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Wild Ginseng Prevents the Onset of High-Fat Diet Induced Hyperglycemia and Obesity in ICR Mice

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Ginseng is a shade-loving perennial herb that is cultivated mainly in Korea, Japan, and China. The ginseng root has been used as a tonic remedy, and its antidiabetic activity has been demonstrated as early as 1920s. Although wild ginseng was anecdotally thought to be superior to cultivated ginseng as far as pharmacological properties were concerned, there have been no prior reports on the antidiabetic effect of wild ginseng. In this study, we investigated the preventative anti-diabetic and anti-obese effects of wild ginseng ethanol extract (WGEE). In the preventive experiment, WGEE co-administered with a high fat diet significantly inhibited body weight gain, fasting blood glucose, triglyceride, and free fatty acid levels in a dose dependent manner. WGEE-treated mice at doses of 250 and 500 mg/kg improved the insulin resistance index by 55% and 61% compared to the high fat diet (HFD) control, respectively. Diameters of white and brown adipocytes were also decreased by 62% and 46% in the WG500-treated group compared to those in HFD fed control mice. Taken together, WGEE has potential as a preventive agent for type 2 diabetes mellitus (and possibly obesity) and deserves clinical trial in the near future.

Key words: Wild ginseng ethanol extract, Anti-diabetic effect, Anti-obese effect, High fat diet, Insulin resistance index

INTRODUCTION

Type 2 diabetes is characterized by high concentrations of glucose in the blood, which is caused by decreased secretion of insulin from the pancreas and decreased insulin action (Cavaghan *et al.*, 2000). This condition is prevalent worldwide and is associated with morbidity and mortality, which are secondary to complications such as myocardial infarction, stroke, and end-stage renal disease. The importance of the tight control of blood glucose in either preventing or delaying the progression of complications is recognized (The Diabetes Control and Complication Trial Research group, 1993; UK Prospective Diabetes Study Group, 1998). Currently available pharmacological agents for type 2 diabetes have a number of limitations, such as adverse effects and high rates of secondary failure (Inzucchi, 2002). Due to these factors, diabetic patients and healthcare professionals are increasingly considering complimentary and alternative approaches, including the use of medicinal herbs with antihyperglycemic activities (Attele *et al.*, 2002).

Ginseng is a low-growing, shade-loving perennial herb of the Araliaceae family, which is cultivated in Korea, China, Japan, and Russia, as well as in the United States and Canada. Ginseng has been used as a tonic remedy in traditional Chinese medicine for several thousand years (Chevallier, 2000). The pharmacological properties of ginseng are mainly attributed to ginsenosides, the active constituents that are found in the extracts of various species of ginseng (Attele *et al.*, 1999). The anti-diabetic properties of ginseng root extracts were demonstrated by Japanese scientists as early as the 1920s (Wang, 1965). Since then, the blood glucose-lowering effects of the ginseng root have been investigated frequently (Sotaniemi *et al.*, 1995; Vuksan *et al.*, 2001; Chung *et al.*, 2001). Although wild ginseng has anecdotally ascertained to be superior to cultivated ginseng in terms of pharmacological properties, there have been no prior reports on the antidiabetic effect of wild ginseng. In this study, the preventative anti-diabetic and anti-obese effects of wild

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ginseng ethanol extract (WGEE) were investigated.

MATERIALS AND METHODS

Plant materials

Wild ginseng (WG, 17-year-old) was obtained from Sansam Nara Corp. (Mapo-gu, Seoul, Korea) and was botanically identified by botanist Dr. Yook at the department of oriental pharmaceutical science, Kyung Hee University. The WG was extracted twice at 80°C in 50% ethanol for 6 h. The extract was then filtered, and the filtrate was concentrated with a vacuum rotary evaporator (EYELA, Japan) under low pressure. The residue was freeze-dried in a freezing dryer (EYELA, Japan) and was stored in a deep freezer until use.

