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Article

Anti-dyslipidemic effects of water extract from the leaves of *Petasites japonicus* subsp. *giganteus* (Akitabuki)

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We studied the anti-dyslipidemic effects of *Petasites japonicus* subsp. *giganteus* (PJG) *in vitro* and *in vivo*. A water extract from the leaves of PJG (PJGL-WE) markedly inhibited lipoprotein production from Caco-2 human colonic carcinoma cells differentiated into intestinal epithelium-like cells and from HepG2 human hepatoma cells. We examined the effects of PJGL-WE on the expression of lipogenic enzymes in both cells at the mRNA level. As a result, PJGL-WE suppressed gene expression involved in transacylation, such as monoacylglycerol acyltransferase 2 and diacylglycerol acyltransferase 2; however, it did not affect enzymes involved in fatty acid synthesis. PJGL-WE also attenuated the expression of microsomal triglyceride transfer protein and apolipoprotein B100, elements required for lipoprotein synthesis. Furthermore, we studied the actions of PJGL-WE on adipose tissue accumulation, intrahepatic lipid content, and plasma parameters *in vivo*. PJGL-WE suppressed mesenteric adipose tissue accumulation and normalized intrahepatic triglyceride content and plasma triglyceride levels in mice fed a high-fat diet. These findings suggested that PJGL-WE attenuated the expression of genes required for lipid and lipoprotein synthesis, thereby suppressing lipid absorption from the small intestine and participating in the improvement of fatty liver and normalization of plasma triglyceride levels.

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Introduction

In developed countries, metabolic syndrome caused by the progressive increase in visceral adiposity has become a serious health problem. The activities of crude drugs or foodstuffs in improving metabolic syndrome have been studied. We previously developed a novel system for evaluating anti-dyslipidemic agents by assessing lipoprotein profiles secreted from HepG2 human hepatoma cells [1], and we found anti-dyslipidemic effects of a fermented extract of rice bran and an ethanol extract from *Brasenia schreberi* using this assay system. Both foodstuffs also were effective for normalizing serum lipid levels and suppressing the accumulation of mes-

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enteric adipose tissue in animals fed a high-fat diet (HFD) [2, 3]. Furthermore, we developed an assay system for lipoprotein profiles from Caco-2 human colonic carcinoma cells differentiated into intestinal epithelium-like cells as an absorption model of lipids in the intestine [4]. *Petasites japonicus* subsp. *giganteus* (PJG) is a subspecies of giant butterbur, a perennial plant of the genus Asteraceae, which is native to Japan and distributed mainly in northern Honshu, Hokkaido and the southern Kurile islands [5]. The petioles are 1–2 m and the diameters of leaves are up to 1.5 m. Petioles of PJG have been utilized as a local food material, and the leaves have been dried and processed for tea-like drinks. However, the biological activities of PJG remain unclear. In the present study, we screened the anti-dyslipidemic activities of petiole and leaf extracts of PJG using our assay systems [1, 4, 6]. Because a water extract from the leaves of PJG (PJGL-WE) was found to markedly reduce lipid release from these cells, we studied the preventive and normalizing effects of the extract against adipose tissue accumulation, intrahepatic lipids content, and plasma parameters in HFD-fed mice.

Materials and Methods

Preparation of PJG extracts

We obtained 7.2 g and 13.4 g of PJG leaf and petiole powders by freeze-drying 100 g of fresh plant materials. The PJG leaf or petiole powders (1 g) were extracted in 50 mL of distilled water or ethanol, and the extracts were collected by centrifugation. The water and ethanol extracts were dried with lyophilization and evaporation, respectively. The procedure yielded 542.5 mg and 226.4 mg of the water and ethanol extracts from leaves, and 448.3 mg and 146.6 mg of the water and ethanol extracts from petioles, respectively.

Chemicals and cell line

Cholesterol-free bovine serum albumin (BSA), lysophosphatidyl choline (LysoPC) from egg yolks, and sodium oleate were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Pluronic L81 was from BASF Japan (Tokyo, Japan) and the human colonic carcinoma cell line, Caco-2 and human hepatoma cell line, HepG2, were obtained from RIKEN Cell Bank (Tsukuba, Japan).

Assessment of lipoprotein profiles from intestinal epithelium-like and hepatoma cells

Caco-2 cells were seeded at a density of 2.5×10^5 cells per well on a 12-well Transwell plate (12 mm diameter and 0.8 μm pore size; Greiner Bio-One, Kremsmünster, Austria) precoated with collagen and were attached to the membrane by culturing for 2 days in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 100 U/mL penicillin―100 mg/mL streptomycin. In Caco-2 cells precultured on ThinCert for 2 days, cells reached a confluent monolayer. Differentiation of Caco-2 cells on ThinCert membranes was induced subsequently by treatments of 5 mM Na butyrate for 4 days, sodium oleate (final 0.75 mM) and LysoPC (final 0.2 mg/mL) were added in the apical medium (FBS-free DMEM 1 mL) of differentiated Caco-2 cells without (control group) or with 0.1 mg/mL Pluronic L81 or four extracts of PJG for 4 days, and secreted lipoproteins in the basolateral medium (DMEM containing 1.0% BSA, 0.5 mL) were subjected to lipoprotein analysis.

