the liver of db/db group treated GBG05-FF showed a significant



decrease on the lipid accumulation (Fig. 4a). The result of the histological analysis of muscle showed that muscle of the non-treated GBG05-FF group significantly decreased myofibers compared to that of the normal group, whereas the mice group treated with GBG05-FF showed the restoration of myofibers by



GBG05-FF. Moreover, through picrosirius red staining, we confirmed that GBG05-FF decreased muscle damage by Type 2 diabetes (Fig. 4b).

Effect of GBG05-FF on expression gene and protein levels and glucose uptake in liver and muscle of type 2 diabetic db/db mice

To confirm the effect of GBG05-FF in glucose uptake mechanism of diabetic mice, quantitative real-time PCR and western blotting analysis were performed. The results show that GBG05-FF increased gene expression of both GLUT2 and GLUT4. Moreover, GLUT2 in the liver and GLUT4 were upregulated by GBG05-FF in the muscles (Fig. 5).

Effect of GBG05-FF on AMPK phosphorylation in type 2 diabetic db/db mice

To confirm the effect of GBG05-FF on AMPK phosphorylation in the liver of diabetic mice, western blotting analysis was performed. The result showed that phosphorylation of AMPK were increased by GBG05-FF in the liver (Fig. 6).

Discussion

In this study, we demonstrated that GBG05-FF decreased hyperglycemia and hyperlipidemia by reducing the blood glucose levels and clarified the mechanism of action in type 2 diabetic C57BKS -db/db mice. Recently, there has been a growing interest in hypoglycemic agents from natural products, in particular, those derived from plants [17]. Therefore, searching for better agents from herbs or natural products that can be used to treat diabetes is necessary.

Ginseng is a well-characterized medicinal herb listed in the classic oriental herbal. Ginseng has diverse pharmacological and therapeutic properties such as immune function, cancer, and cardiovascular diseases, and has shown improved physical, cognitive, and sexual performance [18]. Moreover, many studies have reported that dietary ginseng saponins may have a beneficial effect on hyperlipidemia and obesity as well as reducing total serum cholesterol level [19, 20]. In Asia, there are a variety of commercial ginseng products including white, red, and black ginseng. Especially, black ginseng is produced by a repeated steaming and drying process from three to nine times. Black ginseng possesses better biological activity inducing anti-inflammatory, anti-cancer, anti-stress, and free radical scavenging effects than those of red ginseng [21, 22]. Although black ginseng is pharmacologically effective, the anti-diabetic effect of GBG05-FF has not been examined yet.

First of all, to investigate the major compound that has the effect to lower glucose levels, we were performed using several compounds contained in GBG05-FF profiled from HPLC assay (Fig. 1). GBG05-FF contained ginsenosides such as ginsenoside Rg5, Rk1, and Rh4. These unique ginsenosides are nonexistent in white ginseng. The major ginsenosides including Rb1, Rb2, Rc, and Rd. in white ginseng can be transformed to the minor ginsenoside Rg3. This ginsenoside may also be converted to Rg5 and Rk1 by heating [23]. In particular, delycosylated minor ginsenosides have more pharmaceutical activities than major ginsenosides [24].

Hepatic glucose uptake and glucose production in peripheral tissues are highly important in glucose homeostasis of the body. We proposed that GBG05-FF is effective in inhibiting hepatic gluconeogenesis and promoting glucose uptake of peripheral tissue. Elevated hepatic glucose production is a major cause of hyperglycemia in Type 2 diabetes. G6pase, PEPCK, liver glycogen phosphorylase (LGP), and glycogen synthase (GS) enzymes are important factors related to gluconeogenesis and glycogenolysis in the liver [25]. CPT1 is significant for fatty acid oxidation in the liver [26]. Mitochondrial medium-chain acyl-CoA dehydrogenase (MCAD) are significant for β -oxidation. Glucokinase (GK) and citrate synthase (CS) are related to glucose