Animals and treatment

Male, ICR mice were purchased at 5 week-old from the Biogenomics (Seoul, Korea) and were acclimatized for 1 week before being randomly assigned into the study groups. The animals were housed in individual cages with free access to water in a temperature-controlled facility with a 12:12-h light-dark cycle, and the animals were weighed periodically. During the acclimatization period, each animal was raised on a regular diet (Dyets Inc., Bethlehem, PA) *ad libitum*. At the age of 6 weeks, the ICR mice were randomly divided into five groups; two controls groups and three treatment groups. The control mice continued to receive either a regular diet (RD) or high-fat diet (HFD), and the treatment groups were fed a high-fat diet with either 250 mg/kg or 500 mg/kg of WG (WG250 and WG500) for a 8-week period. As a positive control, metformin was administered with the high-fat diet at a dose of 500 mg/kg (MET500). Hyperglycemia induction diet was purchased from Dyets Inc. (AIN-76 diet #101772, Bethlehem, PA, USA). The nutrition contents of the high fat diet were similar to those of the regular diet except low carbohydrate and the addition of beef tallow (Table I). Body weights were measured weekly, and every other week, blood was collected for blood glucose analysis. At the end of the study, blood was also collected for the determination of plasma insulin and lipid levels, after which they were killed, and brown adipose tissue (BAT) and white adipose tissue (WAT) were immediately removed from interscapular and periepididymal fats, respectively, for morphological examination and mRNA analysis.

Blood sampling and plasma assay

Blood was withdrawn from the orbital venous plexus every other week, using a heparinized capillary tube without anesthesia. The blood samples were placed on ice, centrifuged, and plasma stored at -20°C until assayed. The plasma glucose concentration was determined using the glucose oxidase method (Youngdong Pharmaceutical Co, Korea). The plasma insulin concentration was measured according to the protocol described by the manufacturer of the mouse insulin ELISA kit (Shibayagi Co., Japan). Plasma triglyceride, total cholesterol, HDL cholesterol, and free fatty acid concentrations were determined using commercially available kits (Asan and Youngdong Pharmaceutical Co., Korea).

Table I. Composition of the diets

	Regular diet (g/kg diet)	High fat diet (g/kg diet)
Ingredients		
Casein	200	200
DL-Methionine	3	3
Corn Starch	150	150
Sucrose	500	150
Cellulose	50	-
Corn oil	50	-
Beef tallow	-	400
Mineral mixture[1]	35	35
Vitamin mixture[1]	10	10
Choline bitartrate	2	2
Energy, *kJ/g*	0.9	1.30
Protein, *% kcal/kg*	13.3	13.3
Carbohydrate, *% kcal/kg*	47.4	19.8
Fat, *% kcal/kg*	8.0	65.7
Fiber, *% kcal/kg*	8.0	-
Other	23.3	1.3

[1] AIN 76A Rodent Purified Diet

Oral glucose tolerance test

An oral glucose tolerance test (OGTT) was performed at the end of the treatment. On the test day, animals were fasted for 9 h, and then, glucose (1.5 g/kg) was orally administered to them. Blood glucose and insulin levels were determined from the orbital venous plexus at 0 (before glucose challenge), 30, 60, and 120 min after glucose administration.

Histology and microscopy

After fasting overnight, the mice were deeply anesthetized with urethane (0.9 mL/100 g body weight of 20% solution) and were perfused transcardially with 10% buffered formalin. The mice were killed by decapitation, and BAT and WAT were removed from interscapular and periepididymal fat pads, respectively, and were subsequently embedded in paraffin. Paraffin sections with a thickness of 5 μm were prepared using a microtome (American Optical Company, USA), and these sections were mounted on double-gelatin coated slides. The tissue sections were deparaffinized in 0.01 mol/L citrate buffer (pH 6.0) and then stained with hematoxylin and eosin. Micrographs were taken at ×100 magnification.

RNA extraction and RT-PCR

The total RNA from white adipose tissue was prepared using easy-BLUE (Intron Co., Korea) according to the manufacturer's instructions. One μg of total RNA was reverse transcribed into cDNA using the Moloney murine leukemia virus reverse transcriptase and random hexamers (Promega, USA) as primers. The specific primers were directed against the rat sequence for GLUT-4 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (GLUT4: sense primer 5'-ACA GAA GGT GAT TGA ACA GAC-3', antisense primer 5'-ACC CGT CCA AGA ATG AGT ATC-3' and GAPDH: sense primer 5'-GGA AAG ACA ACG GAC AAA TC-3', antisense primer 5'-GTC ATC TTC TGG AGC ACC TT-3'). The primers were added at a final concentration of 0.5 μM to a 25 μL reaction mixture containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM each dNTP, 0.5-1.5 μL of cDNA, and 2.5 units of Taq DNA polymerase (TaKaRa medicals, Japan).