HepG2 cells (1×10^5 cells) were seeded and precultured in DMEM (1 mL) containing 10% FBS in 24-well plates for 2 days. The cells were washed twice with PBS, and were incubated in FBS-free DMEM (0.5 mL) containing 1.0% BSA and 0.75 mM sodium oleate without (control group) or with 1 mM metformin or 0.1 mg/mL of four extracts of PJG for 2 days. The cell cultures were subjected to analyses of lipoprotein profiles after centrifugation at 15,000 g for 5 min. The separation and determination of major classes of lipoproteins from differentiated Caco-2 cells and

Table 1. Gene-specific primers used for quantitative real-time RT-PCR.

Gene name	Forward	Reverse
ACOX1	5'-TCAACCCGGAGCTGCTTAC-3'	5'-GCTGGAAGTCTGGGTCGTT-3'
APOA1	5'-TGTGTACGTGGATGTGCTCAAAGA-3'	5'-TGTGTACGTGGATGTGCTCAAAGA-3'
APOB100	5'-TCGCCTGCCAAACTGCTTC-3'	5'-CATTGGTGCCTGTGTTCCATTC-3'
DGAT1	5'-GGCCTTCTTCCACGAGTACC-3'	5'-GGCCTCATAGTTGAGCACG-3'
DGAT2	5'-AGTGGCAATGCTATCATCAT-3'	5'-GAGGCCTCGACCATGGAAGAT-3'
GAPDH	5'-GCACCGTCAAGGCTGAGAAC-3'	5'-TGGTGAAGACGCCAGTGGA-3'
FAS	5'-CAGCAGTTCACGGACATGGAG-3'	5'-CGGCACGCAGCTTGTAGTAGA-3'
LDLR	5'-AGTCACAGACGAACTGCCGAGA-3'	5'-CAACGGCTCAGACGAGCAAG-3'
MGAT1	5'-CAGGGCCGATGTCCATTGG-3'	5'-GGGTATGCCAGTCAAAGTAAAGC-3'
MGAT2	5'-CCTTCGGGGAGAATGACCTAT-3'	5'-GAGGGAGATGCCCATGATCTT-3'
MGAT3	5'-ATGGGAGTTGCCACAACCC-3'	5'-CAGAGTGACGTGAAGAGGAGG-3'
MTTP	5'-TCTCTACTCGGGTTCTGGCATTCTA-3'	5'-GCTGCGATTAAGGCTTCCAGTC-3'
PPARα	5'-GCAATGGAACTGGATGACAGTGA-3'	5'-TGCTCTGCAGGTGGAGTCTGA-3'
SCD	5'-TGAACAGTGCTGCCCACCTC-3'	5'-CGGCCATGCAATCAATGAAG-3'

Abbreviations: ACOX, acyl-CoA oxidase; APO, apolipoprotein; DGAT, diacylglycerol; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FAS, fatty acid synthase; LDLR, low-density lipoprotein receptor; MGAT, monoacylglycerol acyltransferase; MTTP, microsomal triglyceride transfer protein; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl-CoA desaturase

Table 2. Nourishment analysis value for the experimental diets.

	HFD	HFD + 2% PJGL-WE
Moisture (g/100 g)	4.4	4.8
Protein (g/100 g)	25.6	25.8
Lipid (g/100 g)	30.9	28.7
Ash (g/100 g)	3.6	4.2
Carbohydrate (g/100 g)	30.4	32.3
Dietary fiber (g/100 g)	5.1	4.1
Energy (kcal/100 g)	598.0	582.0

Abbreviations: HFD, high-fat diet; PJGL-WE, water extract from the leaves of *Petasites japonicus* subsp. *giganteus*

HepG2 cells, respectively, were performed as described previously [1, 4, 6]. Briefly, lipoproteins from 80 µl of culture media were separated using a gel-permeation high-performance liquid chromatography (HPLC) method (LipoSEARCH®; Skylight Biotech, Akita, Japan). The column effluent was then split equally into two lines by a micro-splitter, and each effluent was allowed to react at 37℃ with Diacolor liquid TG-S (TOYOBO, Osaka, Japan) for triglycerides and a Cholescolor liquid kit (TOYOBO, Osaka, Japan) for cholesterol. Triglycerides and cholesterol concentrations in lipoproteins were calculated using frozen serum-based standard material (TOYOBO, Osaka, Japan) as a standard and our in-house computer program [6-9].

