eCAM Evidence-Based Complementary and Alternative Medicine

Looking for New Herbal Treatments for Metabolic Syndrome

Guest Editors: Hyeung-Jin Jang, Jae-Young Um, Hanseok Ko, and Won-Seok Chung

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Editorial Board

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2016, Article ID 7198490, 2 pages
http://dx.doi.org/10.1155/2016/7198490

Editorial

Looking for New Herbal Treatments for Metabolic Syndrome

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Received 12 April 2016; Accepted 3 May 2016

Diabetes, which is one of the metabolic diseases, is a complex and chronic illness to keep maintaining medical and diet care against abnormal glycemic symptoms such as high blood glucose level and insulin resistance. This diabetes, which is inextricably bound up with obesity, can cause decrease of life expectancy, increase of healthcare cost, and weakening of quality of life by risk of acute or long-term complication. In particular, type 2 diabetes mellitus (T2DM) rate of all diabetes reported about over 90%. Also, prevailing population and increasing healthcare budget of diabetes are conservatively estimated to be as much as 366 million people and be as much as 396 billion international dollars in 2030. Even if many clinical doctors and researchers have developed medical care and found therapeutic agent for T2DM, it is difficult to avoid side effects such as hypoglycemia, hyperinsulinemia, diarrhea, and heart failure. Here, this special issue suggested how we accessed alternative treatment or safe therapy for T2DM patients by introducing several research papers using herbal medicines.

Traditional medicine has a history of more than 3000 years in clinical trials including China and Korea. The major use of herbal medicines is for health promotion about diverse disease including T2DM and therapy for chronic, as opposed to life-threatening, conditions. It is the most important reason for which herbal medicines are widely perceived as natural and safe without side effects. The historical factors that the herbal medicine has been prescribed in T2DM patients and evaluated safety against side effects for a long time let our group experimentally test whether hyperglycemia effects by taking herbal medicines are related to glucagon-like peptide-1 (GLP-1) stimulation via taste receptor signaling on enteroendocrine L cells or not. Firstly, our group tested whether prescribed extracts of herbal medicines, *Citrus aurantium, Anemarrhena asphodeloides,* and *Bupleurum falcatum,* were responsible for GLP-1 secretion and taste receptor signaling or not in enteroendocrine L cells using GLP-1 assay and microarray. GLP-1 is one of the incretin hormones and is secreted from intestinal enteroendocrine L cells, which existed mainly in the proximal ileum and colon, and GLP-1 is induced through nutrient sensing taste receptor that responds to sweet, bitter, umami, and fatty acids. These herbal medicines stimulated GLP-1 secretion and upregulated the mRNA of taste receptor genes in L cells. Additionally, we tested whether GLP-1 secretion practically affected hyperglycemia effects on diabetic mice model or not. Diabetic mice exhibited more ameliorated hyperglycemia than control group. However, this GLP-1 secretion by herbal medicines lacks biochemical evidence. It is unknown which components on herbal medicines stimulate GLP-1 and which specific taste receptor signaling among sweet, bitter, umami, and fatty acids was activated by herbal medicines. To overcome these limitations, the study supplemented systemic inhibition study and component analysis on herbal medicine, *Gentiana scabra* extracts. In aspect of systemic inhibition study, pharmacologic inhibitors associated with taste receptor signaling on GLP-1 and calcium imaging study and knockout

mice of α-gustducin applied to mechanism definition of taste receptor signaling of *Gentiana scabra* extracts on *in vivo* study of plasma GLP-1 and insulin measurements. Furthermore, to confirm components in *Gentiana scabra* extracts and to evaluate active compound of GLP-1 secretion, the components of *Gentiana scabra* extractswere analyzed by HPLC-MS and active compound of GLP-1 secretion in L cells was selected by GLP-1 assay.

This issue tried to explain safe application for T2DM patients by regulating hyperglycemia via taste receptor signaling. We provide previous study of therapeutic application via taste receptor signaling for T2DM patients and challenging limitation such as structural assessments. To accurately define specific taste receptor of binding difference according to chemical characters or structure, the components derived from herbal medicines of structural assessment binding taste receptor should be reinforced to cooperate with structural biology areas. Also, for GLP-1 secretagogue of discovery of evidence-based complementary and alternative medicine via taste receptor signaling, development of agonistic component with taste receptor from each herbal medicine, structural assessment of specific taste receptor on discovery agonistic component, and its GLP-1 secretagogue of clinical evaluation need to be supported by future researches.

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2016, Article ID 8674397, 12 pages
http://dx.doi.org/10.1155/2016/8674397

Research Article

Veratri Nigri Rhizoma et Radix (*Veratrum nigrum* L.) and Its Constituent Jervine Prevent Adipogenesis via Activation of the LKB1-AMPKα-ACC Axis *In Vivo* and *In Vitro*

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Received 21 August 2015; Revised 30 November 2015; Accepted 14 March 2016

Academic Editor: Antonella Fioravanti

This study was performed in order to investigate the antiobese effects of the ethanolic extract of Veratri Nigri Rhizoma et Radix (VN), a herb with limited usage, due to its toxicology. An HPLC analysis identified jervine as a constituent of VN. By an Oil Red O assay and a Real-Time RT-PCR assay, VN showed higher antiadipogenic effects than jervine. In high-fat diet- (HFD-) induced obese C57BL/6J mice, VN administration suppressed body weight gain. The levels of peroxisome proliferator-activated receptor gamma (PPARγ), CCAAT-enhancer-binding protein alpha (C/EBPα), adipocyte fatty-acid-binding protein (aP2), adiponectin, resistin, and LIPIN1 were suppressed by VN, while SIRT1 was upregulated. Furthermore, VN activated phosphorylation of the liver kinase B1- (LKB1-) AMP-activated protein kinase alpha- (AMPKα-) acetyl CoA carboxylase (ACC) axis. Further investigation of cotreatment of VN with the AMPK agonist AICAR or AMPK inhibitor Compound C showed that VN can activate the phosphorylation of AMPKα in compensation to the inhibition of Compound C. In conclusion, VN shows antiobesity effects in HFD-induced obese C57BL/6J mice. In 3T3-L1 adipocytes, VN has antiadipogenic features, which is due to activating the LKB1-AMPKα-ACC axis. These results suggest that VN has a potential benefit in preventing obesity.

1. Introduction

Obesity has become a public health dilemma recently, especially in developed countries. According to the report of the World Health Organization, over 1.4 billion of 20-year-old or older individuals worldwide are overweight [1]. Obesity is closely related to chronic diseases such as hyperlipidemia, hypertension, and type 2 diabetes mellitus [2]. Adipogenesis is a process by which undifferentiated preadipocytes are converted into fully differentiated adipocytes, such as fat cells [3]. The mouse preadipocyte cell line 3T3-L1 is one of the best characterized models for studying the conversion process of preadipocytes into adipocytes. Adipogenesis is known as a closely related process to the etiologies of obesity involving several genes and proteins at different stages [4].

During the adipogenesis of 3T3-L1 cells, among the several adipogenic transcription factors, peroxisome proliferator-activated receptor gamma (PPARγ) and CCAAT-enhancer-binding protein alpha (C/EBPα) are known to act as the key regulators [5]. PPARγ acts as a regulator of development of adipocytes and is known as the only factor that can induce the adipocyte-like phenotype in nonadipogenic cell types [6]. C/EBPα, a member of the leucine zipper transcription factor family, plays an important role in the terminal differentiation in adipocytes [7]. These two factors are not expressed in preadipocytes but are activated

during adipocyte differentiation [8]. Adipocyte fatty-acid-binding protein (aP2) acts as cytoplasmic lipid chaperones and plays a role in several lipid signals [9], while resistin, a newly identified adipokine, is secreted by adipocytes and has antagonistic effects on insulin actions [10]. A novel protein, LIPIN1, is primarily expressed in adipose tissues, liver, and skeletal muscles [11]. SIRT1 has been found to suppress adipocyte differentiation and to prevent TG accumulation in white adipose tissue through repression of PPARγ [12]. Similarly, AMP-activated protein kinase (AMPK) activation inhibits adipocyte differentiation and lipogenesis [13, 14].

The fuel-sensing enzyme AMPK is a heterotrimeric protein kinase consisting of three subunits: α, β, and γ [15]. The increased AMP/ATP ratio affects the γ subunit to induce phosphorylation of a threonine residue within the activation domain of the α subunit, by the upstream kinase, liver kinase B1 (LKB1) [16]. AMPK can be activated by inhibition of ATP, that is, hypoxia, ischemia, oxidative stress, and glucose deprivation [15] but, importantly, can be activated by adipokines leptin and adiponectin, the important regulators of energy metabolism [17]. Activation of AMPK results in the repression of ATP-consuming anabolic processes and activation of ATP-producing catabolic processes [17, 18]. AMPK mediates these effects through the phosphorylation of metabolic enzymes, such as acetyl CoA carboxylase (ACC), the rate-limiting enzyme for fatty acid oxidation [19].

Veratrum nigrum L., commonly known as black false hellebore, is a coarse, poisonous perennial herb native to Asia and Europe [20]. The stems and roots of this plant are used under the name of Veratri Nigri Rhizoma et Radix. Due to its ability to cause nausea and vomiting, it is applied to dyspnea in epilepsy or stroke patients in Traditional Korean Medicine. Previous studies report that *Veratrum nigrum* L. is a potential agonist of β2-adrenoceptor [21] and is also able to prevent hepatic ischemia injury in rats [22]. However, the effect of the ethanolic extract of Veratri Nigri Rhizoma et Radix (VN) or its constituent, jervine ((3β,23β)-17,23-epoxy-3-hydroxyveratraman-11-one; Figure 1), on obesity has not been reported to date. Thus, this study was performed to investigate the effects of VN and jervine on obesity *in vivo* and *in vitro*.

2. Materials and Methods

2.1. Sample Collection. The stems and roots of *Veratrum nigrum* L. (Veratri Nigri Rhizoma et Radix), known as "black false hellebore," were obtained from Omniherb (Daejeon, Republic of Korea). A voucher specimen of the plant has been deposited in our laboratory. The Veratri Nigri Rhizoma et Radix was already processed into dried and chopped pieces before purchase. 100 g of Veratri Nigri Rhizoma et Radixslices was extracted for 2 h 20 min using a heating mantle with 1000 mL of 70% aqueous ethanol. The extract was filtered through a 0.22 μm syringe filter, evaporated, and then stored at −20°C until usage.

2.2. Reagents. Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin-glutamine, bovine serum (BS), and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). Insulin, 3-isobutylmethylxanthine (IBMX), dexamethasone (DEX), Oil Red O powder, and 5-amino-4-imidazolecarboxamide riboside (AICAR) were from Sigma Chemical Co. (St. Louis, MO, USA). 6-[4-(2-Piperidin-1-ylethoxy)phenyl]-3-pyridin-4-ylpyrazolo[1,5-a]pyrimidine (Compound C) was obtained from Calbiochem (La Jolla, CA, USA). The antibodies for C/EBPα and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and PPARγ, phospho-LKB1, phospho-ACC, phospho-AMPKα, and AMPKα were obtained from Cell Signaling technology (Beverly, MA, USA). Jervine (PubChem CID: 10098) was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

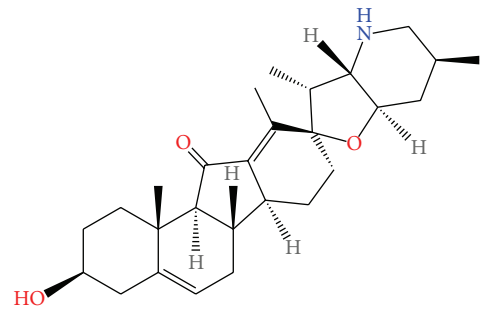

Figure 1: Structure of jervine.

2.3. HPLC Analysis. The chromatographic system consisted of Jasco HPLC-LC-2000 Plus (Tokyo, Japan) equipped with a Jasco MD-2018 Plus Photodiode Array Detector, using the Mightysil RP-18(L) GP column (5 μm, 4.6 × 150 mm, Kanto Chemical Co. Inc., Japan). The column temperature was set to 40°C. The mobile phase consisted of acetonitrile as solvent A and acetic acid in water (0.05%) as solvent B using gradients elution. The initial mobile phase composition was 10% of solvent A, and the following gradient system was used: 10–20% (0–10 min), 20% (10–15 min), 20–35% (15–25 min), 35% (25–30 min), 35–40% (30–35 min), 40% (35–45 min), 40–10% (45–50 min), and 10% of solvent A (50–60 min). The total running time was 60 min, and the flow rate was 1.0 mL/min. Data acquisition was performed in the range of 190–650 nm. The retention times of these compounds were obtained as follows: jervine, 26.3 min.