The PCR conditions were denaturation at 95°C for 40 seconds, annealing at 59°C for 40 seconds, and extension at 72°C for 40 seconds. Initial heating at 95°C for 5 min and final extension at 72°C for 10 min were performed. The RT-PCR product was size fractionated on a 2% agarose gel and was stained with 0.5 μg/mL ethidium bromide. The PCR product of GLUT-4 consisted of 285 base pairs, and GAPDH was amplified as a control gene. The density of the PCR product was measured using a GS-700 imaging densitometer. The level of mRNA was expressed as the ratio of signal intensity for the GLUT-4 gene relative to that of GAPDH.

Statistical analysis

All data were expressed as a mean±S.E.M. For multiple comparisons, an analysis of variance (ANOVA) was carried out, followed by Fishers protected least significant difference test as a post hoc test (StatView, SAS Institute, Cary, USA). A value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Weight gain and feed efficiency

Body weight and food intake were determined once a week. The body weight of the normal mice in the RD group gradually increased as the mice grew during the 8-week trial. In contrast, the body weight of animals on the HFD showed rapid increases during the course of the trial (Fig. 1). Weight gains in RD and HFD control groups during the 8-week period were 11.5±0.3 g and 23.4±0.4 g, respectively (Table II). Subjects fed the HFD and WGEE showed a gradual increase in body weight, but the increase was significantly less than that detected for the HFD control group in spite of continued and prolonged access to the high fat diet (Fig. 1 and Table II). WG250 and WG500 prevented the weight gain by 11% and 16%, respectively, compared to the body weight of the HFD control group. Feed efficiency, calculated by weight gain divided by total food intake during the 8-week period, was compared in order to figure out the relationship between food intake and weight gain. As shown in Table II, weight

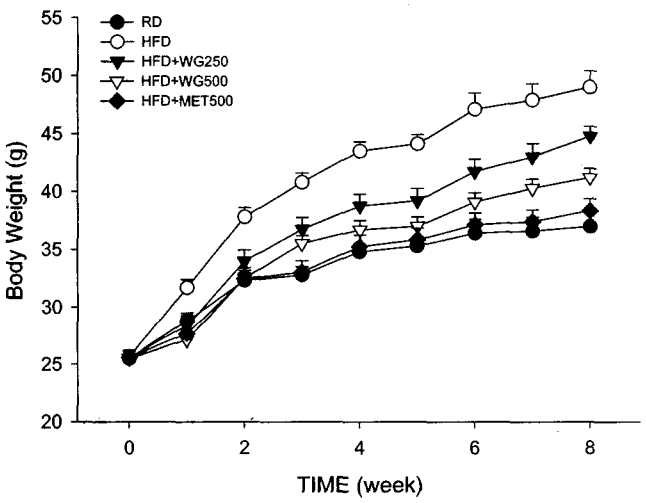

Fig. 1. Body weight of mice consuming regular diet, high fat diet, or high fat diet plus wild ginseng ethanol extract during the 8-week period. Values represent the mean ± SE (n=8). RD, mice consuming regular diet; HFD, mice consuming high fat diet; WG250, mice fed with high fat diet plus 250 mg/kg of wild ginseng ethanol extract; WG500, mice fed with high fat diet plus 500 mg/kg of wild ginseng ethanol extract; MET500, mice fed with high fat diet plus 500 mg/kg of metformin.

Table II. Effect of wild ginseng ethanol extract on weight gain, food intake, and feed efficiency

Group	Dose (mg/kg)	Initial	Final	Weight gain (g/8 wk)	Food intake (g/8 wk)	Feed efficiency ($\times 10^{-3}$)
RD	-	25.5 ± 0.6	37.0 ± 1.3***	11.5 ± 0.3***	1770	6.5
HFD	-	25.6 ± 0.6	49.0 ± 1.4†††	23.4 ± 0.4†††	2483†††	9.4†††
HFD+WG	250	25.0 ± 0.1	43.8 ± 1.2***	18.8 ± 0.3***	2636	7.1***
HFD+WG	500	25.5 ± 0.7	41.2 ± 0.8***	15.7 ± 0.2***	2589	6.1***
HFD+MET	500	25.6 ± 0.1	38.4 ± 0.9***	12.8 ± 0.1***	1800	7.1***