RNA extraction and cDNA synthesis

Total RNA was isolated using a QuickGene RNA cultured cell kit S (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Template cDNA synthesis was performed with 5 µg of total RNA using a PrimeScript RT reagent kit (Takara Bio, Shiga, Japan). In a fluorescence temperature cycler (Chromo4; Bio-Rad Laboratories, Hercules, CA, USA), 2.5% of each RT reaction solution was amplified in 25 µl of 1× SYBR Premix Ex Taq II (Takara Bio, Shiga, Japan) containing 0.2 µM of each primer. Samples were incubated in the thermal cycler for an initial denaturation at 95℃ for 10 s, followed by 40 PCR cycles. Each cycle consisted of 95℃ for 5 s and 60℃ for 30 s. The oligonucleotide primers used in the experiment are indicated in Table 1. To confirm the amplification of specific transcripts, melting curve profiles (cooling the sample to 60℃ and heating slowly to

95℃ with continuous measurement of fluorescence) were produced at the end of each PCR. The relative expression level of each mRNA was normalized by the amount of glyceraldehyde-3-phosphate dehydrogenase mRNA.

Animal experiments

Animal experiments were carried out at the Animal Research Laboratory, Bioscience Education and Research Center, Akita University, Japan. C57BL/6JJcl mice (6-week-old males) were purchased from CLEA Japan (Tokyo, Japan). They were housed in groups of 4 or 5 in wire-topped, polycarbonate cages with wood shavings for bedding in a room at a constant temperature (23 ± 2℃) and humidity (50 ± 10%) with a 12/12 h light–dark cycle (lights on at 7.00 am). The mice had free access to fresh diet and sterile drinking water during the experimental period. They were fed a high-fat diet (HFD32, CLEA Japan, Tokyo, Japan) for 7 days and then divided into two groups: a control group (n = 8) and PJGL-WE group (n = 8). The control and PJGL-WE groups were administered the HFD32 and the HFD32 supplemented with 2% (W/W) of PJGL-WE as test substances, respectively, for 21 days. The actual diet compositions were determined by the Japan Functional Food Analysis and Research Center (Table 2). Food intake was recorded every 2 or 3 days, and body weights were measured every week. At the end of the experimental period, mice were sacrificed under light isoflurane anesthesia after 16 h of fasting. Blood was collected from the abdominal aorta, and heparinized plasma obtained was stored at -80℃ for later analysis. Livers as well as perirenal, mesenteric, and epididymal adipose tissues were excised and weighed. Animal experimentation protocols were previously approved by the Animal Research Committee, Akita University (approval number: a-1-2504). The entire experiment closely followed the university's regulations for animal experimentation, which are in strict accordance with government legislation in Japan.

Intrahepatic triglycerides and cholesterol levels

Liver tissues were homogenized in chloroform/methanol (2:1, v/v), and lipid extracts were prepared using the Folch method [10]. Intrahepatic triglycerides and cholesterol levels were measured using the enzymatic assay kits Cholestest TG and Cholestest CHO (Sekisui Medical, Tokyo, Japan), respectively.

Plasma parameters

Plasma levels of aspartate transaminase, alanine transaminase, and glucose were measured using an automatic analyzer (FUJI DRI-CHEM 3500V; FUJIFILM, Tokyo, Japan). Triglycerides and cholesterol levels in the four major lipoprotein fractions were determined by LipoSEARCH® [6].

Statistical analysis

Data are expressed as the mean ± standard deviation (SD). The significance of differences was analyzed using the Kruskal–Wallis test with Steel (Tables 3 and 4, and Figs. 2, 4 and 5) and multiple comparison tests and the Mann–Whitney U-test (Tables 5 and 6), (BellCurve for Excel, Social Survey Research Information). A value of $P < 0.05$ was considered to be significant.

Results

Effects on lipoprotein secretion and gene expression of differentiated Caco-2 cells

After triglycerides in foodstuffs are hydrolyzed by gastric and pancreatic lipase, the resulting sn-2-monoacylglycerol and free fatty acids are immediately absorbed into intestinal epithelium cells, and are sequentially reconverted to triglycerides by monoacylglycerol acyltransferases (MGATs) and diacylglycerol acyltransferases (DGATs). The synthesized triglycerides are transported to the liver as chylomicrons through in lymphatic system and blood vessels. We briefly measured the effects of four extracts from PJG for