2.4. Animal Experiments. The animal obesity model experiment was conducted based on previous reports [20, 23–25]. Male C57BL/6J mice, weighing 17-18 g at the age of 4 weeks, were purchased from the Dae-Han Experimental Animal Center (Eumsung, Republic of Korea). The animal experiment was proceeded in conditions in accordance with the regulations issued by the Institutional Review Board of Kyung Hee University (confirmation number: KHUASP (SE)-13-012). The mice were maintained for 1 week prior to the experiments in a 12-hour light/dark cycle at humidity of 70% and constant temperature of 23 ± 2°C. The animals were

then divided into four groups (n = 5–7 per group): a normal control group fed normal chow diet (CJ Feed Co. Ltd., Seoul, Republic of Korea), a high-fat diet (HFD) group fed 60% fat HFD (Rodent diet D12492, Research diet, New Brunswick, NJ, USA) for 14 weeks, a VN group and slinti group which were fed HFD for 4 weeks in order to induce obesity and then fed for 10 additive weeks with HFD plus VN or HFD plus slinti (Myungmoon Pharm. Co. LTD., Seoul, Republic of Korea), respectively. Slinti, which consisted of Theae Folium Powder 250 mg and Orthosiphon Powder 150 mg, was used as a positive control according to the antiobese effects reported on our previous studies [20, 24]. The components of the diets are described in S1 Table (in Supplementary Material available online at http://dx.doi.org/10.1155/2016/8674397). The body weight and food intake amount were recorded every other day. At the end of the period of total 14 weeks, the animals were fasted overnight. The next day, they were anesthetized under CO_2 asphyxiation and plasma was separated at 4,000 g for 30 min immediately after blood collection via cardiac puncture. The total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, alanine transaminase (ALT), and creatinine were assessed by Seoul Medical Science Institute (Seoul, Republic of Korea). Animals were killed by cervical dislocation. Subcutaneous white adipose tissues (sWATs) were weighed.

2.5. Cell Culture and Differentiation. 3T3-L1 mouse embryo fibroblasts were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were grown in DMEM plus 10% BS containing penicillin-streptomycin-glutamine solution (100 UI/mL) in a 10 cm dish. After reaching passage 10, the cells were then moved to 6-well plates for final differentiation in DMEM plus 10% FBS containing antibiotics described above. Until 100% confluence (Day 0), the cells were maintained in a cell incubator at 37°C, 5% CO_2, and 95% humidity. Two days after confluence (Day 2), the cells were stimulated to differentiation with differentiation media (DM) composed of DMEM plus 10% FBS and differentiation inducers (MDI: 1 μM DEX, 500 μM IBMX, and 1 μg/mL insulin). After 2 days, the DM was removed and replaced with DMEM plus 10% FBS containing 1 μg/mL insulin (Day 4). After another additional 2 days, the media were replaced with DMEM plus 10% FBS containing 1 μg/mL insulin again (Day 6). The cells were cultured for 2 more days, at which time more than 90% of cells were mature adipocytes with accumulated fat droplets, and then harvested for further experiments (Day 8). The VN or jervine was treated at Day 4, dissolved in the culture media. AICAR or Compound C was administered 30 min before the VN or jervine treatment dissolved in the culture media.

2.6. MTS Assay. The 3T3-L1 preadipocytes were seeded (2×10^4 cell/well) and incubated in DMEM plus 10% FBS for 24 h. Then the cells were incubated in the same media containing an ethanol extract of VN for an additional 48 h. Cell viability was monitored using the cell proliferation MTS kit by the Promega Corporation (Madison, WI, USA) as recommended by the manufacturer. Prior to measuring the viability, the media were removed and replaced with 200 μL of fresh DMEM plus 10% FBS medium and 10 μL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) solution. The cells were then incubated in the incubator for 4 h. The absorbance was measured at 490 nm in a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA) to determine the formazan concentration, which is proportional to the number of live cells.

2.7. Oil Red O Staining. Intracellular lipid accumulation was measured using Oil Red O. The Oil Red O working solution was prepared as described by Ramírez-Zacarías et al. [26]. Briefly, Oil Red O stock solution was prepared Oil Red O (Sigma-Aldrich, St. Louis, MO, USA) dissolved in isopropanol at the concentration of 3.5 mg/mL, and the Oil Red O working solution was prepared 60% Oil Red O stock solution mixed with 40% distilled water. 3T3-L1 adipocytes were harvested 6 days after the initiation of differentiation. Cells were washed twice with phosphate buffered saline (PBS, pH 7.4) and then fixed with 10% neutral formalin for 2 hours at room temperature. After washing with 60% isopropanol, the cells were stained with Oil Red O working solution for 30 min and then were washed 4 times with water in order to remove the unbound dye. The stained cells were observed by an Olympus IX71 Research Inverted Phase microscope (Olympus Co., Tokyo, Japan). Following the microscopic observation, 100% isopropanol was added as an extraction solution to extract the staining dye of cells. The absorbance of the extracted dye was measured spectrophotometrically at 500 nm in a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.8. RNA Isolation and Real-Time RT-PCR. Total cellular RNA was isolated from 3T3-L1 adipocytes using QIAzol lysis reagent (Qiagen Sciences, Maryland, USA). Total RNA was used as a template for first-strand cDNA synthesis performed using a Power cDNA Synthesis Kit (iNtRON Biotechnology, Seoul, Korea) according to the manufacturer's instructions. The Real-Time RT-PCR mixture, with a final volume of 20 μL, consisted of Fast SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 1 μM of a forward primer, 1 μM of a reverse primer, and 0.1 μg of a cDNA sample. The thermal cycling conditions were as follows: holding stage, 10 s at 95°C and 40 cycles of 15 s at 95°C and 1 min at 60°C, and then melt curve stage, 15 s at 95°C, 1 min at 60°C, and 15 s at 95°C. PCR products were measured with a StepOnePlus Real-Time RT-PCR System (Applied Biosystems, Foster City, CA, USA), and the relative gene expression was calculated based on the comparative CT method using a StepOne Software v2.1 (Applied Biosystems, Foster City, CA, USA). The mRNA expression of GAPDH was used as an endogenous control. The target cDNA was amplified using the sense and antisense primers described in S2 Table.

2.9. Western Blot Analysis. After experimental treatment, cells were washed twice with ice-cold PBS and lysed with RIPA lysis buffer, which consisted of 50 mM Tris-HCl

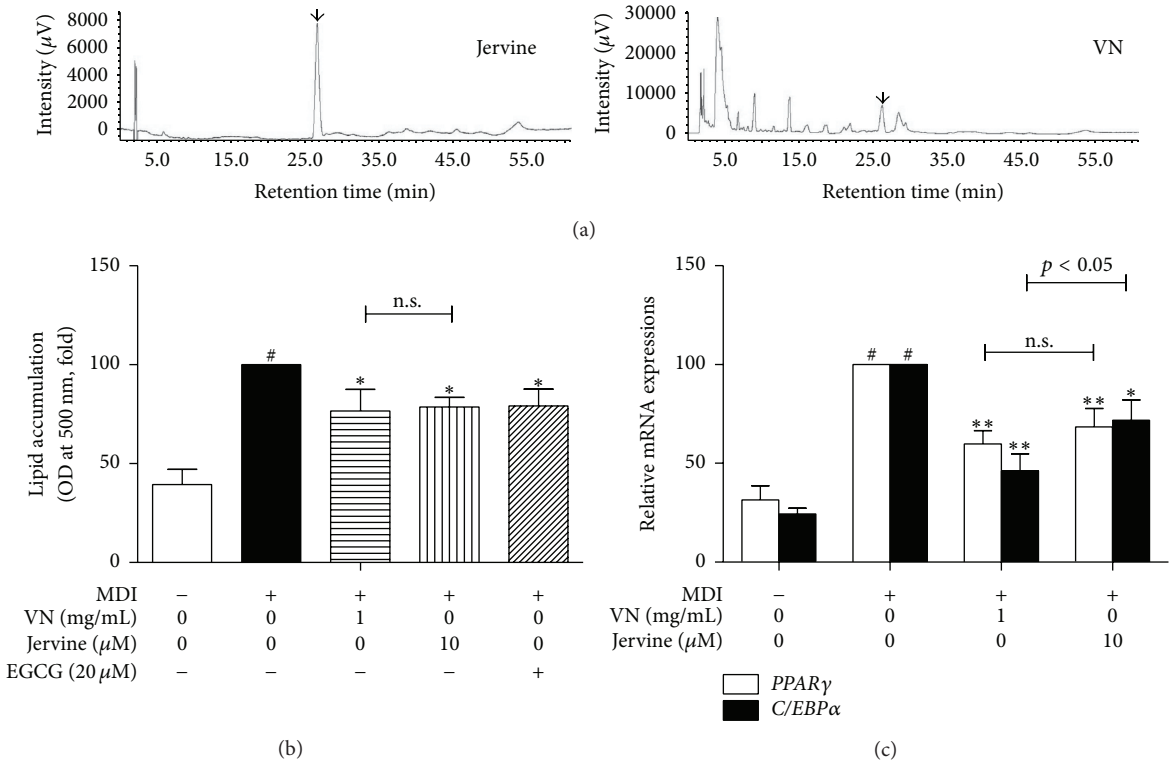

Figure 2: HPLC analysis of VN and effects of VN and its compound jervine on 3T3-L1 adipocytes. (a) HPLC-PDA measurement of VN demonstrated various chromatographic peaks. By comparing chromatographic peaks with reference chromatographic peaks, jervine was identified. (b) The effects of VN and jervine on lipid accumulation during 3T3-L1 adipogenesis were compared by an Oil Red O staining assay. (c) The effects of VN and jervine on adipogenic genes, *PPARγ* and *C/EBPα*, expressions were compared using a Real-Time RT-PCR assay. Data are expressed as mean ± SD of three or more experiments. $^{\#}p < 0.05$ versus MDI-uninduced preadipocytes, $^{*}p < 0.05$, and $^{**}p < 0.01$ versus MDI-induced adipocytes.

(pH 7.5), 0.1% sodium dodecyl sulphate (SDS), 0.1% Triton X-100, 1% Nonidet P-40, 0.5% sodium deoxycholate, 150 mM NaCl, and 1 mM phenylmethylsulfonyl fluoride. Insoluble materials were removed by centrifugation at 13,000 rpm for 20 min at 4°C. The total concentration of extracted proteins was determined using the method of Bradford [27]. The proteins in the supernatants were separated by 8% SDS-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membranes. After blocking with 10 mM Tris, 150 mM NaCl, and 0.05% Tween-20 (TBST) (pH 7.6) containing 5% skim milk for 1 h at room temperature, the membranes were washed with TBST and then incubated with the appropriate primary antibodies against PPARγ, C/EBPα, phospho-AMPKα, AMPKα, phospho-ACC, phospho-LKB1, or GAPDH at 4°C overnight. After washing with TBST, the blots were subsequently incubated with horseradish peroxidase- (HRP-) conjugated AffiniPure Goat anti-rabbit IgG (Jackson ImmunoResearch Lab., West Grove, PA, USA) or HRP-conjugated AffiniPure Goat anti-mouse IgG (Jackson ImmunoResearch Lab., West Grove, PA, USA) in 5% skim milk-TBST at room temperature for 1 h. Protein signals were developed by using the ECL Western Blotting Detection Reagent (Amersham Bioscience, Piscataway, NJ, USA). All experiments were repeated at least three times. PVDF membranes were purchased from Millipore (EMD Millipore Co., Billerica, MA, USA), and the protein assay reagents were obtained from Bio-Rad (Bio-Rad Laboratories, Hercules, CA, USA).

2.10. Statistical Analysis. Data were expressed as the mean ± standard deviation (SD). Significant differences between groups were determined using Student's *t*-test and one-way ANOVA followed by *post hoc* Tukey's multiple comparisons tests. All statistical analyses were performed using SPSS statistical analysis software version 11.5 (SPSS Inc., Chicago, IL, USA). A probability value of $p < 0.05$ was considered as statistical significance.

3. Results

3.1. HPLC Analysis of VN. For qualitative analysis of VN to confirm jervine, we performed an HPLC analysis. HPLC-PDA measurement of the ethanolic extraction of VN demonstrated various chromatographic peaks. Comparing the analyzed chromatographic peaks with reference chromatographic peaks, jervine was identified (Figure 2(a)).

3.2. Comparisons of Antiadipogenic Effects of VN and Its Compound Jervine. As the HPLC analysis identified jervine

as a compound of VN, investigation to compare the two substances was proceeded. Ahead of any further *in vitro* experiments, a cell viability test was performed. As a result, VN did not show any cytotoxicity at the concentration of 0.01–1 mg/mL (Supplementary Figure 1(a)). Due to this result, further assays were performed at concentrations of 0.01, 0.1, and 1 mg/mL. The MTS assay also showed that jervine had no cytotoxicity at the concentration up to 10 μM (Supplementary Figure 1(b)). In order to compare the effects of VN and jervine on lipid accumulation, an Oil Red O assay was performed. The assay result showed that 1 mg/mL of VN had a slightly higher inhibition rate (23.46%) on lipid accumulation than 10 μM of jervine (21.31%), but there were no significant differences between the two (Figure 2(b)). A Real-Time RT-PCR assay was performed to investigate the effects on adipogenic genes *PPARγ* and *C/EBPα*. As in Figure 2(c), VN had higher inhibition rate on both genes and, especially on *C/EBPα* expression, it showed a significantly higher inhibition rate (53.69%) compared to jervine (28.17%). These results suggest that VN and jervine both have antiobese features, while VN might have higher effects than its compound jervine. Therefore, further experiments were performed in order to investigate the effects of VN, not jervine.