Values represent the mean±SE (n=8). †††P< 0.001 vs. RD; ***P < 0.001 vs. HFD
Feed efficiency = [weight gain (g/8 wk)]/[food intake (g/8 wk)]

gain of the HFD control mice was actually due to the increased food intake. However, body weights of WGEE fed mice were significantly reduced despite the even larger increase in food intake compared to the HFD control mice. Feed efficiency of the WG500 fed group was 6.1, which is lower than the value shown for the HFD control group, indicating that WG could be a fascinating drug that allows patients to slim down despite an increase in food intake or reduction of physical activity. On the other hand, body weight of metformin fed mice was similar to that of RD fed mice mainly due to a significant reduction of food intake compared to the HFD control mice.

Insulin resistance index

Plasma glucose was determined every other week and was compared between groups in Fig. 2. Plasma glucose levels were barely increased in the RD fed control group, while a marked increase after the 4th week was observed for mice only fed with the HFD (Fig. 2). WGEE fed mice, however, showed a significant decrease in blood glucose levels in a dose dependent manner when compared to the HFD control mice (Table III). The insulin resistance index, calculated by insulin (μU/mL) × glucose (mM)/22.5, of the HFD control group was 3.3 times higher than that of the RD group, while insulin resistance indices of WG250 and WG500 were significantly reduced by 55% and 61%, respectively, when compared to the HDF control group (Table III). Improvement of insulin resistance in the WG500 fed group was significant and comparable to the MET500 fed group. This result suggests that wild ginseng was able to lower the blood glucose level partially due to the improvement of insulin resistance.

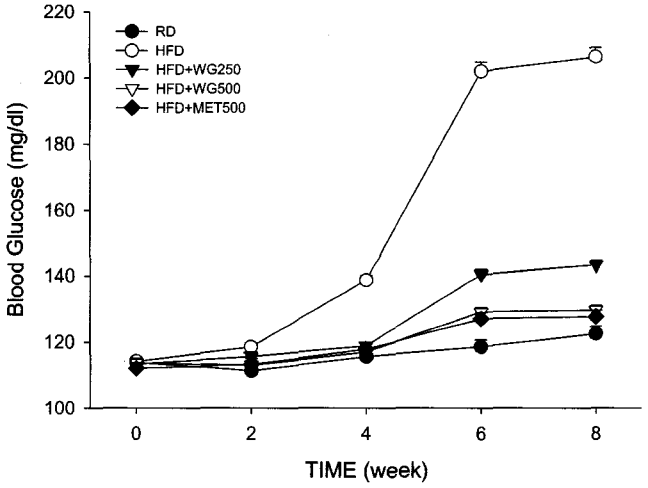

Fig. 2. Plasma glucose levels of regular diet, high fat diet, or high fat diet plus wild ginseng ethanol extract during the 8-week period. Values represent the mean ± SE (n=8). RD, mice consuming regular diet; HFD, mice consuming high fat diet; WG250, mice fed with high fat diet plus 250 mg/kg of wild ginseng ethanol extract; WG500, mice fed with high fat diet plus 500 mg/kg of wild ginseng ethanol extract; MET500, mice fed with high fat diet plus 500 mg/kg of metformin.

Table III. Effect of wild ginseng ethanol extract on plasma insulin, plasma glucose (PG), and insulin resistance index (ISI)

Group	Dose (mg/kg)	Insulin (μU/ml)	PG (mM)	IRI
RD	-	76.3 ± 1.7***	6.8 ± 0.1***	23.1
HFD	-	147.3 ± 2.4†††	11.5 ± 0.2†††	75.1†††
HFD+WG	250	96.0 ± 2.0***	8.0 ± 0.1***	34.0***
HFD+WG	500	91.2 ± 1.9***	7.2 ± 0.1***	29.2***
HFD+MET	500	86.5 ± 1.9***	7.1 ± 0.1***	27.3***

Homeostasis Model Assessment was used to calculate an index of insulin resistance as insulin (μU/mL) x glucose (mM) / 22.5. Values represent the mean ± SE (n=8). †††P< 0.001 vs. RD; ***P < 0.001 vs. HFD

Oral glucose tolerance test (OGTT)

After the 8-week administration of WGEE, OGTT was performed. Glucose challenge dramatically increased the blood glucose levels in HFD fed mice compared to those in RD fed mice, while WGEE treated groups significantly prevented the blood glucose levels from rising, especially at the 30 min time point (Fig. 3A). When the area under the curve (AUC) was compared between groups, WG250- and WG500-treated groups respectively showed 38% and 41% reductions in the area under the curve compared to that in the HFD fed control mice. The insulin response during the OGTT was considerably increased in HFD fed control mice compared to that in RD fed mice, which indicates insulin resistance (Fig. 3B). Plasma insulin levels in WG250 and WG500-treated groups were also markedly decreased by 40% and 52%, respectively.

