

## Research Article

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# Anti-Diabetic Effect of Pectinase-Processed Ginseng Radix (GINST) in High Fat Diet-Fed ICR Mice

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In the present study, we investigate anti-diabetic effect of pectinase-processed ginseng radix (GINST) in high fat diet-fed ICR mice. The ICR mice were divided into three groups: regular diet group, high fat diet control group (HFD), and GINST-treated group. To induce hyperglycemia, mice were fed a high fat diet for 10 weeks, and mice were administered with 300 mg/kg of GINST once a day for 5 weeks. Oral glucose tolerance test revealed that GINST improved glucose tolerance after glucose challenge. Compared to the HFD control group, fasting blood glucose and insulin levels were decreased by 57.8% ( $p < 0.05$ ) and 30.9% ( $p < 0.01$ ) in GINST-treated group, respectively. With decreased plasma glucose and insulin levels, the insulin resistance index of the GINST-treated group was reduced by 68.1% ( $p < 0.01$ ) compared to the HFD control group. Pancreas of GINST-treated mice preserved a morphological integrity of islets and consequently having more insulin contents. In addition, GINST up-regulated the levels of phosphorylated AMP-activated protein kinase (AMPK) and its target molecule, glucose transporter 4 (GLUT4) protein expression in the skeletal muscle. Our results suggest that GINST ameliorates a hyperglycemia through activation of AMPK/GLUT4 signaling pathway, and has a therapeutic potential for type 2 diabetes.

**Keywords:** *Panax ginseng*, Pectinase-processed ginseng radix, High fat diet, AMP-activated protein kinase, Glucose transporter type 4

## INTRODUCTION

Type 2 diabetes, a complex metabolic disease, is increasing rapidly with higher rates of morbidity and mortality [1,2]. It is characterized by insulin resistance and hyperglycemia, which lead to chronic complications such as diabetic nephropathy, neuropathy, and retinopathy [3]. Insulin resistance is a key defect underlying type 2 diabetes [4]. Insulin resistance in skeletal muscle is manifested by decreased insulin-stimulated glucose uptake and results from impaired insulin signaling and multiple post-receptor intracellular defects including impaired glucose transport, glucose phosphorylation, and reduced glucose oxidation and glycogen synthesis [5]. Therefore, numer-

ous studies have focused on development of therapeutic agents able to maintain normal levels of blood glucose by increasing glucose clearance in peripheral tissues such as skeletal muscle and adipose tissue [6,7].

Ginseng is a commonly used traditional Chinese medicine. The root of ginseng has been used for treatment of various diseases in Asian countries for thousand years. Modern pharmacological research has found that ginseng exerts anti-diabetic, anti-cancer, anti-inflammatory, and anti-oxidant effects [8-11]. The main bioactive components in ginseng are ginsenosides. Hitherto, ginsenosides Rb<sub>1</sub>, Rg<sub>1</sub>, Rg<sub>3</sub>, Rh<sub>2</sub>, Re, and IH-901 have been docu-

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mented in many studies and found the anti-diabetic effects of ginsenosides [12-16]. To develop safer and more effective anti-diabetic agents from ginseng radix, ginseng radix was treated with pectinase enzyme and obtained pectinase-processed ginseng radix (GINST). Here, we investigate the anti-diabetic activity and action mechanism of GINST using ICR mice fed a high fat diet.

## MATERIALS AND METHODS

### Chemicals

Pectinase was purchased from the DSM food specialties (ZAE La Baume, Servian, France). Antibodies against AMP-activated protein kinase (AMPK), phospho-AMPK, acetyl-CoA carboxylase (ACC), phospho-ACC, glucose transporter 4 (GLUT4) were from Cell Signaling Technology (Beverly, MA, USA), and anti-actin, anti-insulin, and anti-goat IgG-HRP were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Protein extraction and western blotting detection kits were from Intron Biotechnology Inc. (Beverly, MA, USA). Bio-Rad protein assay kit was from Bio-Rad Laboratories (Hercules, CA, USA). Polyvinylidene difluoride membranes were from Millipore (Bedford, MA, USA). Other reagents and chemicals were of analytical grade.

### Preparation of pectinase-processed ginseng radix

GINST was obtained from Ilhwa Co. Ltd. (Guri, Korea). Briefly, the ginseng radix (containing with 30% to 40% moisture) was extracted from 40% to 50% ethanol and concentrated with a speed-vac, then incubated with an enzyme solution containing pectinase at 55°C for 24 h.