3.3. VN Has Beneficial Effects on HFD-Induced Obese C57BL/6J Mice. To investigate the antiobesity effects of VN *in vivo*, an animal experiment was performed as described in the materials and methods section. As shown in Figure 3(a), VN treatment significantly suppressed body weight gain (12.37 ± 1.04 g) when compared to the HFD group (27.49 ± 1.01 g), which was even greater than the positive control, slinti group (20.59 ± 1.25 g). Furthermore, the weight of the sWATs between the VN group and HFD group showed significant difference (Figure 3(b)). The blood serum analysis revealed the beneficial effects of VN on total cholesterol, triglyceride, and LDL-cholesterol levels (Figures 3(c), 3(d), and 3(e)). In particular, the serum LDL-cholesterol level of the VN group was highly downregulated compared to that of the HFD group. In addition, in spite of the concern on toxic features of VN, the VN group did not show any toxicity in the liver and kidney as proved by the serum ALT and creatinine levels (Supplementary Figure 2).

3.4. VN Inhibits Lipid Accumulation in 3T3-L1 Adipocytes. Next, to investigate the effects of VN on adipocyte differentiation, the lipid accumulation was measured using the Oil Red O staining method. As in Figures 4(a) and 4(b), VN significantly suppressed lipid accumulation at the dose of 0.1 and 1 mg/mL, suggesting its antiadipogenic effect. Epigallocatechin-3-gallate (EGCG), a green tea compound previously reported to show antiobese features [28], was used as a positive control.

3.5. VN Modulates Adipogenic Gene Expressions in 3T3-L1 Adipocytes. Among the several related factors in adipogenesis, PPARγ and C/EBPα especially are well known as the two major regulators in managing adipogenesis [29]. Figure 5(a) shows that VN treatment suppressed *PPARγ* and *C/EBPα* gene expression at 0.1 and 1 mg/mL. Further investigations on protein levels were performed in order to confirm the antiadipogenic effects of VN. As shown in Figure 5(b), VN treatment successfully downregulated the protein levels of PPARγ and C/EBPα.

We also examined the effects of VN on adipogenic genes *aP2*, *resistin*, and *adiponectin*. The downstream target genes of *PPARγ* and *C/EBPα*, such as *aP2* and *adiponectin*, are involved in maintaining the adipocyte phenotype [30], and *resistin* has been reported as a link between obesity and diabetes [10]. These three adipokines were also downregulated by VN treatment, at a dose-dependent manner (Figure 5(c)).

LIPIN1 is an adipokine which has an important role in the regulation of cellular lipid and energy metabolism [31]. As in Figure 5(d), *LIPIN1* was suppressed by VN at a dose-dependent manner.

These results suggest the beneficial effects of VN on obesity, as it suppresses adipogenic factors expressed during the differentiation of 3T3-L1 adipocytes, at both the mRNA and protein levels.

3.6. VN Activates Phosphorylation of the LKB1-AMPKα-ACC Axis in 3T3-L1 Adipocytes. Next, we investigated whether VN can influence the SIRT1-AMPK axis. SIRT1, one of the seven mammalian orthologs (SIRT1–SIRT7), is a conserved NAD^+-dependent protein deacetylase [32], which is known to suppress adipogenesis [12]. *SIRT1* was upregulated by the VN treatment, but only at the highest concentration of 1 mg/mL (Figure 6(a)). As SIRT1 is a closely related factor to AMPKα in obesity, we assessed the effects of VN on AMPKα and its upstream and downstream targets, LKB1 and ACC.

As our hypothesis, VN treatment could induce the phosphorylation of AMPKα (Figure 6(b)). AMPKα is a key player in energy homeostasis, and its activation results in inhibition of adipocyte differentiation [13] and lipogenesis via increased ACC phosphorylation [14]. AMPKα phosphorylation was successfully activated in the VN treated cells. However, interestingly, VN treatment failed to activate phosphorylation of both the AMPK upstream kinase LKB1 and the AMPK downstream target ACC (Figure 6(b)). The Western blot results suggested that VN treatment activates phosphorylation of AMPK directly, without affecting the phosphorylation levels of LKB1 or ACC.

AICAR is an AMPK agonist and, in contrast, Compound C acts as an inhibitor of AMPK. Sullivan and colleagues reported that AICAR was able to activate AMPK in a time- and dose-dependent manner and therefore inhibit lipogenesis [14]. On the other hand, Compound C, also known in the name dorsomorphin, is reported to be the only available agent that is used as a cell-permeable AMPK inhibitor [33]. As in Figure 6(c), AICAR treatment upregulated AMPKα phosphorylation while Compound C was able to suppress the phosphorylation of AMPKα. VN treatment could not boost up the effect of AICAR but, on the other hand, it was able to show compensation to the AMPKα inhibition of Compound C and highly upregulated the phosphorylation of AMPKα. These results confirm the antiadipogenic effects of VN, supposably by its ability to activate AMPKα phosphorylation.

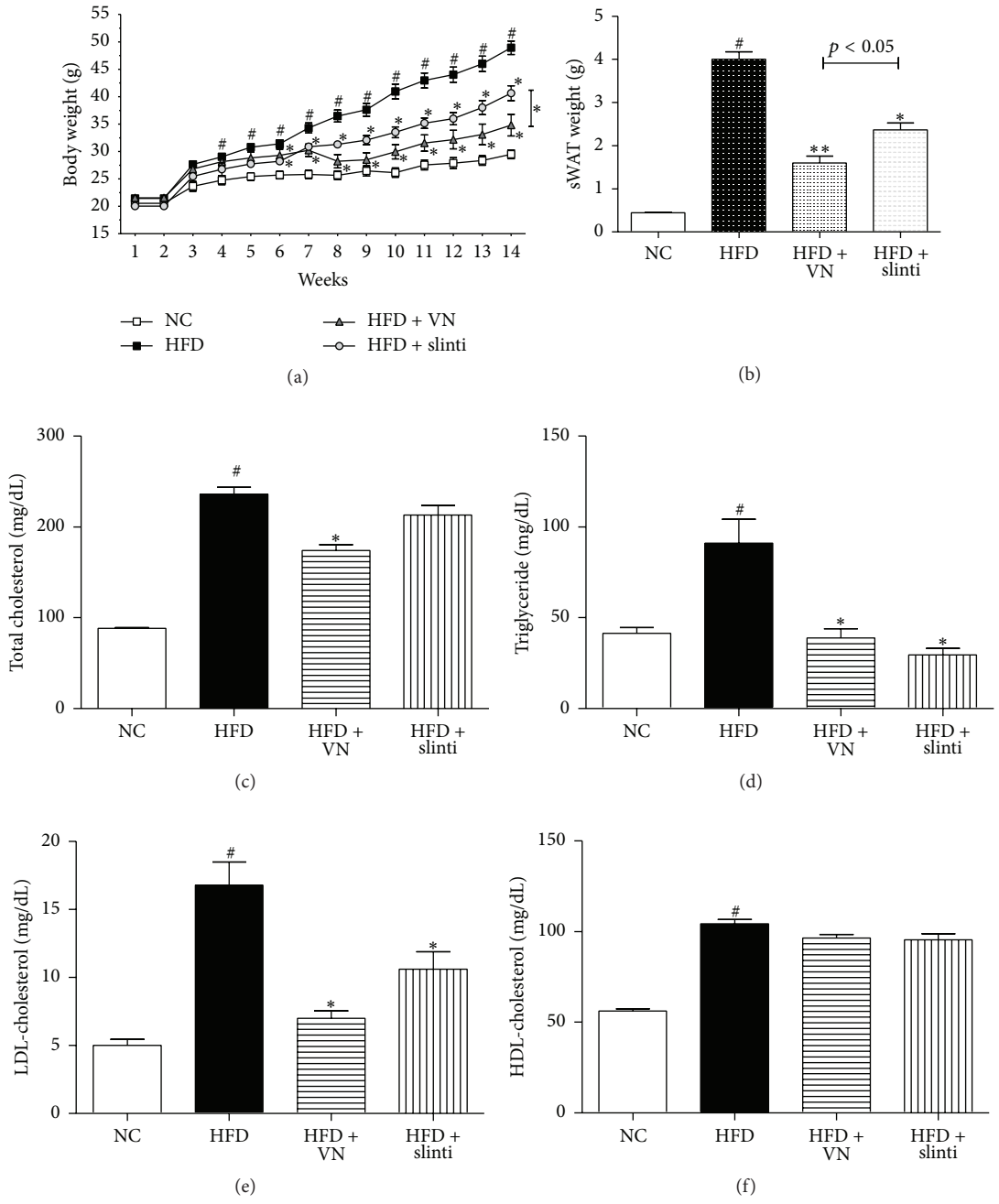

Figure 3: Effect of VN in HFD-induced obese mice. (a) The body weight changes of the NC group, HFD group, HFD + VN group, and HFD + slinti group were measured every week. (b) The subcutaneous adipose tissue weights were measured after the termination of the experiment. The serum levels of (c) total cholesterol, (d) triglyceride, (e) LDL-cholesterol, and (f) HDL-cholesterol were measured. Data are expressed as mean ± SD (n = 5–7). $^{\#}p < 0.05$ versus NC group, $^{*}p < 0.05$, and $^{**}p < 0.01$ versus HFD-induced obese group.

4. Discussion

In this study, we have evaluated the effects of VN and its constituent jervine on obesity using the *in vivo* HFD-induced obese mouse model and the *in vitro* 3T3-L1 adipocyte model, for the first time.

Obesity is a chronic metabolic disorder caused by an imbalanced energy intake-expenditure status [34]. The prevalence of obesity is growing; in the year 2008, the worldwide obesity has nearly doubled since 1980 [1]. Current medications for the treatment of obesity include mixed noradrenergic-serotonergic agents (sibutramine) [35] and absorption-reducing agents (orlistat) [36]. However, these two drugs show adverse effects at high frequencies. For example, sibutramine is reported to cause cardiac arrhythmias, constipation, and headache with only minimum weight

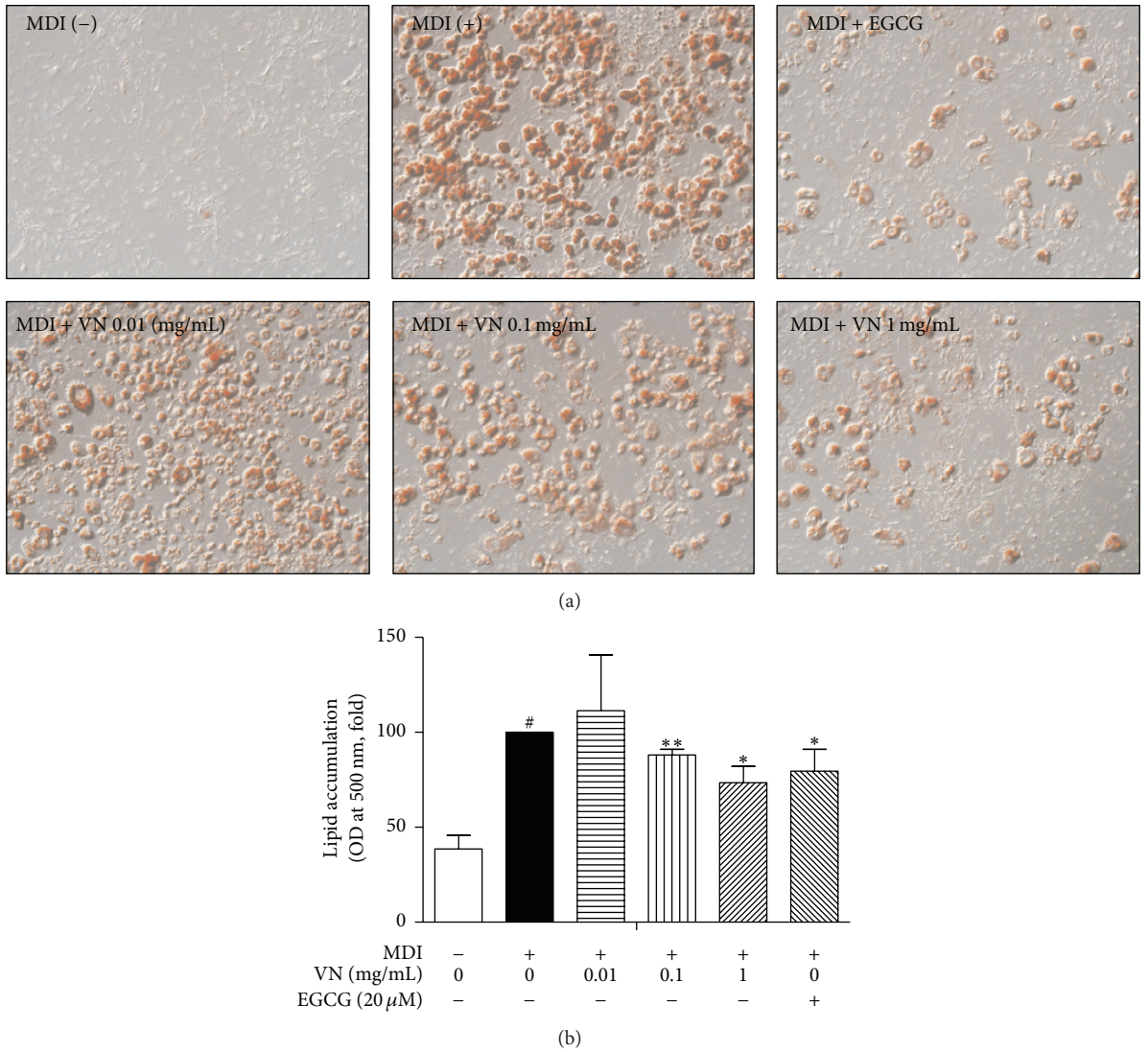

Figure 4: Effect of VN on lipid accumulation during 3T3-L1 adipocyte differentiation. (a) The lipid droplets were observed at the magnification of 100x. (b) The lipid content was quantified by resolving the Oil Red O stain in isopropanol and measuring absorbance at 500 nm. EGCG was used as a positive control. Data are expressed as mean ± SD of three or more experiments. $^{\#}P < 0.05$ versus MDI-uninduced preadipocytes, $^{*}P < 0.05$, and $^{**}P < 0.01$ versus MDI-induced adipocytes.

loss [35], and orlistat can show steatorrhea and lipid-soluble-vitamin-deficiency [36]. Due to the limits of currently available drugs, the necessity for new drugs for the treatment of obesity is rapidly growing, and the interest in natural products especially is increasing.