Plasma lipid levels

The effects of WGEE on plasma lipid levels were examined at the end of the treatment. The plasma lipid levels in HFD fed mice were dramatically increased compared to the levels in RD fed mice except for the HDL-cholesterol (HDL-C) level, which is supposed to be higher than 40 mg/dL for males and 50 mg/dL for females (Table IV). In the HFD control group, plasma triglyceride (TG) was increased by 1.7-fold (106 to 181 mg/dL), LDL-cholesterol increased by 4.2-fold, free fatty acid increased by 2.0-fold, and total cholesterol (TC) increased as reflected in the increase in LDL-cholesterol (LDL-C) concentration compared to those in the RD group. WG250- and WG500-treated groups, however, showed considerably reduced levels of TG, TC, LDL-C, and free fatty acid (15%, 32%, 50%, and 32% inhibition in the WG500 fed group), while they showed an increased level of HDL-C compared to that in HFD fed control mice (164% in WG500 fed group) in a dose dependent manner. Metformin remarkably

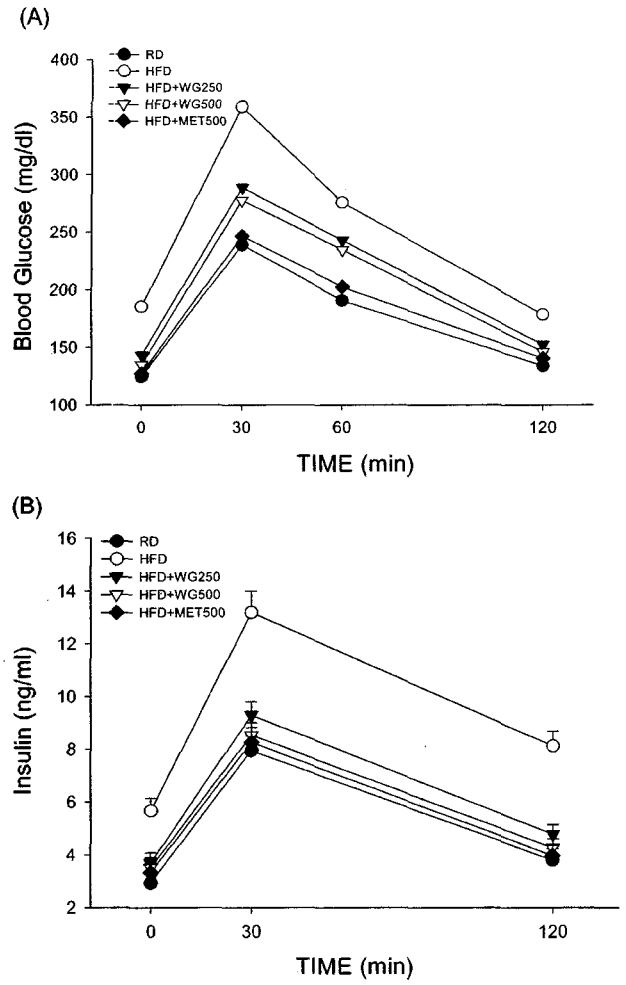

Fig. 3. Plasma glucose (A) and insulin (B) responses to an oral glucose challenge (1 g/kg) after 9 h of food deprivation at week 8 in high fat and regular diet fed mice. Values represent the mean ± SE (n=8). RD, mice consuming regular diet; HFD, mice consuming high fat diet; WG250, mice fed with high fat diet plus 250 mg/kg of wild ginseng ethanol extract; WG500, mice fed with high fat diet plus 500 mg/kg of wild ginseng ethanol extract; MET500, mice fed with high fat diet plus 500 mg/kg of metformin.

improved high fat diet induced dyslipidemia, and all lipid-related plasma parameters in metformin fed mice were comparable to those in RD fed mice.