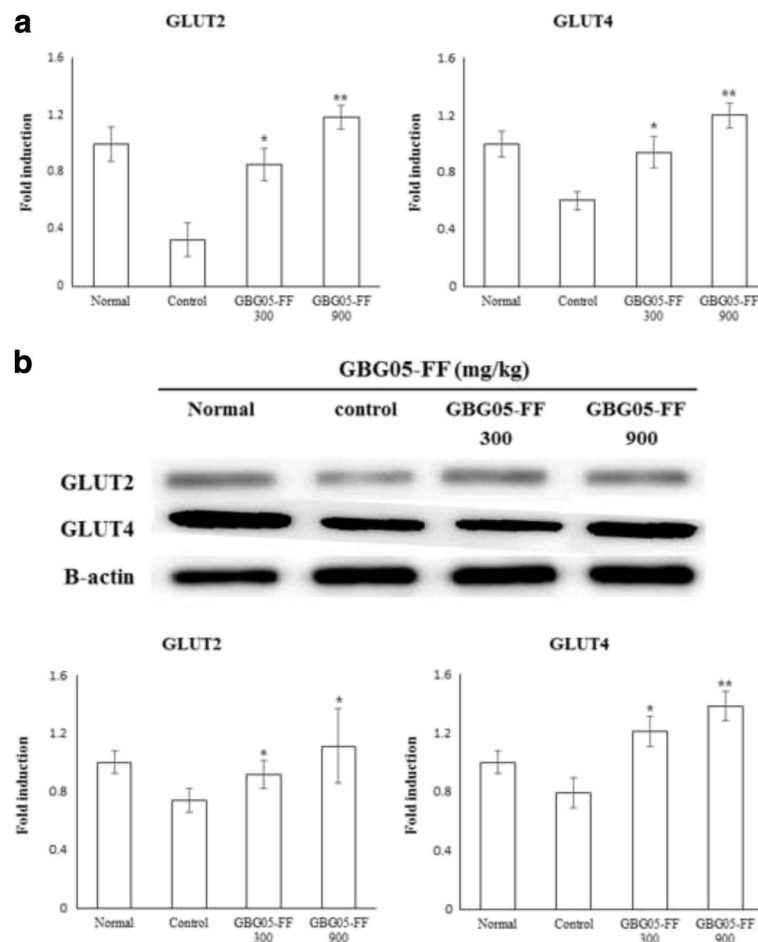


Fig. 5 Effects of GBG05-FF on the gene expression and the protein expression of GLUT2 in liver (**a**), and the gene expression and protein expression of GLUT4 in muscle (**b**) of mice. Normal: dbh, Control: db/db, GBG05-FF 300: db/db fed the GBG05-FF 300 mg/kg, and GBG05-FF 900: db/db fed the GBG05-FF 900 mg/kg. Each value represents the mean \pm SEM ($n = 10$ per group) in three independent experiments. * $P < 0.05$, ** $P < 0.01$: Significant difference vs. control group

oxidation. [27]. Our results suggest that GBG05-FF suppressed the expression of G6Pase, PEPCK, and LGP in the liver tissue (Fig. 7), whereas it increased the expression of GS in the liver tissue and the expression of CPT1 α , CS, GK, and MCAD in the muscle tissue. In addition, GBG05-FF increased the expression of SIRT4 and SIRT6, which belong to the sirtuin family of proteins that regulate insulin secretion and glucose homeostasis (Figs. 7 and 8) [28, 29]. Taken together, our results suggest that GBG05-FF significantly decreased the lipid accumulation and muscle damage by Type 2 diabetes (Fig. 4).

AMPK is a phylogenetically conserved serine/threonine protein kinase that has been proposed to act as a target for the prevention of the metabolic syndrome, characterized in hypertension, obesity, dislipidemia and insulin resistance, eventually leading to type 2 diabetes. In particular, AMPK is an $\alpha\beta\gamma$ heterotrimer, has a main function in regulating gluconeogenesis, and glucose

uptake in surrounding tissues and is mediated by GLUT4 [30]. AMPK phosphorylation can directly phosphorylate CREB-regulated transcription coactivator 2 on Ser171, which would be antagonistic to the induction of gluconeogenic genes [31]. β -Cells are designed to sense blood glucose and other secretagogues to adjust insulin secretion according to the needs of the organism [32]. Glucose enters β -cells by facilitating diffusion through the glucose transporter (GLUT2 in rodents; mainly GLUT1 in humans) and is retained inside the cell through its phosphorylation by glucokinase [12]. This glucose sensor mechanism regulates glycolytic flux to the plasma glucose concentration in hepatocytes [33]. In contrast, glucose uptake in surrounding tissues is mediated by GLUT4 [30], which is the major glucose transporter of muscle and adipose tissues and facilitates intracellular glucose delivery from extracellular milieu, thus augmenting glucose uptake. GLUT4 mRNA and protein content can decrease in the peripheral tissues