Veratrum nigrum L. is a medicinal plant used in Traditional Chinese and Korean Medicine native to Asia and Europe. In the plant, mainly the stem and root of *Veratrum nigrum* L., Veratri Nigri Rhizoma et Radix, are administered internally as an emetic medicine in cases of strokes or epilepsies or also topically treated in order to kill parasites or to stop pruritus [37]. But because of its toxicology, Veratri Nigri Rhizoma et Radix is not widely used, as it is difficult to prepare a safe yet effective dose [38]. Therefore, only few reports on Veratri Nigri Rhizoma et Radix are currently published. Among those studies, none has reported the effects of VN on obesity or adipogenesis.

Jervine ($C_{27}H_{39}O_3N$), a steroidal alkaloid derived from the *Veratrum* genus [39], which is reported to have antitumor effects [40, 41], was detected as an active compound of VN by the HPLC analysis. Jervine and VN both successfully suppressed lipid accumulation and expressions of adipogenic genes *PPARγ* and *C/EBPα* in 3T3-L1 adipocytes. However, the antiadipogenic effects of VN were higher than jervine, and thus further investigations were performed in order to assess the effects of VN.

As the basic *in vitro* experiments preceded suggested positive effects on obesity, an *in vivo* experiment was carried on using C57BL/6J mice. As expected, VN had beneficial effects on obesity in the animal model, too. The weight

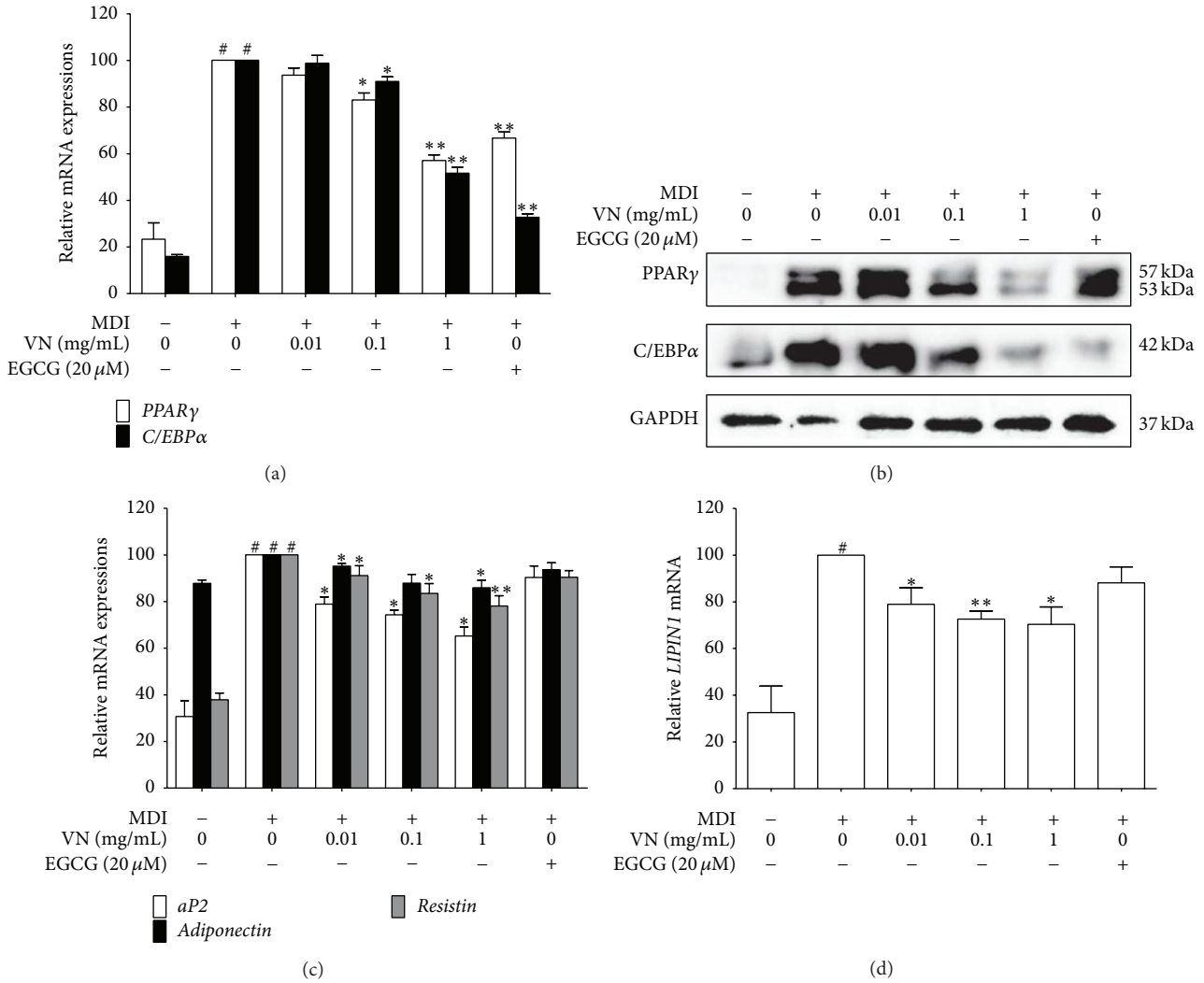

Figure 5: Effect of VN on adipogenesis-related factors in 3T3-L1 adipocytes. The mRNA expression levels of (a) *PPARγ* and *C/EBPα*, (c) *aP2*, *adiponectin*, and *resistin*, and (d) *LIPIN1* were measured by the Real-Time RT-PCR assays. (b) The expressions PPARγ and C/EBPα were measured using a Western blot assay. GAPDH was used as an endogenous control. EGCG was used as a positive control. Data are expressed as mean ± SD of three or more experiments. $^{\#}P < 0.05$ versus MDI-uninduced preadipocytes, $^{*}P < 0.05$, and $^{**}P < 0.01$ versus MDI-induced adipocytes.

gains and sWAT weights were significantly suppressed in the VN administered group. Serum analyses also confirmed the beneficial effects of VN on obesity. On the other hand, ALT and creatinine, the barometers measuring liver and kidney toxicity, respectively, were not negatively affected but showed lower levels than the HFD group. These results are conflict to the formerly known toxicity of *Veratrum nigrum* [38, 42]. The *in vivo* results do not only prove the beneficial effects of VN in obesity, but the toxicity-safe dosage of VN also shows potential application to human treatment as well, leading to expansion of the limited oral use of VN.

Based on the positive *in vivo* results on obesity, we then performed more experiments back at the cell level, in order to find out which responsible mechanism was giving the beneficial effects. First, an Oil Red O staining assay showed suppressed lipid accumulation by VN treatment. In addition, the mRNA levels of adipogenic genes including *PPARγ*, *C/EBPα*, *aP2*, *adiponectin*, *resistin*, and *LIPIN1* were downregulated. The suppression of the genes suggested the inhibiting effect on adipogenesis by VN treatment. PPARγ and C/EBPα are well known as important regulators of adipogenesis [5–7], while adipose-derived adipokines, aP2, adiponectin, and resistin possess their roles in adipocytes in lipid signaling [9], glucose regulation [43], and insulin resistance [10], respectively. On the other hand, *LIPIN1* is a candidate gene for lipodystrophy [11]. In addition to these genes, the level of *SIRT1*, the NAD^{+}-dependent protein deacetylase [12], which is able to suppress adipogenesis, was significantly upregulated by VN at the highest dose of 1 mg/mL. The elevated *SIRT1* expression suggested the effects of VN on the SIRT1-AMPKα axis, which is a key factor in the etiology of obesity.

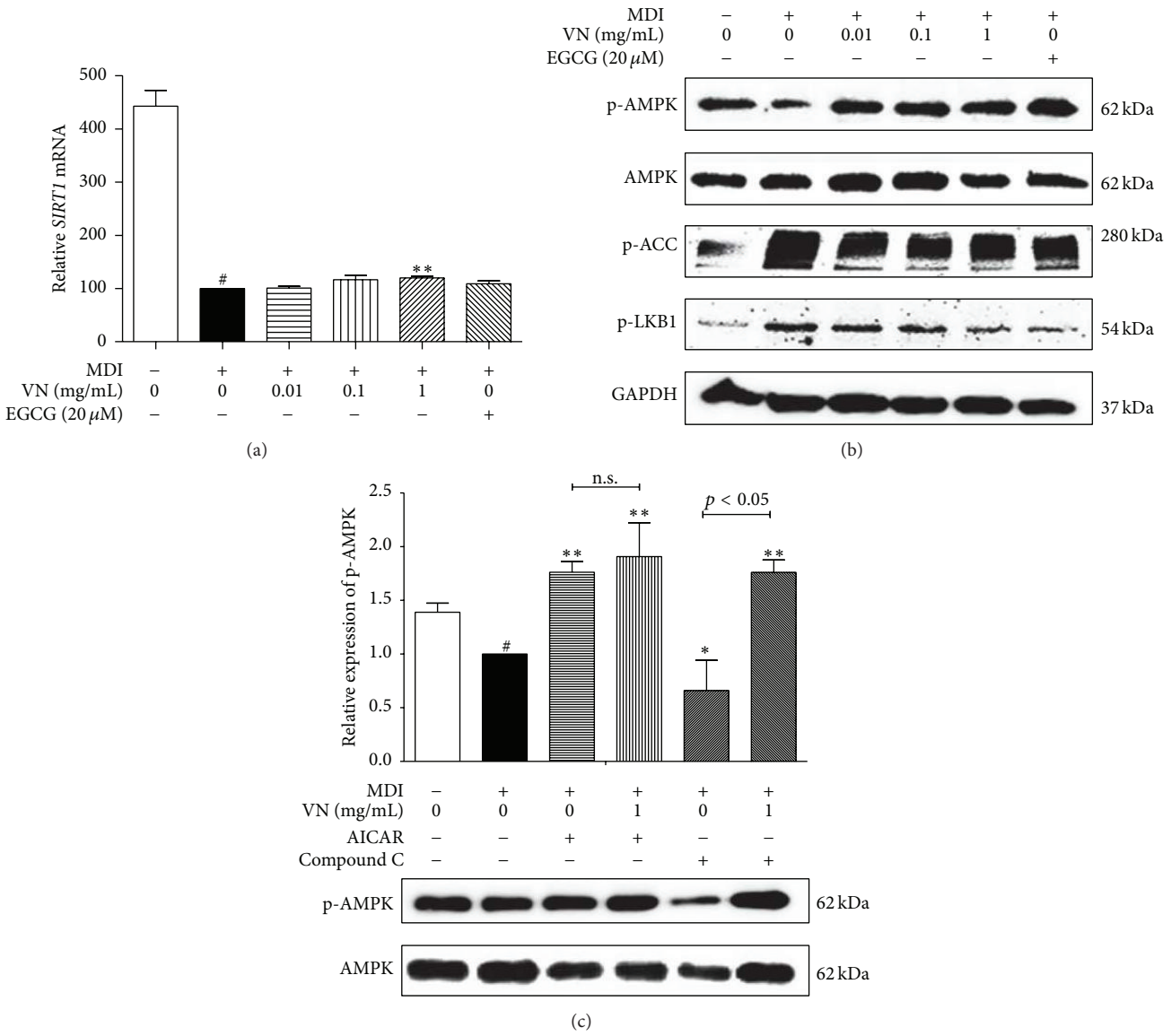

Figure 6: Effect of VN on AMPKα pathway-related factors in 3T3-L1 adipocytes. (a) The mRNA expression level of SIRT1 was measured by the Real-Time RT-PCR assay. (b) The expressions of p-AMPKα, p-ACCα, and p-LKB1 were measured using a Western blot assay. (c) The effects of VN on the AMPKα modulation when administered with AMPK activator AICAR or AMPK inhibitor Compound C were evaluated by a Western blot assay. AMPKα was used as an endogenous control for p-AMPKα measurement. GAPDH was used as an endogenous control. EGCG was used as a positive control. Data are expressed as mean ± SD. of three or more experiments. $^{\#}p < 0.05$ versus MDI-uninduced preadipocytes, $^{*}p < 0.05$, and $^{**}p < 0.01$ versus MDI-induced adipocytes.