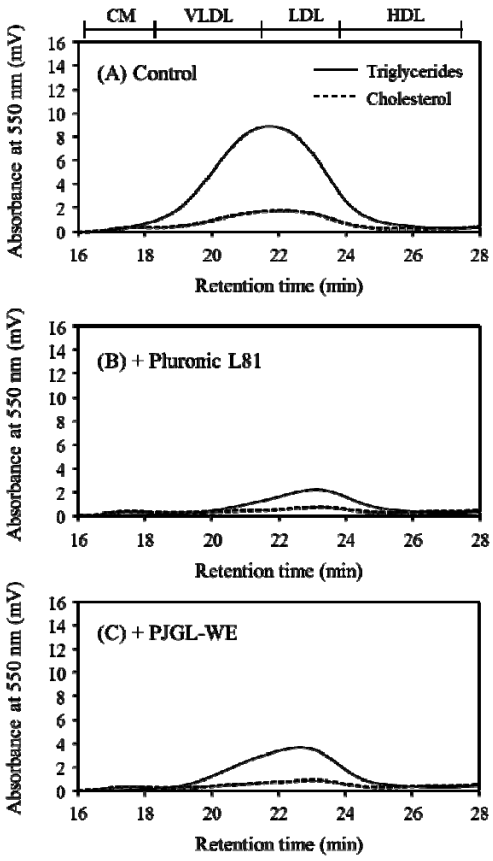

Fig. 1. Effects of PJGL-WE on the lipoprotein profiles of differentiated Caco-2 cells. Differentiated Caco-2 cells were cultured in FBS-free DMEM (1 mL) containing (A) 0.75 mM sodium oleate and 0.2 mg/mL LysoPC, (B) plus 0.1 mg/mL Pluronic L81 or (C) plus 0.1 mg/mL PJGL-WE for 4 days, and the levels of triglycerides and cholesterol in the culture medium were determined. The data are representative of four independent experiments.

lipoprotein production from Caco-2 cells that had differentiated into human intestinal epithelium-like cells. Triglycerides and cholesterol contents in four major lipoproteins—chylomicron, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL)—from differentiated Caco-2 cells are shown in Fig. 1, and data are summarized in Table 3. Pluronic L81, an inhibitor of lipoprotein secretion in differentiated Caco-2 cells [11] and some extracts decreased triglycerides and/or cholesterol release without impairing cell viability. Water and ethanol extracts from leaves of PJG at 0.1 mg/mL markedly suppressed triglyceride release from differentiated Caco-2 cells by 47.1% and 61.6%, respectively, compared with those of the control group. As strong activity for prevention of triglyceride release was detected in a water extract from leaves of PJG (PJGL-WE), we examined whether the extract affects the expression of various genes required for lipid and lipoprotein synthesis in differentiated Caco-2 cells by quantitative real-time RT-PCR (Fig. 2). Apolipoproteins (APOs) are classified into five major classes, APOA-E. APOA1, a subclass of APOA, is a constituent of HDL and is involved in HDL metabolism [12]. APOB100, which is encoded by the

Table 3. Effects of extracts from PJG on triglyceride and cholesterol secretion from differentiated Caco-2 cells.

	Control	Pluronic L81	Leaf		Petiole	
			Water	Ethanol	Water	Ethanol
Cell number ($\times 10^5$)	2.7 ± 0.3	2.7	2.7 ± 0.2	2.5 ± 0.3	2.6 ± 0.1	2.7 ± 0.1
Triglycerides (μg/10^6 cells)						
Total	34.4 ± 2.6	8.3 ± 0.8 *	16.2 ± 2.8 *	21.2 ± 1.7 *	26.2 ± 5.1 *	33.1 ± 12.9
CM	1.3 ± 0.2	0.1 ± 0.1 *	0.4 ± 0.2 *	0.2 ± 0.1 *	1.2 ± 0.5	0.4 ± 0.2 *
VLDL	23.5 ± 2.1	3.4 ± 0.6 *	9.5 ± 2.0 *	13.0 ± 1.2 *	17.2 ± 3.5 *	22.1 ± 10.1
LDL	8.3 ± 0.3	3.8 ± 0.3 *	4.8 ± 0.7 *	6.7 ± 0.7	6.4 ± 1.0	9.1 ± 2.3
HDL	1.4 ± 0.1	1.0 ± 0.1 *	1.3 ± 0.2	1.3	1.4 ± 0.1	1.5 ± 0.2
Cholesterol (μg/10^6 cells)						
Total	3.0 ± 0.3	1.5 ± 0.2 *	2.3 ± 0.3 *	3.3 ± 0.3	2.9 ± 0.5	3.2 ± 1.0
CM	0.3 ± 0.1	0.2	0.3 ± 0.1	0.3 ± 0.1	0.4	0.2
VLDL	1.6 ± 0.2	0.5 ± 0.1 *	0.8 ± 0.1 *	1.5 ± 0.1	1.4 ± 0.3	1.7 ± 0.7
LDL	0.7	0.5 ± 0.1	0.5 ± 0.1	1.0 ± 0.1 *	0.7 ± 0.2	1.0 ± 0.2
HDL	0.4	0.4	0.6	0.6 ± 0.1	0.4 ± 0.1	0.4

Data are expressed as the mean ± standard deviation (n = 4, *P < 0.05 vs control group).