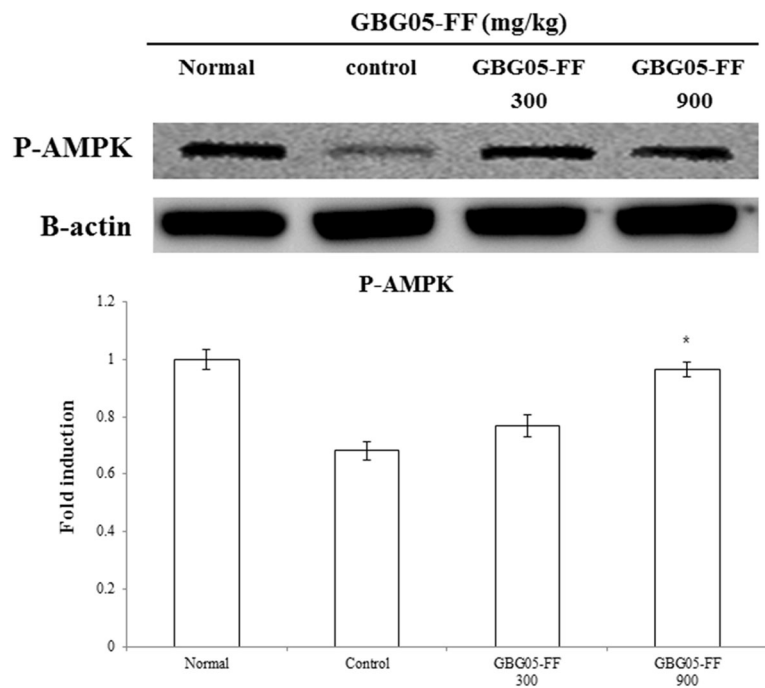


Fig. 6 Effects of GBG05-FF on the phosphorylation of AMPK in livers of mice. Normal: dbh, C2ntrol: db/db, GBG05-FF 300: db/db fed the GBG05-FF 300 mg/kg, and GBG05-FF 900: db/db fed the GBG05-FF 900 mg/kg. Each value represents the mean \pm SEM ($n = 10$ per group) in three independent experiments. * $P < 0.05$, ** $P < 0.01$: Significant difference vs. control group

and may be one of the reasons for insulin resistance [34]. To evaluate the effect of GBG05-FF on GLUT2 and GLUT4 expression in the surrounding tissue and AMPK protein in the hepatic tissue, quantitative RT-PCR and Western blot analysis were used in our study. As shown

in Fig. 5, GBG05-FF significantly increased GLUT2 and GLUT4 mRNA and protein level compared to the control group. As expected, the data shows that GBG05-FF significantly induced AMPK phosphorylation in hepatic tissues and increased the expression of GLUT2 in liver