Previous studies have reported the detailed role of SIRT1-AMPKα axis in obesity. According to Ruderman et al., AMPKα is suggested to play a central role in metabolic syndromes [44]. Other numerous studies also link the phosphorylation of AMPK to obesity in 3T3-L1 models [3, 8, 24, 45, 46]. Several genetic rodent models with a metabolic syndrome phenotype, such as *ob/ob* mice or *fa/fa* rats, show decreased AMPK activity [47], and when the decreased AMPK activity is restored by AICAR, they showed improved glucose homeostasis [48, 49]. Sirtuins, a group of histone/protein deacetylases, are regulated by the NAD^+/NADH ratio. SIRT1 is the most well-known member of this family, which is reported to respond to changes in energy expenditure [47], which is similar to AMPK. Other studies revealed that SIRT1 can activate AMPK by deacetylating LKB1, the upstream kinase of AMPK [50, 51], and vice versa AMPK can activate SIRT1 by increasing the NAD^+/NADH ratio [52]. Therefore, these previous reports suggest the important role of SIRT1-AMPKα axis, or circle, in obesity.

The Real-Time RT-PCR result showing the upregulation of the antiadipogenic gene *SIRT1* by VN treatment suggested the possible effects of VN on the SIRT1-AMPKα circle. As we expected, a Western blot analysis confirmed the effect

of VN on AMPKα phosphorylation, subsequently to the previous results. VN treatment also suppressed the expression of PPARγ and C/EBPα at the protein levels. Unlike AMPKα, however, the phosphorylation of ACC and LKB1, the upstream and downstream enzymes of AMPKα, respectively, were not upregulated as we expected. These results were conflict to our former researches, in which the protein expressions of p-ACC or p-LKB1 were successfully elevated by treatments showing antiobese features [8, 24]. LKB1, also known as serine/threonine kinase 11, is a protein kinase encoded from the *LKB1* gene [53]. Originally known as a tumor suppressor, LKB1 is also related to obesity due to its role as an upstream factor of the energy homeostasis regulator, AMPK [54]. The downstream target of AMPK, ACC, is dephosphorylated by AMPK inhibition [55], and activation of AMPK leads to inhibition of cholesterol synthesis by direct phosphorylation of ACC [56]. However, recent studies report that p-LKB1 [57] or p-ACC [58] is not essential in the cascade of AMPK phosphorylation.

In contrast to the p-ACC and p-LKB1 expressions, the effect of VN on AMPKα activation was surely confirmed, as we coadministered the AMPK activator AICAR and the AMPK inhibitor Compound C with VN. AICAR and Compound C were able to activate or attenuate the phosphorylation of AMPKα. In addition, the treatment of VN was able to restore the inhibited AMPKα phosphorylation by Compound C, nearly up to the AMPK level by AICAR activation. These cotreatment results suggest the effect of VN on the LKB1-AMPKα-ACC axis, by solely affecting the AMPK activation only.

Our results on sole phosphorylation of AMPK in the LKB1-AMPK-ACC pathway suggest that the antiadipogenic features of VN resulted from direct activation of AMPK by VN and proceed through SIRT1 activation, which leads to inhibition of PPARγ and C/EBPα. However, the detailed mechanism for how VN regulates adipogenesis regarding sole activation of AMPK within the LKB1-AMPK-ACC axis requires further investigation.

5. Conclusions

In summary, our results demonstrated that VN contains jervine, and they both can attenuate lipid accumulation during 3T3-L1 adipogenesis. VN showed beneficial effects on obesity in a HFD-induced obese C57BL/6J mouse model. In 3T3-L1 adipocytes, VN was able to attenuate adipogenic factors and upregulate SIRT1 and AMPKα phosphorylation, suggesting its ability to activate the SIRT1-AMPKα circle. These results led to further investigations involving the LKB1-AMPKα-ACC axis. VN treatment was able to compensate for the action of the AMPK inhibitor, Compound C. These results suggest the potential of VN as an AMPKα axis-modulating antiobese agent.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (NRF-2011-0012113).

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2016, Article ID 6828514, 10 pages
http://dx.doi.org/10.1155/2016/6828514

Research Article

Antihyperglycemic and Antiobesity Effects of JAL2 on *db/db* Mice

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Received 14 October 2015; Revised 30 January 2016; Accepted 8 February 2016

Academic Editor: Yew-Min Tzeng

Lonicera japonica Thunb. (LJT) and *Rehmannia glutinosa* Libosch. (RGL) have been used traditionally as a herbal medicine in Korean medicine. Using LC/Q-TOF was performed to profile the two herbal medicines and the mixture of LJR and RGL (JAL2, ratio 1 : 1). We performed oral glucose tolerance test (OGTT) and plasma GLP-1 and insulin secretion by multiplex assays to investigate antidiabetic effects of LJT, RGL, and JAL2 in *db/db* mice, the mice model of type 2 diabetes mellitus (T2DM). Also, the antiobesity-related factors such as plasma peptide YY (PYY), triglyceride, total cholesterol, HDL, LDL, and weight of liver, epididymal, and retroperitoneal fat tissue were investigated. Through the multiplex assay, it was found that JAL2 treatment more efficiently attenuated high levels of blood glucose by stimulating GLP-1 secretion and reduced LDL concentration and weight of liver and retroperitoneal fat tissue compared to LJT or RGL treated separately. These results suggest that the JAL2 has antidiabetes and antiobesity effects in T2DM mice model.

1. Introduction

Obesity, characterized by excess accumulated body fat, may result in a negative effect on health and lead to reduction in life expectancy [1]. Currently, the number of people afflicted with obesity has increased due to nutritionally rich diets, physical inactivity, and genetic susceptibility [2]. Obesity is associated with lots of diseases, that is, type 2 diabetes mellitus (T2DM), cardiovascular disease, obstructive sleep apnea, certain types of cancer, osteoarthritis, and asthma [1, 3]. Particularly, T2DM may be the most serious among them [4, 5].

The symptoms of high blood glucose level include frequent urination, increased thirst, and increased hunger; diabetes has become one of the principle causes of morbidity and mortality [6]. T2DM, the far more common type of diabetes, is the state of insulin resistance whose cells fail to respond to insulin properly [6, 7].

Insulin resistance and common property such as hypertension, hyperlipidemia, and abdominal obesity are common phenomenon in T2DM and obesity [8]. The prevalence rate of people who have both T2DM and obesity has rapidly increased all over the world every year, and the rates of increase show no signs of slowing [1, 2, 9]. Even though T2DM and obesity are a risk factor for cardiovascular disease, most of the treatment of metabolic syndrome only focuses on the reduction of blood glucose levels or the regulation of body weight.

Glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) are gastrointestinal (GI) hormone that both are secreted from enteroendocrine L cells [10, 11]. GLP-1 has been thoroughly studied as a potential attenuating hyperglycemic agent because its functions induce glucose-dependent insulin secretion from pancreatic β cells and insulin sensitivity levels [10, 12]. PYY acts to regulate appetite by inhibiting gastric motility and raising the water absorption in the colon [11, 13]. Retarding gastric emptying and acting as satiety factors of both GLP-1 and PYY have physiologically significant functions of relevance to obesity and T2DM.

Lonicera japonica Thunb. (LJT) and *Rehmannia glutinosa* Libosch. (RGL) occupy the highest proportion of the nine

herbal medicines that make up the Yangkyuksanhwa-tang. Yangkyuksanhwa-tang, prescribed to patients with diabetes in Korea, consists of nine herbal medicines: LJT, RGL, Forsythiae Fructus, Gardeniae Fructus, Menthae Herba, Anemarrhena Rhizome, Gypsum Fibrosum, Schizonepetae Herba, and Ledebouriellae Radix [14]. Methanol extract of *Lonicera japonica* flower has been investigated for having suppression effect of body weight gain and body fat increase [15]. Aqueous extract of RGL has been closely studied for stimulating the expression of proinsulin gene in T2DM rats [16].

In this study, we investigated the mixture of LJT and RGL (JAL2, 1 : 1) and whether 30% ethanol extracts of JAL2 attenuates hyperglycemia and reduces the body weight via stimulating GI hormones such as GLP-1 and PYY, respectively, using *db/db* mice, which has the characteristics of T2DM. This study provided the important information of the JAL2 effects to enhance GLP-1 and PYY secretion and the possibility that the herbal medicine mixture may be used as a therapeutic agent of T2DM and obesity.

2. Materials and Methods

2.1. Preparation of 30% Ethanol Extract of LJT, RGL, and JAL2. LJT, RGL, and both herbal medicine mixture were purchased from Hamsoapharm (Hamsoa Pharmaceutical Co., Ltd., Seoul, South Korea). The herb was cut down in a proper size and extracted as follows: each medicinal herbal medicine and the mixture were performed using reflux extraction with 30% ethanol (30% EtOH) for 3 h at 75°C. Filtration and evaporation were then conducted with a rotary vacuum evaporator (N-N series, EYELA, Japan) at 55°C. The solution was freeze-dried for 24 h at −80°C and lyophilized.

2.2. Analysis of LJT, RGL, and JAL2 Extracts by Using LC/Q-TOF. LC/Q-TOF was conducted at the Korea Basic Science Institute (KBSL, Seoul, Korea). Chromatographic separation of the two herbal medicines and JAL2 by Agilent 1290/6550 (Agilent Technologies, Waldron) was performed using a Waters BEH C_{18} column (2.1 mm × 150 mm ID, 1.7 μm, Agilent). The mobile phase consisted of solvent A (water) and solvent B (acetonitrile), both containing 0.1% formic acid. The flow rate of the mobile phase was 300 μL/min and the gradient program was as follows: 0–15 min (0–40% B), 15–20 min (40–95% B), and 20–27 min (95–0% B), after which the column was equilibrated with 0% B for 5 min. Samples of 1 μL were injected into the column using an autosampler. The HPLC system was interfaced to the MS system, an Agilent 6550 Accurate-Mass Q-TOF (Agilent Technologies, Santa Clara) equipped with a Dual AJS ESI source operating in positive ion mode. Mass spectra were acquired at a scan rate of 1 spectrum/s with a mass range of 50 to 1,000 *m/z*.

2.3. Animal. The *db/db* mice were used as a murine model exhibiting hyperglycemia and overweight which are typical phenotypes of T2DM and obesity. Seven-week-old male *db/db* mice with a C57BL/6 background were obtained from the Daehan Bio Link (Daehan Bio Link Co., Ltd., Eumseong-gun, Chungcheongbuk-do, South Korea). All animal studies were performed according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Kyung Hee University (confirmation number: KHUASP(SE)-14-046). The animals were housed in the animal room where the condition of a 12 h light-dark cycle at moderate temperature (21–23°C) and humidity levels (55–60%) are maintained. All mice were acclimated for one week before the experiment. During the acclimation period, all mice were fed with the standard rodent chow (28.507% of protein, 13,496% of fat, and 57.996% of carbohydrates (LabDiet, St. Louis, MO)) and water *ad libitum*. After acclimation period, eight-week-old mice ($n = 5$ per each group) were divided into five groups: control (saline treatment), 280 mg/kg/day of metformin treatment, 30 mg/kg/day of LJT treatment, 30 mg/kg/day of RGL treatment, and 30 mg/kg/day JAL2 treatment group.

2.4. Oral Glucose Tolerance Test (OGTT). At the seventh week of the experiment, all mice were fasted for 16 h before the OGTT. Each group of mice was orally administrated saline, metformin, LJT, RGL, or JAL2, after which 5 g/kg of glucose was orally administrated. Using an Accu-Chek Performa device (Roche Diagnostics, Mannheim, Germany), the blood glucose levels were measured from the tail vein at 6 time points: before the glucose gavage (time point 0 min), 10 min after the glucose gavage (time point 10 min), 20, 40, 90, and 120 min.

2.5. Mouse Metabolic Hormones Multiplex Assay. After OGTT, all mice were allowed to rest for four days to prevent hypotensive or hemorrhage shock. After resting phase, all mice were fasted for 16 h before the blood sampling. Each mouse group was orally administrated saline, metformin, LJT, RGL, or JAL2 just before the glucose gavage (2 g/kg). The blood samples were collected from the tail vein of each mouse at 5 time points (before the glucose time point: 0 min, 10 min after the glucose gavage: 10 min, 20, 30, and 40 min) and were transferred to an EDTA-coated 1.5 mL microcentrifuge tube containing a dipeptidyl peptidase- (DPP-) IV inhibitor (EMD Millipore Co., Billerica, MA) and a protease inhibitor cocktail (Roche Diagnostics) to protect against GLP-1 degradation and blood coagulation, respectively. Collected blood samples were centrifuged at 1,000 ×g for 10 min at 4°C. The plasma samples were carefully transferred to the fresh tube. The Milliplex Map Kit Mouse Metabolic Hormones Magnetic Bead Panel: GLP-1, insulin, and PYY, (EMD Millipore Co., Billerica, MA) was performed as described in the manufacturer's guidelines. The experimental plates were run using the MAGPIX instrument (Bio-Rad, Hercules, CA) and data were extracted with Luminex xPONENT software. Using Bio-Plex Manager software (Bio-Rad), preliminary data was checked and analyzed.