### Analysis of ginsenosides in pectinase-processed ginseng radix

An Acquity liquid chromatograph (Waters, Milford, MA, USA) equipped with gradient pump, autosampler, and diode array detection was used. An Acquity UPLC BEH C18 reversed-phase column (100×1.0 mm, i.d., 1.7 μm) was used. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B). The gradient elution was used as follow: 0-3 min, 5% B; 10 min, 15% B; 12 min, 30% B; 15 min, 35% B; 20 min 60% B. The column temperature was kept constant at 35°C, and the flow rate was 0.5 mL/min.

### Animals

Five-week-old ICR mice were purchased from Orient Bio (Seoul, Korea). All animals were acclimatized to the laboratory environment for 1 week before the experi-

ment. Mice were allowed to freely access to drinking water and food under constant room temperature (22±2°C) and humidity (50±10%) conditions with an automatic 12 h light and dark cycle and experimental protocol was approved by the Institutional Animal Ethics Committee of the Kyung Hee University. Mice were randomly divided into three groups as following; group fed a regular diet (RD), group fed a high fat diet (HFD; Research diet, New Brunswick, NJ, USA), group treated with 300 mg/kg of GINST (GINST). In the treatment group, mice were orally administered once a day for 5 wk after freely access to HFD for 5 wk.

### Oral glucose tolerance test

At the end of the experiment, the ICR mice were fasted for 12 h prior to the experiment. Glucose (1.5 mg/kg) was orally administered at 0 min, and the blood was withdrawn from the orbital venous plexus at 0, 30, 60 and 90 min after glucose administration. Plasma level was determined by the glucose oxidase method [13].

### Determination of serum parameters

At the end of treatment, mice were anaesthetized by diethyl ether and blood samples were collected by cardiac puncture. Blood samples were centrifuged at 3,000×g for 15 min at 4°C, and plasma glucose level was determined using commercial kit (Stanbio Laboratory, Boerne, TX, USA) and automatic analyzer (SMARTLAB, Mannheim, Germany). The plasma insulin concentration was determined using a mouse insulin enzyme immunoassay kit (Shibayagi, Gunma, Japan).

### Histological analysis

For hematoxylin-eosin staining, the pancreas tissue was removed and fixed in 10% neutral buffered formalin. The tissues were subsequently embedded in paraffin and sectioned with 5 μm thickness (Leica, Wetzlar, Germany), and stained with hematoxylin-eosin for microscopic assessment (Olympus, Tokyo, Japan). To examine the insulin contents in pancreas, immunohistochemistry technique was used. The sections were deparaffinized in xylene and rehydrated through a graded ethanol series. Antigen retrieval was performed by 0.1% trypsin. To block nonspecific binding of immunoglobulin, the sections were incubated with normal serum blocking solution for 30 min at room temperature. Goat anti-insulin IgG (1:75) were applied overnight at 4°C, then the tissue sections were incubated with donkey anti-goat IgG-HRP (1:200) for 30 min at room temperature. Positive control was visualized DAB peroxide substrate solution for 5 to 10 min,

and tissues were counterstained with hematoxylin.

### Western blot analysis

Total protein extracts were prepared using a protein extraction kit and insoluble protein was removed by centrifugation at 13,000 g for 20 min. Protein concentrations in cell lysates were measured using a Bio-Rad protein assay kit. For Western blotting, 40 µg of protein was separated by 8% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. The membrane was further incubated with the indicated primary antibody, followed by secondary antibody conjugated with horseradish peroxidase. Protein bands were detected using an enhanced chemiluminescence Western blotting detection kit and then exposed to X-ray film.

### Statistical analysis

Results were represented as the mean±SE. Comparison between groups was made by ANOVA and analyzed by post-hoc test. Differences of  $p < 0.05$  were considered to be statistical significant.

## RESULTS AND DISCUSSION

### Effect of pectinase on ginsenosides profile

Ginseng is a well-known medicinal plant widely used in oriental societies as one of the most valuable medicines. Ginseng has a glucose lowering effect probably