Abbreviations: CM, chylomicron; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein

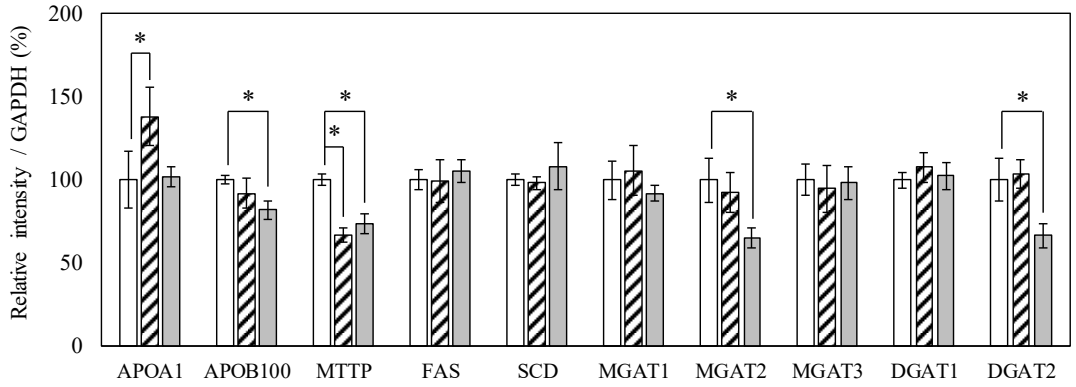

Fig. 2. Effects of PJGL-WE on the mRNA expression levels of genes involved in lipid and lipoprotein synthesis in differentiated Caco-2 cells. Differentiated Caco-2 cells were incubated with 0.75 mM sodium oleate and 0.2 mg/mL LysoPC (control group, white bars), plus 0.1 mg/mL Pluronic L81 (hatched bars), or plus 0.1 mg/mL PJGL-WE (gray bars) for 4 days, and mRNA expression levels were analyzed by quantitative real-time RT-PCR. Data are expressed relative to control group and represent mean ± SD (n = 4, $*P < 0.05$ vs control group).

APOB gene, is a structural component of triglyceride-rich lipoproteins and is necessary for triglyceride transport in the body [13]. Microsomal triglyceride transfer protein (MTTP), localized to the lumen of the endoplasmic reticulum in the intestine and liver [14], has been identified as a factor necessary for the assembly of APOB100. Pluronic L81 is known to inhibit lipoprotein production by suppressing the expression of MTTP, required for the assembly and secretion of VLDL by the liver and chylomicron by the intestine [11]. Even in this study, Pluronic L81 suppressed MTTP expression in differentiated Caco-2 cells. PJGL-WE suppressed APOB100 and MTTP by 81.9% and 73.6%, respectively, compared with those of the control group; however, it did not affect the gene expression of APOA1. PJGL-WE also did not affect the gene expression levels of fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD), which are involved in fatty acid synthesis. MGATs catalyze the first step in triglyceride synthesis involved in dietary absorption by enterocytes. Three isoforms of MGAT enzymes, known as MGAT1, MGAT2, and MGAT3, have been identified so far. Among them, MGAT2 catalyzes the synthesis of diacylglycerol from free fatty acids and sn-2-monoacylglycerol, the two major hydrolysis products of dietary fat. DGATs catalyze the final step in mammalian triglyceride synthesis, which merges the MGAT and glycerol-3-phosphate pathways. In mammals, there are two isoforms of DGAT enzymes, DGAT1 and DGAT2 [15]. In this study, PJGL-WE also attenuated the expression of genes encoding MGAT2 and DGAT2 by 65.3% and 66.5%, respectively, compared with the control group.

Effects on lipoprotein secretion and gene expression of hepatoma cells

Triglycerides and cholesterol are re-synthesized from chylomicron and free fatty acids in the liver and then released as lipoproteins. Therefore, we studied the actions of PJGL-WE on lipoprotein profiles from HepG2 cells; representative profiles are shown in Fig. 3 and data are summarized in Table 4. Triglyceride levels in the culture medium of HepG2 cells were elevated after stimulation with 0.75 mM sodium oleate (sodium oleate:BSA ratio 5:1) for 2 days [16]. Metformin was used as positive control because it is known to reduce cellular lipid levels by activation of AMP-activated protein kinase (AMPK) [17].

Metformin at 1 mM and PJGL-WE at 0.1 mg/mL markedly suppressed triglyceride release from HepG2 cells by 36.0% and 36.6%, respectively, compared with the control group. Because PJGL-WE showed intensive reductions in triglyceride release, we examined whether PJGL-WE at 0.1 mg/mL affected the expression of various genes required for lipid and lipoprotein synthesis in HepG2 cells by quantitative real-time RT-PCR as well as in differentiated Caco-2 cells (Fig. 4).