2.6. Measurements of Plasma Triglycerides and Cholesterol Levels. After eight weeks of treatment, whole blood of each mouse was collected via the retroorbital plexus to BD Vacutainer® Plus Plastic K_2 EDTA tubes (BD Biosciences, San Jose, CA) and centrifuged 1,000 ×g for 20 min at 4°C. The supernatant was transferred to a fresh tube for determination.

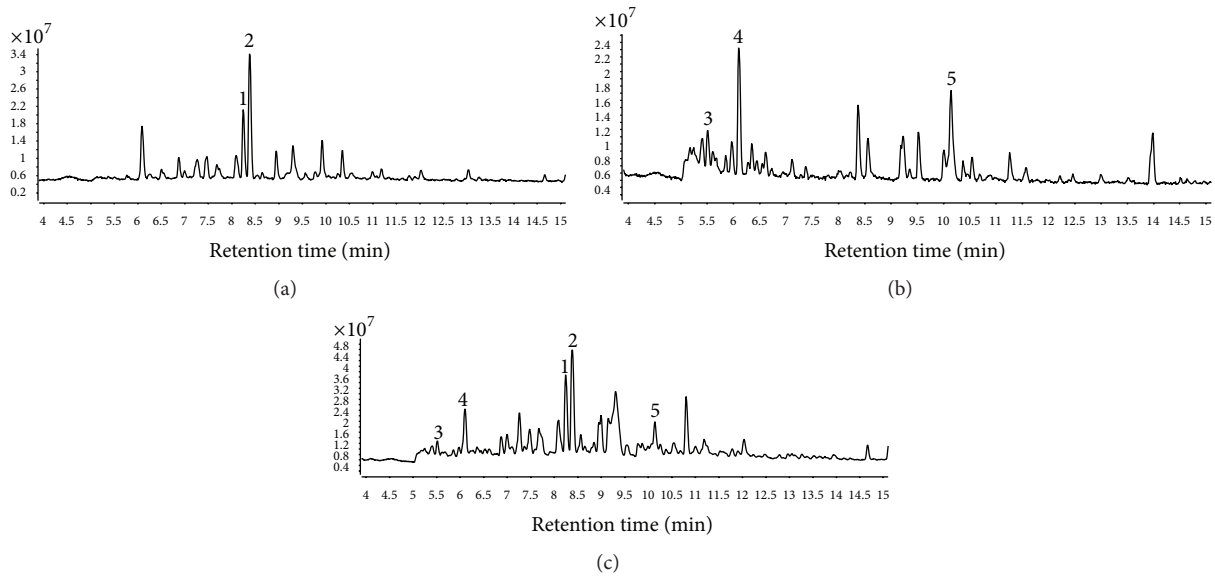

Figure 1: LC/Q-TOF profiling of LJT, RGL, and JAL2. To profile LJT, RGL, and the mixture of LJT and RGL (JAL2), LC/Q-TOF was performed. (a) Sweroside (1) and loganin (2) were identified in the LJT extract. (b) In the RGL extract, rehmannioside D (3), leonuride (4), and rehmaionoside A/B (5). (c) All aforementioned components were identified in JAL2.

Table 1: Characterization of LJT, RGL, and JAL2 compounds by using LC/Q-TOF.

Peak number	Compound	Formula	RT	Mass	$(M + H)^+$ m/z	Sample
1	Sweroside	$C_{16}H_{22}O_9$	8.389	358.1264	359.1337	LJT/JAL2
2	Loganin	$C_{17}H_{26}O_{10}$	8.243	390.1526	391.1599	LJT/JAL2
3	Rehmannioside D	$C_{27}H_{42}O_{20}$	5.511	686.2342	687.2342	RGL/JAL2
4	Leonuride	$C_{15}H_{24}O_9$	6.107	348.142	349.1493	RGL/JAL2
5	Rehmaionoside A/B	$C_{19}H_{34}O_8$	10.143	390.2254	391.2326	RGL/JAL2

Plasma TGs, TC, LDL, and HDL levels were analyzed at the Seoul Medical Science Institute (SCL, Seoul, South Korea).

2.7. Measurements of Liver, Retroperitoneal Fat, and Epididymal Fat Tissue Weights. On the day of the experiment, all groups of mice were sacrificed and the livers and epididymal fat and retroperitoneal fat tissues of each mouse were enucleated and weighed.

2.8. Statistical Analysis. All data are represented as mean ± SEM. IBM SPSS Statistics 22 software (IBM Corporation, Armonk, NY) and Graphpad Prism 5 software (Graphpad Software, San Diego, CA) were used for statistical analysis and graphics. The statistical significance of area under curve (AUC), blood profiling, and weight of tissues graphs was measured by Mann-Whitney *U* test (one-tailed). The generalized estimating equation for repeated-measures was used to analyze the blood glucose levels and GI hormones levels to detect group-by-time interactions. Using the Mann-Whitney *U* test was done for the intergroup comparison of OGTT and plasma hormones at each time point. A group-by-time interaction was found by the generalized estimating equation (GEE).

3. Results

3.1. LC/Q-TOF Analysis of 30% EtOH Fraction of LJT, RGL, and JAL2. LC/Q-TOF was performed to profile the two herbal medicines and herbal medicines mixture (Figure 1). Among the several prominent peaks, sweroside and loganin were identified as the highest peak in the LJT extract (Figure 1(a)). In the RGL extract, rehmannioside D, leonuride, and rehmaionoside A/B were identified (Figure 1(b)). All compounds mentioned above were determined in the JAL2 extract (Figure 1(c)). Table 1 contains more detailed information about JAL2 including the LJT and RGL extract metabolites.

3.2. Hyperglycemia Attenuating Effects of LJT, RGL, and JAL2 on db/db Mice. The hyperglycemic attenuating effects of LJT, RGL, or JAL2 were investigated using the T2DM rodent model, *db/db* (Figure 2). A group-by-time interaction was noted. Blood glucose levels in most groups were peaked at 20 min from the glucose gavage process. The blood glucose levels of the LJT-treated mice showed variation tendencies similar to those of the saline-treated mice (Figure 2(a)). The RGL-treated group, compared to the saline-treated

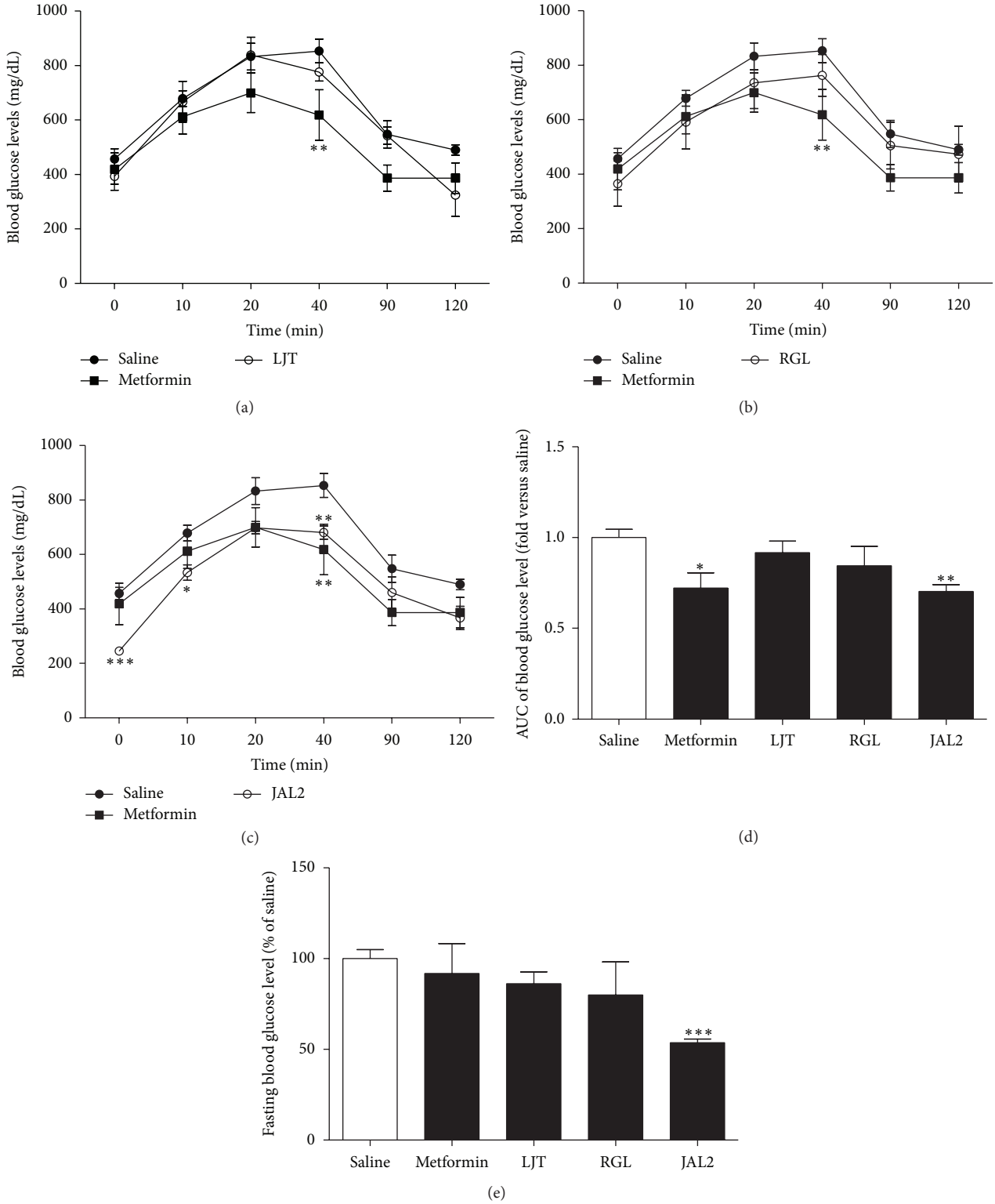

Figure 2: Effects of LJT, RGL, and JAL2 on blood glucose levels change and fasting blood glucose level. To test regulatory effect of LJT (a), RGL (b), and JAL2 (c), LJT, RGL, and JAL2 were orally administrated just before glucose gavage (5 g/kg) to the *db*/*db* mice. Metformin used as a positive control. (d) The variation of blood glucose levels of each group is indicated by a bar graph. (e) Fasting blood glucose level of each group is indicated by a bar graph. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, compared with saline-treated group by Mann-Whitney U test. Results are represented as means ± SEM. $n = 5$ for each group.

group, showed maintaining lower blood glucose levels at all times without significance (Figure 2(b)). Mice treated with JAL2 were found to exert the highest hypoglycemic effect compared to the saline-treated mice (Figure 2(c)). Metformin, known to lower blood glucose levels, was also shown to compare the efficiency with those of LJT, RGL, and JAL2 (Figures 2(a)–2(c)). AUC for representing the blood glucose levels changes during OGTT was presented to make a comparison between the groups treated with saline, met, LJT, RGL, or JAL2 (Figure 2(d)). There was a trend toward decreased fasting blood glucose levels by LJT and RGL. In the JAL2-treated group, the mixture of LJT and RGL, the fasting blood glucose levels significantly decreased compared to the saline-treated group (Figure 2(e)).

3.3. Regulation Effects of LJT, RGL, and JAL2 on the GI Hormones Secretion. To investigate the regulation effects of LJT, RGL, and JAL2 on the GI hormones secretion, GLP-1, insulin, and PYY, plasma was separated from the collected blood samples obtained at each time point (0, 10, 20, 30, and 40) (Figure 3). For plasma GLP-1, insulin, and PYY, a group-by-time interaction was found by the GEE. Plasma GLP-1 concentration levels were increased in all herbal medicines and met treated mice compared to the saline-treated mice: specifically, the mice are treated with JAL2 or met significantly more secreted GLP-1 20 min after glucose gavage process (2 g/kg) (Figure 3(b)). Therefore, insulinotropic action may be led by the stimulated GLP-1. Insulin secretion was increased by all herbal medicines at all times, but metformin-treated mice less secreted insulin compared to the saline-treated mice 10 min after glucose gavage process (Figure 3(d)). PYY secretion was also increased by all herbal medicines and metformin compared to the saline-treated mice. Interestingly, each LJT- or RGL-treated mouse demonstrated significant increased PYY secretion at 30 and 40 min, respectively, after glucose gavage process (Figure 3(e)). The concentration of PYY was significantly increased both 30 and 40 min after glucose gavage process in the JAL2-treated mice including LJT and RGL (Figure 3(f)).

3.4. Effects of LJT, RGL, and JAL2 on Blood Profiles in db/db Mice. At the end of the treatment, whole blood of each mouse was collected from which plasma was isolated for investigating the effects of the LJT, RGL, and JAL2 on the obesity-related blood factors: plasma triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) levels. Each LJT or RGL treatment did not result in significantly reducing the obesity associated factors, while the JAL2 significantly reduced TC and LDL-c levels (Figure 4).

3.5. Effects of LJR, RGL, and JAL2 on Liver and Fat Tissues Weight. To investigate the effects of LJT, RGL, and JAL2 on the liver and fat tissues weights, each mouse was sacrificed, after which the liver and fat tissues of each mouse were collected and weighed just after blood collection (Figure 5). Single-treatment of the LJT or RGL did not result in significantly reducing the weights of the liver and fat tissues. However, the weights of the liver and retroperitoneal fat tissues were significantly decreased by mice with JAL2 treatment.