Fat mass and morphology

The body weight of HFD fed mice increased by 32% compared to the RD fed control mice. Notably, there were 3.1- and 1.8-fold increases in the masses of WAT and BAT of HDF mice, respectively, compared to their respective controls. Histological analysis of WAT and BAT further confirmed this result and indicated that the increase in the HFD white and brown adipocytes size [4.9- and 7.2-fold (cell diameter); Fig. 4] results mainly from the accumulation of lipids. The diameters of white and brown adipocytes decreased by 62% and 46% in the WG500-treated group (Table V).

GLUT-4 mRNA expression

At the end of the treatment, the semi-quantitative analysis of glucose transporter-4 (GLUT-4), which mediates the rate limiting step of glucose utilization in skeletal muscles and white adipose tissue, was performed using RT-PCR. It is very unlikely that GLUT-4 reduction in adipose tissue would be established in a 14-week old HFD fed mice when glucose utilization and the rate of lipid accretion was increased (Cousin *et al.*, 1992). In fact, our results showed that the white adipose tissue of HFD control mice showed a 3-fold increase in the GLUT-4 mRNA level (Fig. 5), while GLUT-4 contents in the WG fed group were significantly reduced compared to that of the HFD control mice in a dose dependent fashion. The expression level of GLUT-4 mRNA in the WG500 fed group was similar to that of MET500 fed mice, which indicates the over expression of GLUT-4. Increased utilization of glucose and increased fat size caused by the high fat diet were ameliorated by down regulation of GLUT-4 shown in WGEE- and metformin-fed mice.

A survey of the phytochemistry of *Panax quinquefolius L.* (North American ginseng) collected from wild populations in Ontario, Quebec, Maine, Vermont, and Wisconsin was recently reported (Assinewe *et al.*, 2003). Reverse-phase HPLC was used to determine the natural variation levels of ginsenosides Rg1, Re, Rf, Rb1, Rc, Rb2, and Rd and their total in leaf, stem, and root of authentic wild-grown material. There was no statistical difference in mean

Table IV. Effect of wild ginseng ethanol extract on plasma lipid levels

Group	Dose (mg/kg)	TG (mg/dL)	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	NEFA (μEq/dL)
RD	-	106 ± 2	162 ± 4	85 ± 2	56 ± 3	695 ± 5
HFD	-	181 ± 3†††	308 ± 4†††	39 ± 1†††	233 ± 5†††	1362 ± 8†††
HFD+WG	250	162 ± 2***	221 ± 3***	61 ± 2***	128 ± 4***	944 ± 5***
HFD+WG	500	153 ± 2***	210 ± 2***	64 ± 1***	116 ± 4***	922 ± 5***
HFD+MET	500	116 ± 3***	172 ± 3***	78 ± 1***	71 ± 2***	724 ± 4***

Values represent the mean±SE (n=8). †††P< 0.001 vs. RD; **P<0.01, ***P < 0.001 vs. HFD
BG, blood glucose; TG, triglyceride; TC, total Cholesterol; HDL-C, HDL-cholesterol; LDLC, LDL- cholesterol; NEFA , nonesterified fatty acid. LDL-C (mg/dl) = TC-HDL-TG / 5