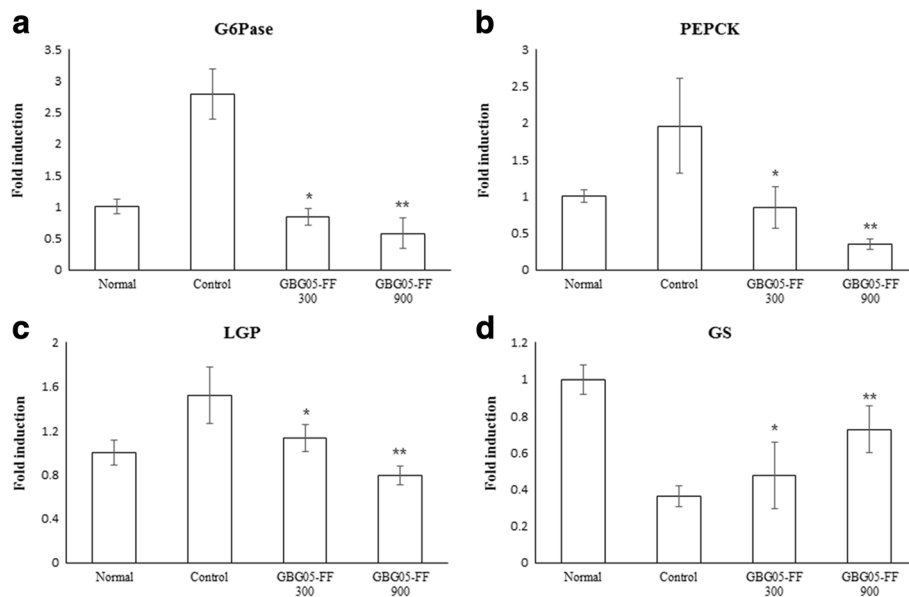


Fig. 7 Effects of GBG05-FF on the expression of G6Pase (a), PEPCK (b), LGP (c) and GS (d) in the liver of mice. Normal: dbh, Control: db/db, GBG05-FF 300: db/db fed the GBG05-FF 300 mg/kg, and GBG05-FF 900: db/db fed the GBG05-FF 900 mg/kg. Each value represents the mean \pm SEM ($n = 10$ per group) in three independent experiments. * $P < 0.05$, ** $P < 0.01$: Significant difference vs. control group