4. Discussion

Abdominal obesity, raised fasting plasma glucose, and high TG levels are medical conditions followed by metabolic syndrome, related to the risk of developing cardiovascular disease (CVD) and diabetes [17–19]. Constantly, many researchers have studied to reduce the risk of metabolic disorder such as attenuating high blood glucose levels or reducing body mass index (BMI). Due to the side effects of the drugs, the purpose of a single target disease like antiobesity or antidiabetes has been investigated [20]. For example, diguanides, thiazolidinedione, and alpha glucosidase inhibitors, used for antidiabetic agents, result in renal failure, hypoglycemia, liver toxicity, abdominal distention, and abnormal weight gain. On the other hand, antiobesity agent, sibutramine, causes the cardiac vascular disease [21–23].

Therefore, oriental medicine has begun to attract the attention because of its excellent efficacy and safety compared to current drugs [24]. In this study, we investigated how the LJT, RGL, and JAL2 affected obesity and T2DM related factors such as body weight, body compositions, blood glucose level changes, and GI hormones on *db/db* mice, which have both phenotypes of obesity and T2DM [25]. Mice treated with JAL2 showed less increase changes of blood glucose levels and significant reduction of fasting blood glucose level compared to the saline-treated mice and metformin-treated mice, known to be a T2DM agent (Figures 2(c) and 2(e)). Metformin treatment did not affect fasting blood glucose levels compared to saline-treated *db/db* mice, which may be associated with an improvement in glycocalyx barrier properties [26].

In recent year, there is new trend of drugs that excite to release GLP-1 secretion or inhibit the GLP-1 degradation. GLP-1, a GI hormone, regulates the insulin secretion, appetite, and gut motility. Also, the insulin sensitivity of pancreatic beta-cells is enhanced by GLP-1 [27–29]. GLP-1 secretion in mice treatment with JAL2 was significantly increased (Figure 3(b)). Although the significance was not determined, a trend toward higher levels of plasma GLP-1 secretion during the feeding periods of LJT and RGL was determined. The two herbal medicines and JAL2 led to increase of GLP-1 secretion in *db/db* mice, thereby stimulating the insulin secretion (Figures 3(b) and 3(d)) [10]. The maintained lower fasting blood glucose levels and significantly stimulated GLP-1 secretion may prove the improvement in insulin resistance capabilities of JAL2 treatment in *db/db* mice. Another GI hormone, PYY, known to be a pancreatic peptide YY_{3-36}, plays a role in decreasing appetite through inhibiting gastric motility [11, 30]. The two herbal medicines and JAL2 increased PYY secretion and had significance (Figure 3). The results of this study demonstrate the possibility of the JAL2 as a weight controller via reducing appetite.

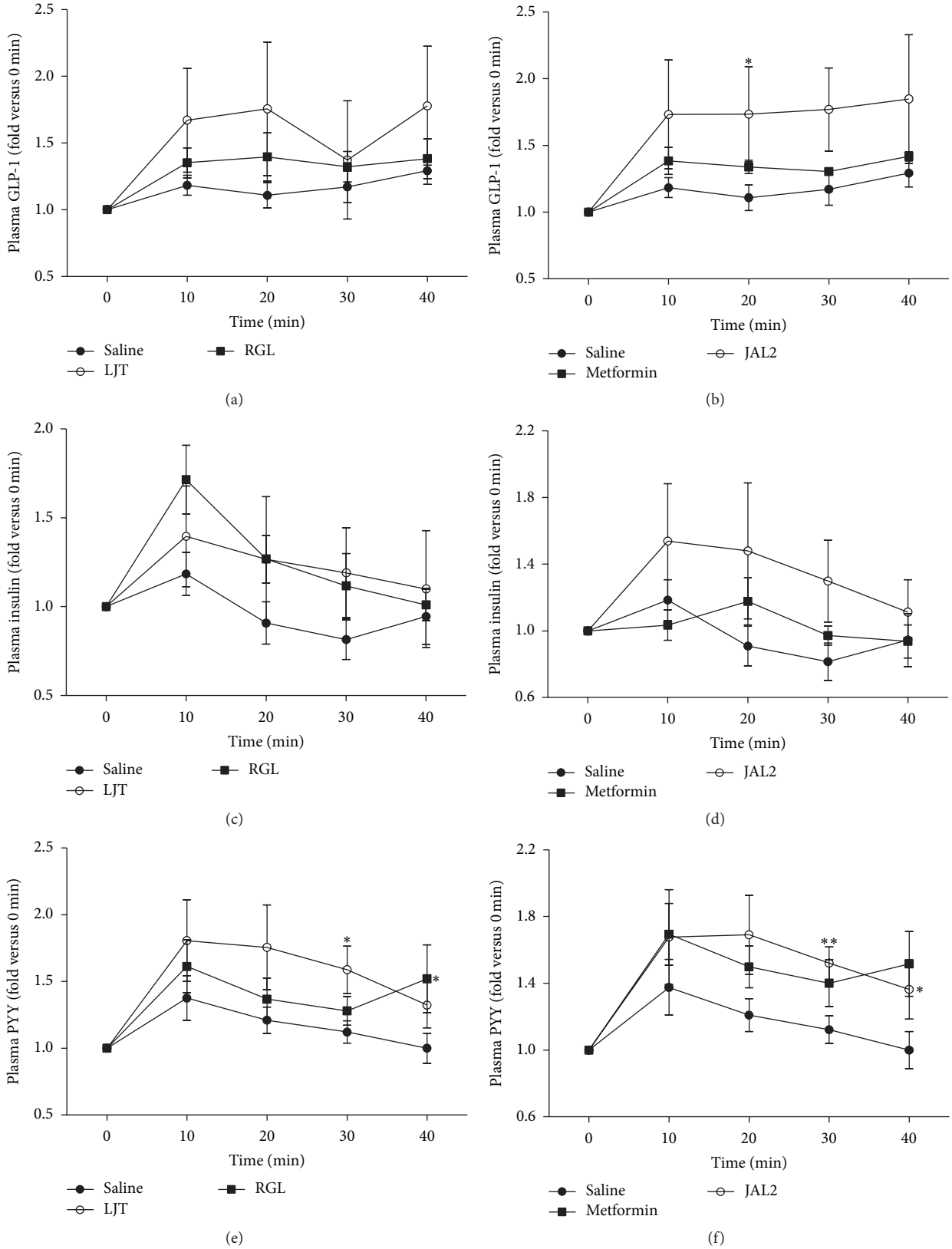

Figure 3: Plasma GI hormones change by LJT, RGL, and JAL2 administration. To test the stimuli effect of LJT, RGL or JAL2 was given to *db/db* mice. Before glucose administration (2 g/kg), LJT, RGL, or JAL2 was orally administrated. (a-b) Plasma GLP-1 secretion variation levels after glucose gavage. (c-d) Plasma insulin secretion variation levels after glucose gavage. (e-f) Plasma PYY secretion variation levels after glucose gavage. Metformin was used for positive control. $^{*}P < 0.05$; $^{**}P < 0.01$, compared with saline-treated group by Mann-Whitney U test. Results are represented as means ± SEM. $n = 5$ for each group.

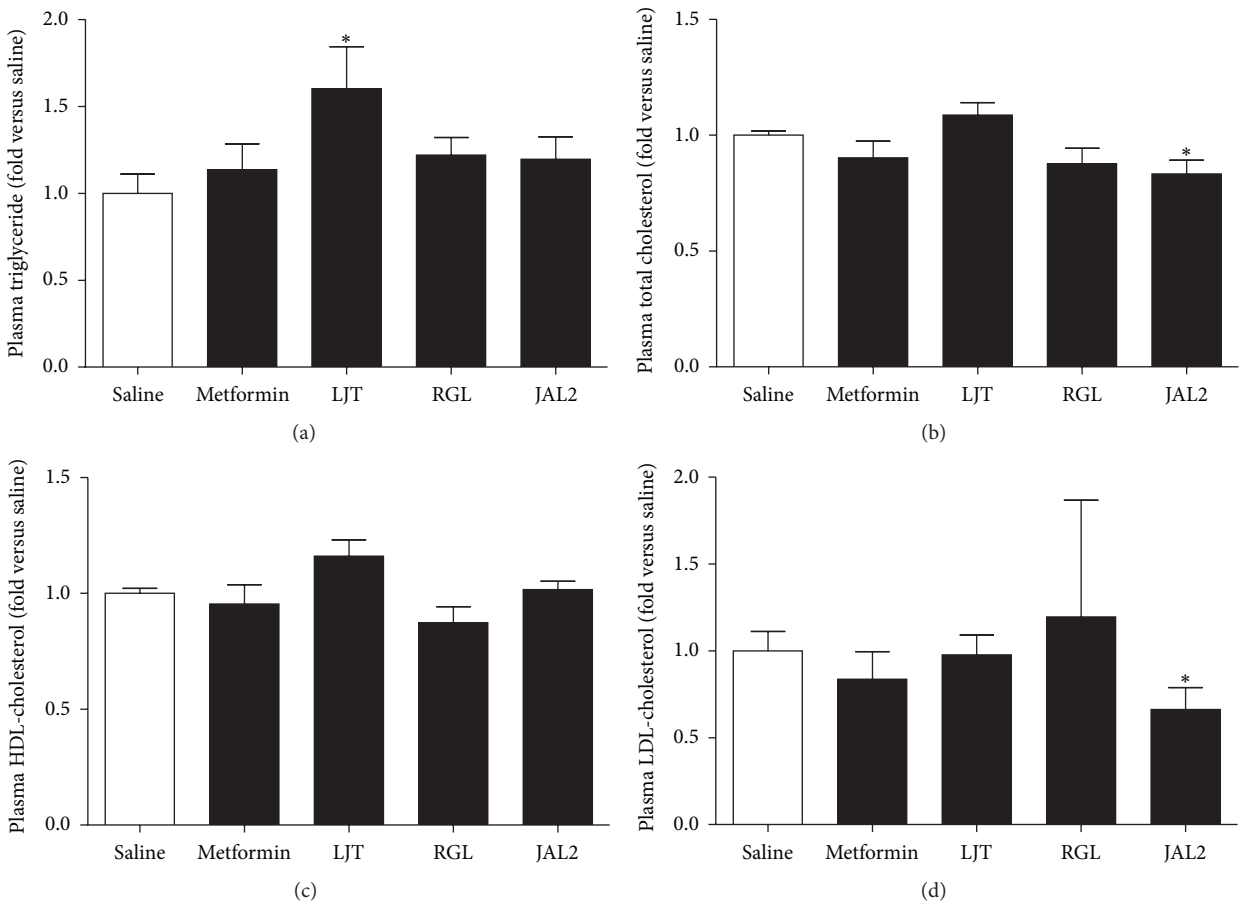

Figure 4: Plasma concentration of TGs, TC, HDL, and LDL in response to LJT, RGL, and JAL2. To study antiobesity effects of LJT, RGL, and JAL2 on obesity-related factors: TGs (a), TC (b), HDL (c), and LDL (d). $^{*}P < 0.05$ compared with saline-treated group by Mann-Whitney U test. Results are represented as means ± SEM. $n = 5$ for each group.

TG, relevant to the accumulation of lipid repository in the liver, is concerned with metabolic syndrome and T2DM [31]. However, plasma TG levels were significantly increased in LJT-treated *db/db* mice (Figure 4(a)). A study reported that pioglitazone, an antidiabetic agent, successfully restored the insulin secretory capacity of pancreatic β-cells of obese *db/db* mice, but TG levels were not affected when compared to nontreated group [32]. Also, there was a report that metformin showed a side effect by increasing TG levels in T2DM patients [33]. It demonstrates that diabetes improving effect may be largely associated with TG levels. Lower total and LDL-cholesterol levels were found in the JAL2-treated group compared to saline-treated group (Figures 4(b) and 4(d)). The results indicate an improvement in lipid accumulation in *db/db* mice by JAL2.

Obesity, a medical condition in the excessive accumulation of body fat, is designated by BMI and total cardiovascular risk factors [34]. We investigated the effects of LJT, RGL, and JAL2 by weighing the liver, retroperitoneal fat, and epididymal fat tissues (Figure 5). Function of the liver is essential for maintaining blood glucose levels by supplying glucose as an energy source to the organs [35]. However, the rise of obesity or diabetes causes nonalcoholic fatty liver disease (NAFLD) which is a condition that causes serious liver complications [36]. For the liver, the weight of the liver tissue was significantly reduced in JAL2-treated mice (Figure 5(a)). Also, in retroperitoneal fat tissue, JAL2 significantly decreased the weight of retroperitoneal fat tissue compared to saline-treated group (Figure 5(c)). Epididymal fat, considered as a key marker of the alteration of white adipose tissue, is one of several adipose depots that compose the visceral fat, places inside the abdominal cavity [37–39]. The significance was not determined; only mice treated with JAL2 demonstrated decreasing trends toward the weights of the epididymal fat tissue (Figure 5(b)).