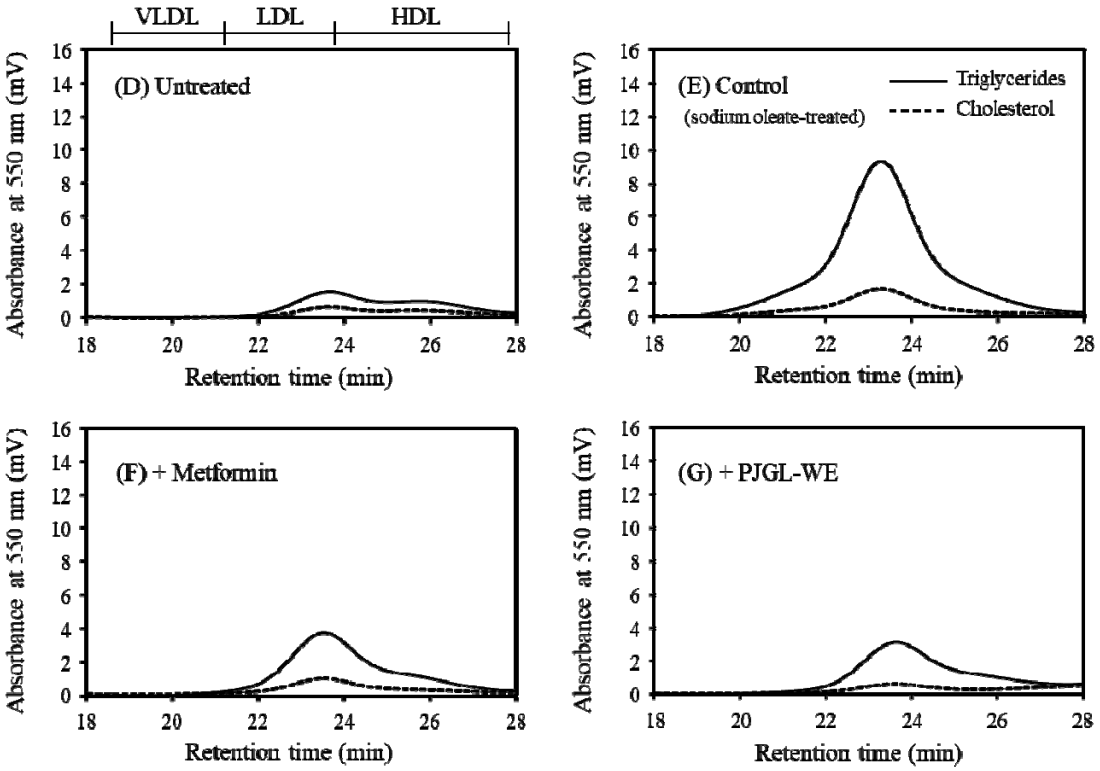

Fig. 3. Effects of PJGL-WE on lipoprotein profiles of HepG2 cells. Following HepG2 cell preculture in DMEM (1 mL) containing 10% FBS for 2 days, the cells were cultured in FBS-free DMEM (0.5 mL) containing (D) 1.0% BSA alone, (E) with 0.75 mM sodium oleate, (F) 0.75 mM sodium oleate plus 1 mM metformin, or (G) 0.75 mM sodium oleate plus 0.1 mg/mL PJGL-WE for 2 days, and the levels of triglycerides and cholesterol in the culture medium were determined. The data are representative of four independent experiments.

Table 4. Effects of extracts from PJG on triglycerides and cholesterol secretion from HepG2 cells.

	Untreated	Control (sodium oleate-treated)	Metformin	Leaf		Petiole	
				Water	Ethanol	Water	Ethanol
Cell number ($\times 10^5$)	5.2 ± 0.3	4.5 ± 0.2	4.9 ± 0.1	5.0 ± 0.4	5.3 ± 0.4 *	4.3 ± 0.5	5.2 ± 0.9
Triglycerides (μg/10^6 cells)							
Total	2.9 ± 0.2 *	16.1 ± 0.7	5.8 ± 0.2 *	5.9 ± 0.1 *	14.4 ± 0.5	13.2 ± 0.2 *	16.5 ± 0.3
VLDL	0.4 ± 0.1 *	6.4 ± 0.2	1.3 ± 0.1 *	1.0 ± 0.1 *	7.6 ± 0.3 *	4.9 ± 0.8 *	8.6 ± 0.2 *
LDL	1.5 ± 0.1 *	8.3 ± 0.5	3.5 ± 0.1 *	3.3 ± 0.1 *	5.6 ± 0.3 *	7.0 ± 0.4 *	6.7 ± 0.1 *
HDL	1.0 ± 0.1 *	1.4 ± 0.1	1.1 ± 0.1 *	1.6	1.2 ± 0.1	1.3 ± 0.1	1.2
Cholesterol (μg/10^6 cells)							
Total	2.6 ± 0.2 *	10.2 ± 0.6	4.8 ± 0.2 *	5.0 ± 0.1 *	7.9 ± 0.3 *	8.7 ± 0.5 *	9.1 ± 0.1 *
VLDL	0.1 *	0.5 ± 0.1	0.2 *	0.1 *	1.1 ± 0.3 *	0.4 ± 0.1	1.2 ± 0.1 *
LDL	1.5 ± 0.1 *	8.3 ± 0.5	3.5 ± 0.1 *	3.3 ± 0.1 *	5.6 ± 0.3 *	7.0 ± 0.4 *	6.7 ± 0.1 *
HDL	1.0 ± 0.1 *	1.4 ± 0.1	1.1 ± 0.1 *	1.6	1.2 ± 0.1	1.3 ± 0.1	1.2

Data are expressed as the mean ± standard deviation (n = 4, *P < 0.05 vs control group).