due to increasing insulin secretion, ameliorating insulin resistance or both. However, anti-diabetic efficacy of ginseng is not remarkable and often ambiguous, and thus most of physician would hesitate to recommend the ginseng to diabetic patients as a supplement. Therefore, in a series of investigations to develop anti-diabetic agent with higher efficacy, ginseng radix was processed with pectinase enzymes. When ginsenoside profile was analyzed by ultra performance liquid chromatography, ginseng radix and GINST showed quiet different profiles and therefore differential effects were expected. As shown in Fig. 1, the saponin peaks in untreated ginseng radix (ginsenoside Rb<sub>1</sub>, Rc, Rb<sub>2</sub> and Rd) were decreased during the enzyme treatment. One day after pectinase treatment, these four ginsenosides were difficult to identify in the chromatogram. On the other hand, IH-901 was appeared during the enzyme process (Fig. 1B). IH-901 is a final metabolite of ginsenoside and may be one of active components for pharmacological activity of GINST. Although *Panax ginseng* is known to have anti-diabetic activity, the active ingredient is not yet fully identified. Recently, we reported that IH-901 is the one to decrease the fasting blood glucose levels in C57BL/KsJ db/db mice *via* enhancing insulin secretion and improving insulin resistance [13,17]. Having these results we attempt to explore whether GINST shows anti-diabetic effect in ICR mice fed a high fat diet [18,19].

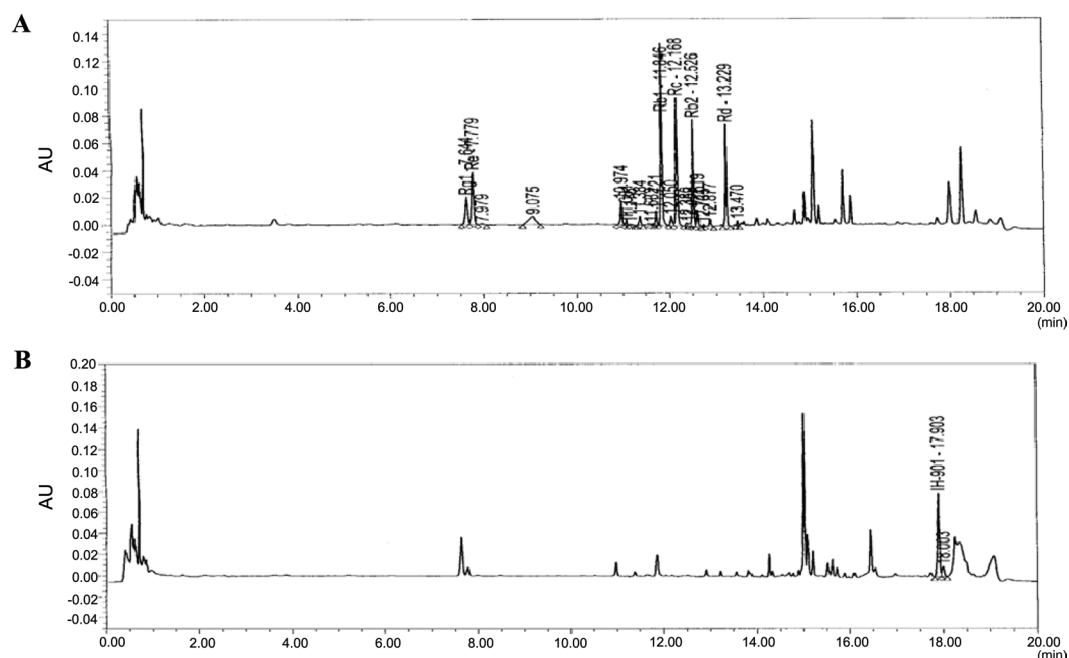
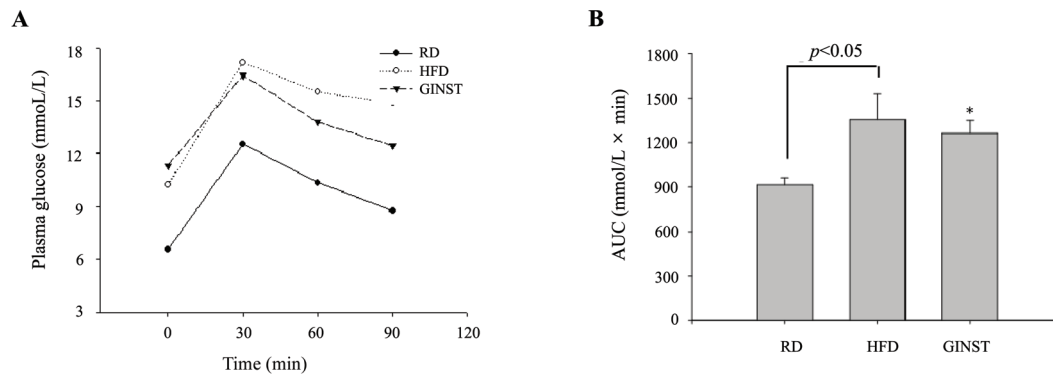


Fig. 1. Ultra performance liquid chromatography profiles of ginseng radix (A) and pectinase-processed ginseng radix (B).