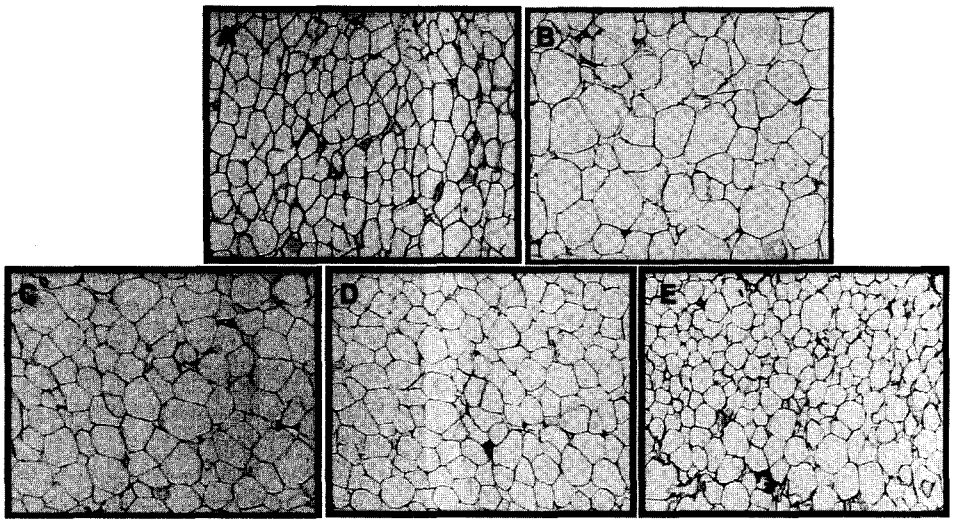

Fig. 4. HE staining of periepididymal fat. A, RD; B, HFD; C, HFD+ WG250; D, HFD+WG500; E, HFD+MET500.

Table V. Effect of wild ginseng ethanol extract on area and diameter of adipose tissues

Group	Dose (mg/kg)	Epididymal fat		Interscapular fat	
		Area (μm^2)	Diameter (μm)	Area (μm^2)	Diameter (μm)
RD	-	739.4 ± 11.7	36.9 ± 2.3	287.3 ± 6.1	17.3 ± 1.5
HFD	-	9283.2 ± 45.3†††	182.3 ± 4.2†††	4321.3 ± 32.2†††	121.9 ± 4.3†††
HFD+WG	250	4773.1 ± 24.3***	131.4 ± 2.3***	2638.3 ± 23.0***	94.2 ± 2.6***
HFD+WG	500	3553.4 ± 24.3***	88.9 ± 3.2***	2346.1 ± 20.7***	75.3 ± 2.6***
HFD+MET	500	1027.5 ± 11.3***	47.4 ± 2.9***	634.7 ± 8.3***	29.4 ± 2.1***

Values represent the mean±SE (n=8). †††P< 0.001 vs. RD; ***P < 0.001 vs. HFD

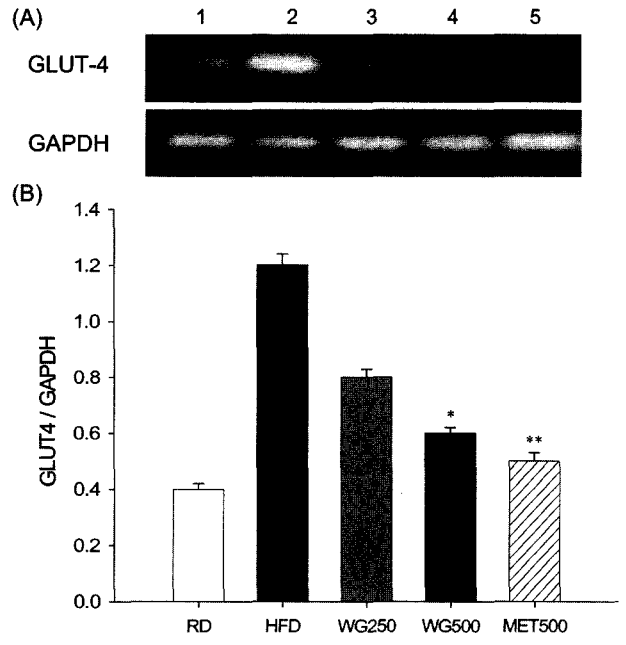

Fig. 5. Expression of GLUT-4 mRNA in WAT. (A) A representative RT-PCR of GLUT-4 and GAPDH. Lane 1: RD; Lane 2: HFD; Lane 3: HFD+WG250; Lane 4: HFD+WG500, Lane 5: HFD+MET500 (B) Densitometric quantitation of GLUT-4 expression normalized by GAPDH. **P<0.01, *P<0.05 *vs.* HFD.

ginsenoside content between wild and cultivated *P. quinquefolius* roots at 4 years of age. Our preliminary study, however, showed that there are significant deifferences in ginsenoside profiles between wild and cultivated Korean ginseng (data not shown). One of the striking differences was that the ratio of protopanaxadiol (PPD) and protopanaxatriol (PPT) was higher in wild ginseng ethanol extract than that in cultivated ginseng (2.35 vs. 1.81), which suggests that there is phytochemical and possibly pharmacological justifications for wild crafting. We do not yet know whether antidiabetic and antiobesity activities of wild ginseng ethanol extract were ascribed to relatively higher contents of PPT. Further studies related to the anti-metabolic activity of wild ginseng to corresponding ingredients is required.

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