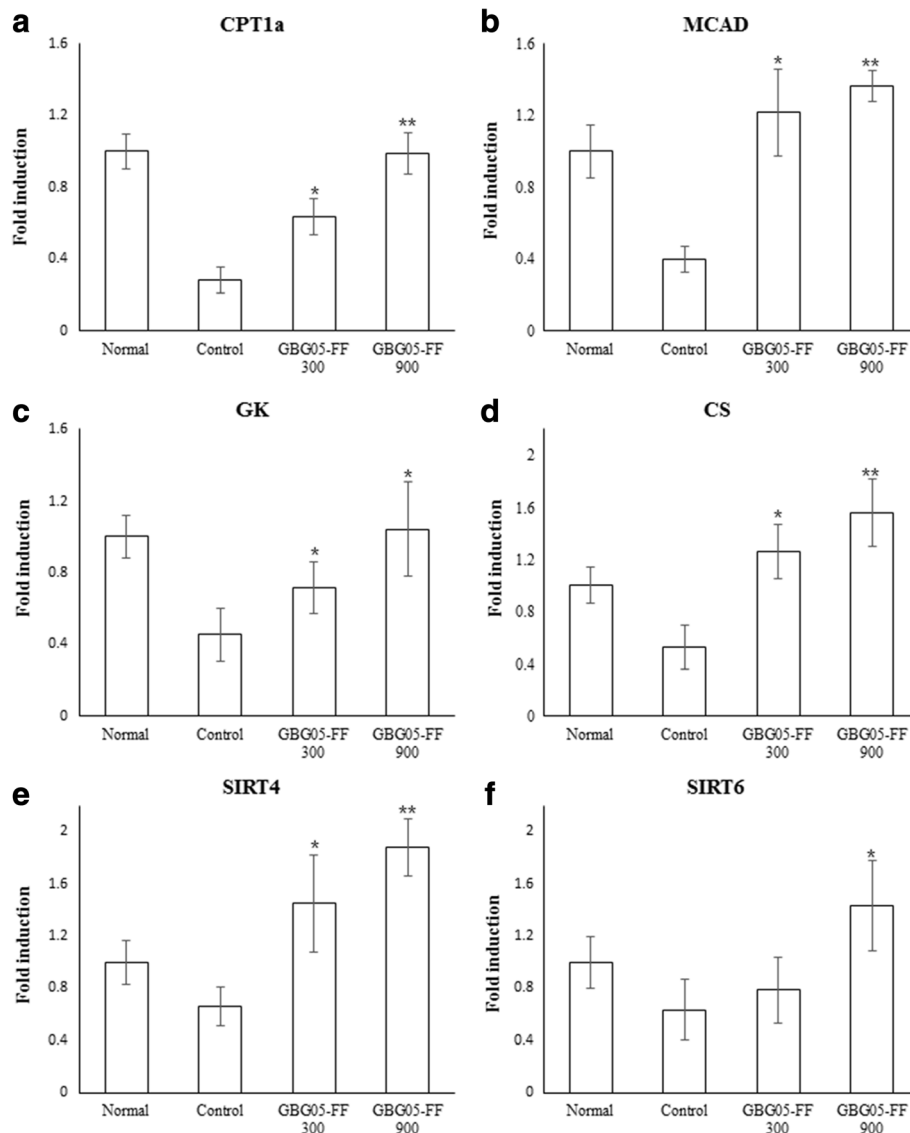


Fig. 8 Effects of GBG05-FF on the expression of CPT1a (a), MCAD (b), GK (c), CS (d), SIRT4 (e) and SIRT6 (f) in the muscle of mice. Normal: dbh, Control: db/db, GBG05-FF 300: db/db fed the GBG05-FF 300 mg/kg, and GBG05-FF 900: db/db fed the GBG05-FF 900 mg/kg. Each value represents the mean \pm SEM ($n = 10$ per group) in three independent experiments. * $P < 0.05$, ** $P < 0.01$: Significant difference vs. control group

and GLUT4 in skeletal muscle and abdominal fat of db/db mice compared to the control group. These results indicate that GBG05-FF effectively inhibited AMPK-regulated gluconeogenesis and enhanced glucose uptake in surrounding tissues by upregulating the expression of GLUT2 and GLUT4.

Conclusion

We have proved in this study that GBG05-FF regulates hyperglycemia and hyperlipidemia in mice. And the results confirmed that GBG05-FF possessed anti-diabetic activities in db/db mice. As a result of its fewer side effects, especially GBG05-FF has high potential in regulating glucose metabolism. Our data prove that GBG05-

FF has multiple targets in the hypoglycemic mechanism, such as suppressing gluconeogenesis through AMPK activation in the liver and affecting glucose uptake of surrounding tissues by upregulating the expression of GLUT2 in liver and GLUT4 in muscle. These findings suggest that black ginsengs might play an important role in the development of promising functional foods and drugs from the viewpoint of the chemical composition and biological activities of black ginsengs with a distinction from those of white and red ginsengs.

Abbreviations

AMPK: 5'adenosine monophosphate-activated protein kinase; ANOVA: One-way analysis of variance; CHO: Cholesterol; CPT1a: Carnitine palmitoyltransferase 1a; CS: Citrate synthase; DAB: 3,3-Diaminobenzidine

tetrahydrochloride; G6pase: Glucose 6-phosphatase; GBG05-FF: Black ginseng ethanol extract; GK: Glucokinase; GLUT: Glucose transporter; HbA1c: Glycated hemoglobin; LGP: Liver glycogen phosphorylase; MCAD: Mitochondrial medium-chain acyl-CoA dehydrogenase; NAFLD: Nonalcoholic fatty liver disease; OGTT: Oral glucose tolerance test; PEPCK: Phosphoenolpyruvate carboxykinase; RT-PCR: Reverse transcription polymerase chain reaction; SPSS: Statistical Package for the Social Sciences; TG: Triglyceride

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Availability of data and materials

Data will not be available on a public repository because participants were not asked to consent for this.

Authors' contributions

Wonkwang-Oriental Medicines Research Institute did the biology part of the experimental work, while International Ginseng and Herb Research Institute did the experimental chemistry work. All authors took part in planning the study design, supervising the study design, contributed to the analysis and writing of the manuscript, edited the paper, reviewing it critically for important intellectual content and provided the final version. Two authors shared co-first authorship. All authors contributed to and have approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

"Not applicable" in this section.

Ethics approval

All the animal experiments were performed in accordance with the guidelines established by the Animal Ethics Committee of Wonkwang University. This experiment was approved by the committee (Approval No. WKU15-101).

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