**Fig. 2.** Plasma glucose responses to an oral glucose challenge (1.5 g/kg) after 12 h food deprivation in ICR mice. Plasma glucose response to an oral glucose challenge (A), and the area under the curve of plasma glucose concentration versus time (B). Values are mean±SE ( $n=5$ ) and \* $p<0.05$  compared to high fat diet control group. RD, regular diet; HFD, high fat diet; GINST, pectinase-processed ginseng radix.

**Table 1.** Effects of pectinase-processed ginseng radix (GINST) on body weight and metabolic parameters

|                 | RD        | HFD                    | GINST      |
|-----------------|-----------|------------------------|------------|
| Body weight (g) | 38.16±1.3 | 43.2±1.4 <sup>##</sup> | 41.2±1.6*  |
| Glucose (mM)    | 6.3±0.5   | 15.2±3.4 <sup>##</sup> | 6.4±1.3**  |
| Insulin (U/ml)  | 18.7±1.4  | 33.3±2.4 <sup>##</sup> | 23.0±2.9** |
| HOMA-IR         | 5.3±0.7   | 22.6±5.0 <sup>##</sup> | 7.2±2.2**  |

Data are mean±SE ( $n=5$ ). Homeostasis model assessment was used to calculate an index of insulin resistance as insulin ( $\mu\text{U}/\text{mL}$ ) $\times$ glucose (mM)/22.5. \* $p<0.05$  and \*\* $p<0.01$  compared to high fat diet (HFD) control group. <sup>##</sup> $p<0.01$  compared to regular diet (RD) control group

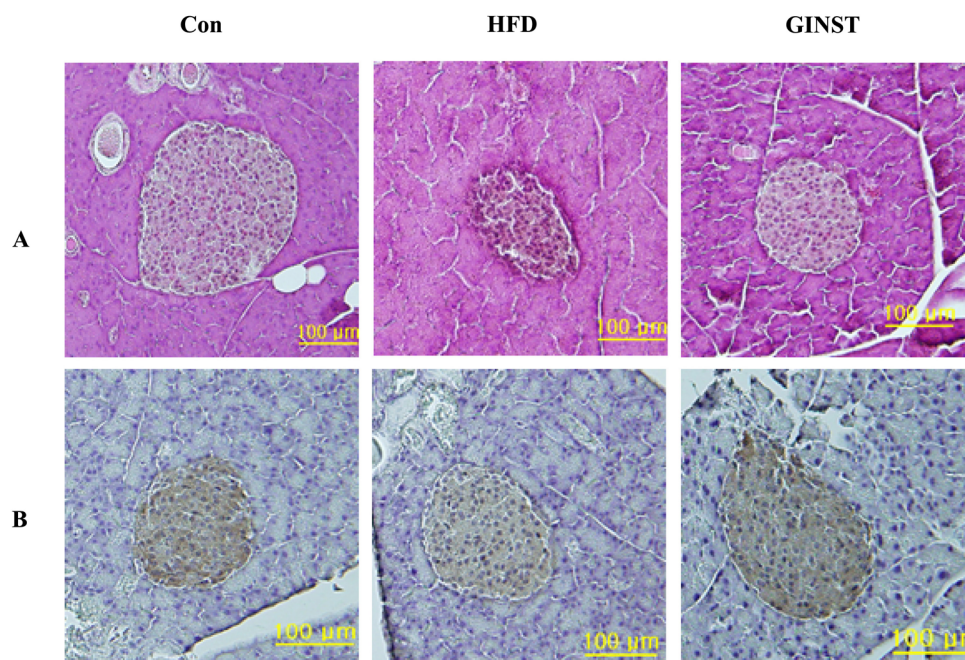
### Effects of GINST on hyperglycemia and insulin resistance induced by high fat diet