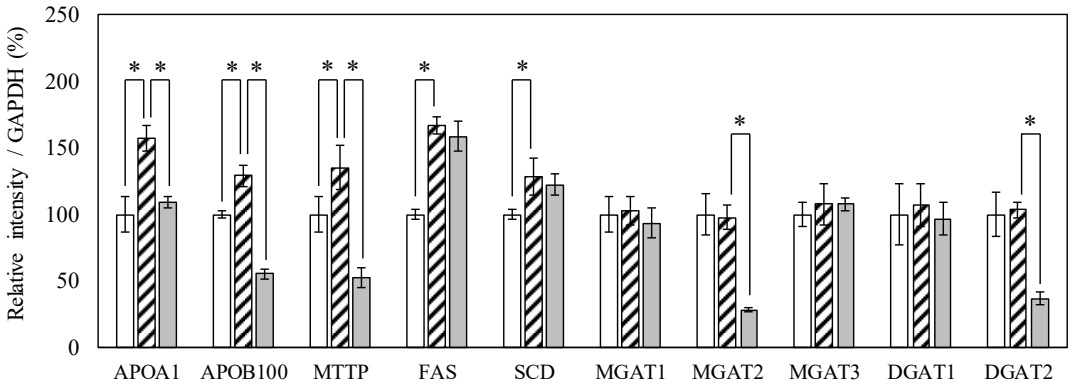

Fig. 4. Effects of PJGL-WE on the mRNA expression levels of genes involved in lipid and lipoprotein synthesis in HepG2 cells. HepG2 cells were incubated alone (untreated group, white bars), with 0.75 mM sodium oleate (control group, hatched bars) or with 0.75 mM sodium oleate plus 0.1 mg/mL PJGL-WE (gray bars) for 2 days, and mRNA expression levels were analyzed by quantitative real-time RT-PCR. Data are expressed relative to untreated group and represent mean ± SD (n = 4, **P* < 0.05 vs control group).

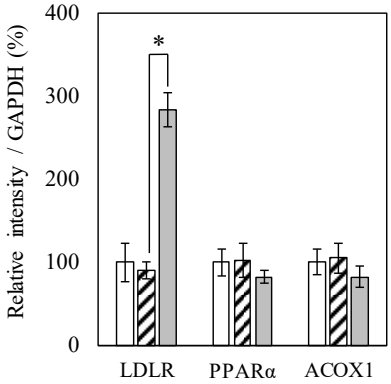

Fig. 5. Effects of PJGL-WE on mRNA expression of LDLR and β-oxidization elements in HepG2 cells. HepG2 cells were incubated alone (untreated group, white bars), with 0.75 mM sodium oleate (control group, hatched bars) or with 0.75 mM sodium oleate plus 0.1 mg/mL PJGL-WE (gray bars) for 2 days, and mRNA expression was analyzed by quantitative real-time RT-PCR. Data are expressed relative to the untreated group and represent mean ± SD (n = 4, **P* < 0.05 vs control group).

Sodium oleate elevated the gene expression levels of APOA1, APOB100, MTTP, FAS, and SCD in HepG2 cells. PJGL-WE did not affect the gene expression levels of FAS and SCD, which are involved in fatty acid synthesis. However, PJGL-WE suppressed the enhancement of APOA1 by sodium oleate at the mRNA level and attenuated APOB100 and MTTP gene expression by 42.8% and 38.8%, respectively, compared with the levels of the control group. Furthermore, PJGL-WE significantly reduced the gene expression levels of MGAT2 and DGAT2 by 29.0% and 35.4%, respectively, compared with the control group. To confirm the effects of PJGL-WE on lipoprotein incorporation and the fatty acid β-oxidation system in HepG2 cells, we also examined the mRNA levels of the LDL receptor (LDLR) and β-oxidization elements. The peroxisome proliferator-activated receptors (PPARs), which have an important role in fatty acid metabolism. The PPAR family contains three distinct isotypes: α, β, and γ [18]. PPARα controls the expression of numerous genes related to lipid metabolism in the liver, including genes involved in mitochondrial β-oxidation, peroxisomal β-oxidation, fatty acid uptake and/or binding, and lipoprotein assembly and transport [19-21]. Acyl-CoA oxidase 1 is the first and a rate-limiting enzyme of PPARα-regulated and peroxisome proliferator-inducible fatty acid β-oxidation systems [22-25]. PJGL-WE remarkably elevated the expression of LDLR compared with the levels in the control group; however, there was little change in

the gene expression levels of PPARα and acyl-CoA oxidase 1 (Fig. 5).

Anti-dyslipidemic effects against HFD-fed mice

To confirm the anti-dyslipidemic effects of PJGL-WE *in vivo*, we investigated the influences of PJGL-WE on body weight, liver weight, adipose tissue weight, intrahepatic lipids content (Table 5), and plasma parameters (Table 6) in HFD-fed mice. PJGL-WE did not affect the body or liver weights; however, oral administration of PJGL-WE markedly suppressed the accumulation

Table 5. Effects of PJGL-WE on tissue weight and intrahepatic lipids content in HFD-fed mice.