To determine the effect of multiple oral administration of GINST on glucose tolerance, Oral glucose tolerance test was carried out at the end of the experiment. As shown in Fig. 2, glucose challenge significantly increased the blood glucose levels in the HFD control group, whereas GINST-treated group significantly suppressed the blood glucose levels from rising during 90 min after glucose challenge (Fig. 2A). Compared to the HFD control group, area under the curve was reduced by 7% ( $p<0.05$ ) (Fig. 2B). Body weight and metabolic parameters related to diabetes are shown in Table 1, body weight was increased by 11.3% in the HFD control group compared to the RD group. When compared to HFD control group, final body weight was lowered by 4.6% in GINST group. Fasting plasma glucose, insulin and the insulin resistance index (HOMA-IR) [20] levels in GINST group were significantly decreased by 57.8% ( $p<0.05$ ), 30.9% ( $p<0.01$ ), and 68.1% ( $p<0.01$ ), respectively, compared to the HFD control group (Table 1). Histological analysis of the pancreas from the HFD control group revealed a

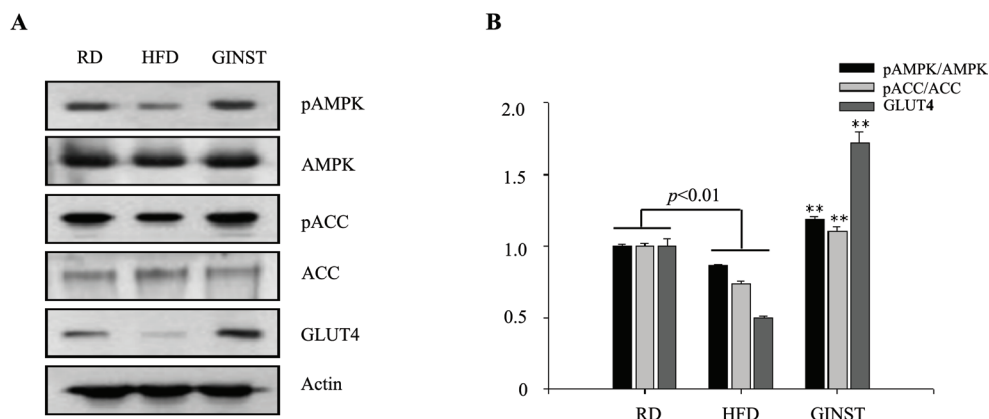
degeneration of islets, whereas mice treated with GINST preserved the islets architecture (Fig. 3A). In addition, insulin contents in GINST-treated group were restored when compared to the HFD control group (Fig. 3B). These results suggest that GINST may have beneficial effects on hyperglycemia and insulin resistance induced by HFD, and IH-901 might be responsible for these effects.

### Effects of GINST on protein expression of AMPK and GLUT4

Resistance to the insulin action in skeletal muscle is a major risk factor in type 2 diabetes mellitus [5,21]. Skeletal muscle plays a crucial role in maintaining systemic glucose metabolism, accounting for 85% of whole body insulin-stimulated glucose uptake [22]. In skeletal muscle, insulin stimulates glucose uptake by increasing the translocation of glucose transport molecules, mainly GLUT4, from intracellular vesicles to the cell surface [23]. Therefore, elucidation of the signaling pathways governing contraction-induced increases in skeletal muscle glucose uptake may provide new pharmacological targets for the treatment of individuals with type 2 diabetes. AMPK is one of the most important factors for cellular energy balance and is recognized as a potential therapeutic target in the prevention and treatment of type 2 diabetes [24-26]. AMPK is emerging as a signaling intermediary that controls the use of glucose and fatty acids in skeletal muscle [27,28]. Increased GLUT4 expression or translocation to the plasma membrane can be regulated by the activation of AMPK through an insulin-independent mechanism [29]. Thus, in the present study, we examined whether GINST activates AMPK through phosphorylation in the skeletal muscle. As shown in Fig. 4, GINST stimulates the phosphorylation of AMPK in the



**Fig. 3.** Effect of pectinase-processed ginseng radix on pancreas morphology. Panel A represents H&E staining and panel B represents insulin immunostaining ( $\times 200$ ). Con, control; HFD, high fat diet; GINST, pectinase-processed ginseng radix.



**Fig. 4.** Effects of pectinase-processed ginseng radix on pAMPK, pACC, and GLUT4 protein expression measured via Western blot analyses (A) and their densitometric analyses (B). RD, regular diet; HFD, high fat diet; GINST, pectinase-processed ginseng radix.

skeletal muscle. GLUT4 protein expression in the skeletal muscle was also markedly enhanced in GINST-treated group. Taken together, these results suggest that GINST lowered plasma glucose level through ameliorating insulin resistance via AMPK signal pathway in the skeletal muscle of high fat diet-fed ICR mice, and GINST can be a health functional food or therapeutic agent for type 2 diabetic patients.

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