	Control group	PJGL-WE group	P-value
Food intake (g/day/mouse)	2.8	2.6	-
Body weight (g)	31.1 ± 0.7	29.8 ± 0.2	0.135
Body weight gain (g/21days)	7.1 ± 0.6	5.6 ± 0.3	0.064
Tissue weight			
Liver (mg)	1214.7 ± 37.7	1118.8 ± 30.1	0.071
Perirenal adipose tissue (mg)	591.4 ± 40.0	538.8 ± 38.0	0.358
Mesenteric adipose tissue (mg)	482.4 ± 41.2	385.5 ± 12.1 *	0.049
Epididymal adipose tissue (mg)	1341.0 ± 108.2	1198.5 ± 58.7	0.282
Intrahepatic lipid content			
Triglycerides (mg/g)	57.7 ± 12.7	25.9 ± 8.5 *	0.007
Cholesterol (mg/g)	3.0 ± 0.6	3.4 ± 0.5	0.201

Data are expressed as the mean ± standard deviation (n = 8, *P < 0.05 vs control group).

Table 6. Effects of PJGL-WE on plasma parameters in HFD-fed mice.

	Control group	PJGL-WE group	P-value
Triglycerides (mg/dL)			
Total	66.2 ± 30.2	39.9 ± 14.5 *	0.041
CM	9.6 ± 3.8	4.3 ± 2.5 *	0.005
VLDL	47.3 ± 25.3	24.3 ± 10.8 *	0.048
LDL	7.3 ± 1.9	5.9 ± 1.4	0.119
HDL	2.0 ± 0.5	2.5 ± 0.6	0.104
Cholesterol (mg/dL)			
Total	123.0 ± 13.5	125.8 ± 11.5	0.208
CM	0.6 ± 0.2	0.4 ± 0.1	0.088
VLDL	5.0 ± 1.9	4.3 ± 1.2	0.405
LDL	22.8 ± 3.8	23.7 ± 5.1	0.639
HDL	94.7 ± 11.1	97.4 ± 7.4	0.562
AST (U/L)	57.7 ± 28.7	51.5 ± 9.6	0.287
ALT (U/L)	35.5 ± 11.8	25.4 ± 10.5	0.577
Glucose (mg/dL)	196.1 ± 26.9	123.1 ± 18.7 *	0.047

Data are expressed as the mean ± standard deviation (n = 8, *P < 0.05 vs control group).

of mesenteric adipose tissue and intrahepatic triglyceride content by 79.9% and 44.9% respectively, compared with the control group. Furthermore, intakes of PJGL-WE normalized the plasma total-, chylomicron-, and VLDL-triglyceride levels and glucose levels in HFD-fed mice but did not affect plasma cholesterol levels or those of AST and ALT, which are plasma liver disease markers (Table 6).

Discussion

In recent years, various food components with the ability to suppress increases in blood triglyceride levels have been studied extensively. Examples include diacylglycerol [26-28], globin digest [29, 30], medium-chain fatty acids [31, 32], tea catechins [33, 34], EPA [35], DHA [35], oolong tea polyphenols [36], β-conglycinin [37], resistant maltodextrin [38, 39], and others. In the present study, we found a unique mechanism of PJGL-WE, which acts as an anti-dyslipidemic agent not previously reported in the other food components mentioned above. PJGL-WE attenuated the expression of MGAT2 and DGAT2 and consequently suppressed the synthesis of triglycerides *in vitro* by blocking transacylation not but free fatty acids synthesis. Furthermore, PJGL-WE also suppressed lipoprotein synthesis and secretion by attenuating the expression of MTTP and APOB100. Based on these mechanisms, PJGL-WE suppressed lipid absorption from the small intestine and participated in suppressing adipose tissue accumulation, improvement of fatty liver, and normalization of plasma triglyceride levels in HFD-fed mice. In addition, the results of RT-PCR analysis revealed that PJGL-WE remarkably induced LDLR levels in hepatic cells, which are responsible for the incorporation of chylomicron and LDL. The upregulation of LDLR in the liver by PJGL-WE may promote the collection of these lipoproteins and is one cause for normalizing plasma lipoprotein levels in HFD-fed mice. Thus, this study elucidated the biological activities of PJGL-WE, which may be a good candidate for development as an anti-dyslipidemic agent, with therapeutic potential in hypertriglyceridemia and fatty liver disease. However, further chemical and pharmacological investigations are required to isolate and identify the active principles responsible for these effects and to prove its safety and efficacy. At present, work is in process to isolate the active compounds responsible for the observed effects. Finally, *Petasites japonicus* is known to contain pyrrolizidine alkaloids, which possess strong hepatic toxicity [40], and it will be necessary to ensure these are absent before the intake of *Petasites japonicus* extract can be considered for human use.

Conflict of interest

The authors declare that they have no conflict of interest.

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