In conclusion, most of the drugs that aim to treat metabolic syndrome only improve one symptom such as antidiabetes or antiobesity. In this study, diabetic and obesity-related factors studies proved that JAL2 (the mixture of LJT and RGL) possessed a synergistic effect of LJT and RGL, which showed significantly improved glucose tolerance by stimulating GLP-1 secretion and inhibited the undue accumulation of body fat. Although the exact mechanism of synergy between the two herbs still requires further studies, our

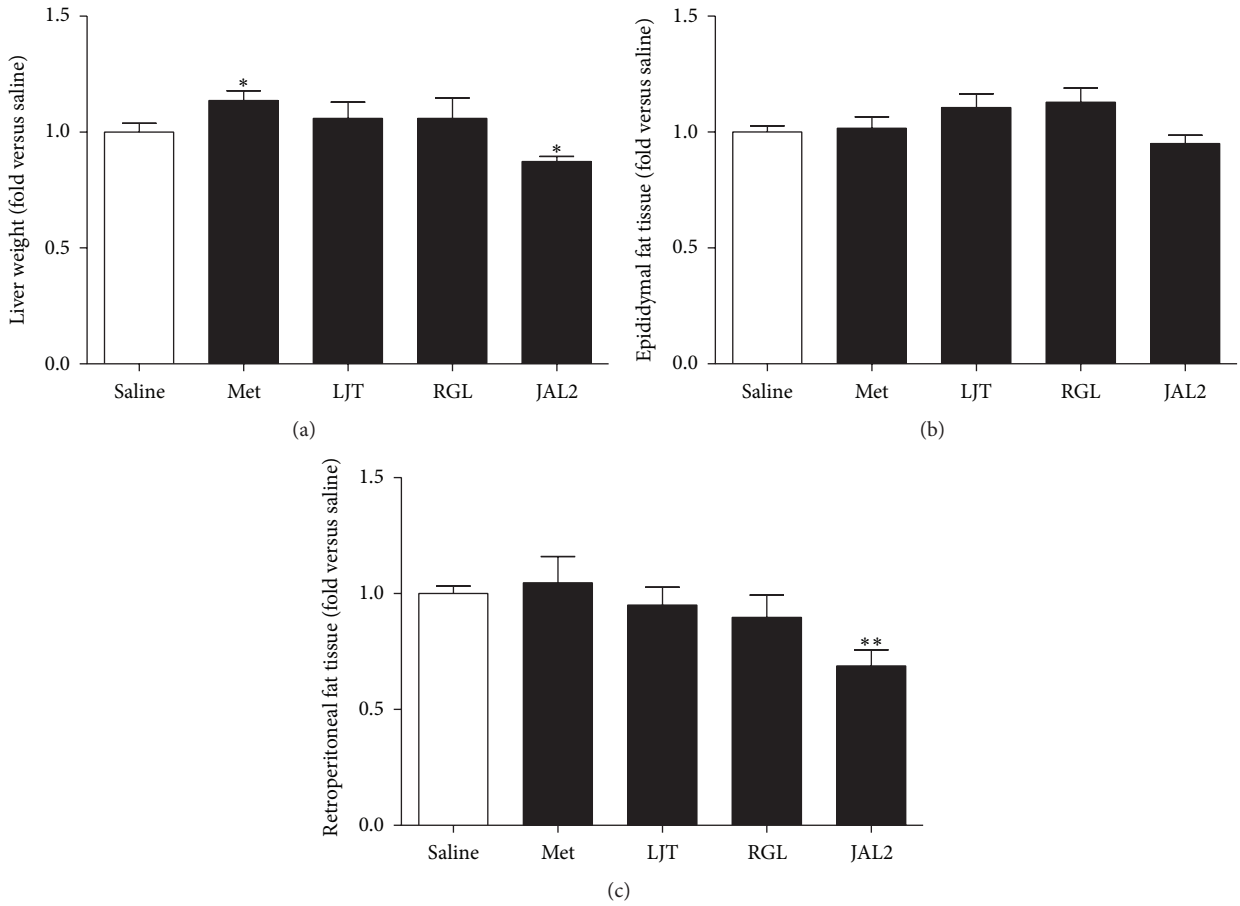

Figure 5: Weight of liver, epididymal, and retroperitoneal fat tissue of each experimental group. Another obesity-related factors, weight of liver (a), epididymal fat (b), and retroperitoneal fat tissue (c), of each group were measured. The significance of each experimental group was compared to the saline-treated group by Mann-Whitney U test. $^{*}P < 0.05$; $^{**}P < 0.01$. Results are represented as means ± SEM. $n = 5$ for each group.

results recommend that JAL2 might be helpful in attenuating high blood glucose levels and reducing accumulated body fat in patients with obese-T2DM and prediabetic patients.

Conflict of Interests

The authors declare that there is no conflict of interests.

Authors' Contribution

In-Seung Lee and Ki-Suk Kim contributed equally to this work.

Acknowledgment

This research was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (Grant no. HI13C22960000).

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2016, Article ID 9417362, 8 pages
http://dx.doi.org/10.1155/2016/9417362

Research Article

The Preventive Effect of Zuogui Wan on Offspring Rats' Impaired Glucose Tolerance Whose Mothers Had Gestational Diabetes Mellitus

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Received 21 September 2015; Revised 15 December 2015; Accepted 18 January 2016

Academic Editor: Won-Seok Chung

In this experiment, we used streptozotocin (STZ) to establish a model of gestational diabetes mellitus (GDM) rats, where Zuogui Wan was given to GDM rats. After pregnancy, offspring rats were divided into 4 groups: control group, high fat and sugar as the control group, GDM group, and Zuogui Wan GDM group. Rats in high fat and sugar as the control group, GDM group, and Zuogui Wan GDM group were fed with high fat and sugar diet. Rats in control group were fed the basic diet. The means of 2hPG were higher than 7.8 mmol·L^{-1} and lower than 11.1 mmol·L^{-1} on the rats of GDM group on week 15, and IGT models were successful. Body weight, abdominal fat weight, the ratio of abdominal fat weight and body weight, fasting plasma glucose, 2hPG, insulin, leptin, total cholesterol, and low density lipoprotein (LDL) of Zuogui Wan GDM group were significantly lower than GDM group. The level of adiponectin in Zuogui Wan GDM group was significantly higher than GDM group. And we concluded that giving Zuogui Wan to GDM rats can have a preventive effect on the offsprings' IGT induced by high fat and sugar diet.

1. Introduction

In the 1990s, British professor Barker [1] put forward "the fetal origins of adult disease" hypothesis. The hypothesis proposed that cardiovascular disease, hypertension, abnormal glucose metabolism, and other chronic diseases are caused by an adverse environment for the early fetus. Specifically, an adverse intrauterine environment prompts the early fetus to make adaptive adjustments to its own metabolism and the organizational structure of organogenesis. This adaptation will lead to permanent changes in tissue structure and function, which will then lead to adult diseases. From then on, the study of the origin of health and disease has become a worldwide research interest [2–5]. Traditional Chinese medicine theories in Huangdi Neijing believe that "kidney essence is stored in the Kidney," and "the kidney is responsible for birth, growth and reproduction." The kidney is the inborn origin of the body. If kidney function is poor in the mother, then the offspring will likely develop many diseases when growing up. We believe that the theory of traditional Chinese medicine is consistent with "the fetal origins of adult disease" hypothesis. Zuogui Wan is a prescription from a Chinese medicine book "Jingyue Quanshu," whose effect is to nourish Yin and tonify the kidney. There are 8 ingredients in Zuogui Wan: prepared rhizome of adhesive *Rehmannia*, Rhizoma Dioscoreae, Barbary Wolfberry fruit, *Cornus officinalis*, China dodder, Colla Cornus Cervi, tortoise shell glue, and medicinal *Cyathula* root. In this experiment we gave Zuogui Wan to GDM rats and supplemented the inborn origin of the body in fetus to GDM rats. The results

demonstrated that Zuogui Wan can prevent IGT induced by high fat and sugar diet in offspring rats.

2. Materials

2.1. Experimental Animals. Experimental Wistar rats (female = 100, male = 100) were all adult rats with a body weight between 200 g and 250 g. The experimental animals were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. (license number: SCXK (BJ) 2012-0001). The conduct of the experiments was in accordance with international zoology ethical standards.

2.2. Medicines and Reagent. Zuogui Wan is a prescription from a Chinese medicine book "Jingyue Quanshu," made of prepared rhizome of adhesive *Rehmannia*, Rhizoma Dioscoreae, Barbary Wolfberry fruit, *Cornus officinalis*, China dodder, Colla Cornus Cervi, tortoise shell glue, and medicinal *Cyathula* root with a 8 : 4 : 4 : 4 : 4 : 4 : 4 : 3 ratio. Ingredients were purchased from Beijing Tong Ren Tang and then verified by the Department of Medicine of Beijing University of Chinese Medicine as genuine. We used ceramics to decoct and extract Chinese herbal medicines. The mass concentration was $1\,g{\cdot}mL^{-1}$ of dried herbs. Streptozotocin (STZ) was produced by American Sigma Corporation (batch: B64927). We adjusted the STZ solution to acidity (pH = 4.2) by $0.1\,mol{\cdot}L^{-1}$ citric acid buffer solution, which was purchased from Beijing fraternity Port Company. Uric sugar test paper was purchased from Uritest Guilin Medical Electronic Sales Co., Ltd. (batch: 56130184). Chloral hydrate was purchased from Tianjin Fucheng Chemical Reagent Factory. Triglyceride detection kit and cholesterol detection kit were purchased from Beijing Wan Tai Derui Diagnostic Technology Co., Ltd. (batch: ZL2103AA02T and batch: ZL2105AA31).

2.3. Feed. A basic diet (ID = 1022) and high fat and sugar diet (protein 20%, carbohydrate 20%, and fat 60%) were all purchased from Beijing, China Huafukang Biological Technology Co., Ltd. (license number: SCXK (BJ) 2009-0008).

2.4. Instruments. Instruments used in this study included blood sugar detector (Johnson stable fold easily type LF033/V02), glucose test strips (Johnson Lot3354358), automatic biochemical analyzer (Toshiba TBA-40PR), −80°C ultralow temperature refrigerator (Plus Value, Thermo, USA), 10 mL disposable venous blood collection (Hunan Sanli Industrial Co., Ltd.), and 100 μL–1000 μL micro sample adding device (Dragonlab Company, USA).

3. Methods

3.1. Model Preparation

3.1.1. Model Preparation for Pregnant Rats. 100 female rats were fed for one week under the following conditions: 20–22°C, 30% ~65% for relative humidity, 150~300 Lx for illumination, and 12 : 12 day and night ratio. During the adaptive feeding period, saline ($20\,mL{\cdot}kg^{-1}$) was administered to rats by gavage once a day. One week later, 100 sets of rat cages were fixed in shelves of hanging type. Meanwhile, a tray was placed under each rat cage. 100 female and 100 male rats were put together in cages in which the female/male ratios were 1 : 1. At the same time, two methods were used to determine pregnancy of female rats. Firstly, after 12 hours, the method of pessary in tray was used to observe whether there were pessaries (ivory and solid jelly). Then, rats with pessary in tray were taken to do vaginal smears. If sperm had been found by microscopic examination [4], then they were labeled as pregnant rats (0 d). A total of 34 pregnant rats were detected in this experiment. Then, the pregnant rats were taken out; 23 were fed with high fat and sugar diet, and 11 were fed with basic diet.

3.1.2. Establishment of Model of Rats of GDM. The 34 pregnant rats taken from the preparation stage (the first day of pregnancy) were fasted for 12 hours. After that, 23 of them were injected peritoneally with dissolved STZ (40 mg/kg) and labelled as Zuogui Wan GDM group ($n = 12$) and GDM group ($n = 11$) and the remaining 11 rats were labelled as normal pregnant group and received an injection of sodium citrate buffer solution. The 23 rats were given normal water and high fat and sugar diet after 4 hours of giving STZ. The film forming standard of gestational diabetes was fasting blood glucose after 72 hours of the injection of STZ ≥ 11.1 mmol/L or random blood glucose ≥ 16.7 mmol/L and urine glucose > ++. Rats of 4-day pregnancy in Zuogui Wan GDM group and GDM group were all in accordance with film forming standard of GDM [6].

3.2. Grouping and Treatment

3.2.1. Grouping and Treatment on Mother Rats. The 34 pregnant rats were divided into three groups: Zuogui Wan GDM group ($n = 12$), GDM group ($n = 11$), and normal pregnancy group ($n = 11$). Rats in both Zuogui Wan GDM group and GDM group were fed with high fat and sugar diet. Rats in normal pregnancy group were fed basic diet. Zuogui Wan decoction (i.e., $1\,g{\cdot}mL^{-1}$, $20\,mL{\cdot}kg^{-1}$) was administered once a day to rats in Zuogui Wan GDM group by gavage for 19 days. Saline ($20\,mL{\cdot}kg^{-1}$) was administered by gavage once a day to rats in GDM group and normal pregnancy group for 19 days.

3.2.2. Grouping and Treatment on Offspring Rats. 20 to 22 days after pregnancy, the pregnant rats gave birth to the first-generation rats (offspring for short). As can be seen from Figure 1, after 21 days of breastfeeding, one male newborn rat was selected randomly from every brood of Zuogui Wan GDM group and GDM group, and the names of these two groups were unchanged (one brood of offspring in GDM group did not have any males, and two broods of offspring in Zuogui Wan GDM group did not have any males). Two male newborn rats were randomly selected from every brood of normal pregnancy group (one brood of offspring control group did not have any males); the two groups were named as control group and high fat and sugar as the control group. At

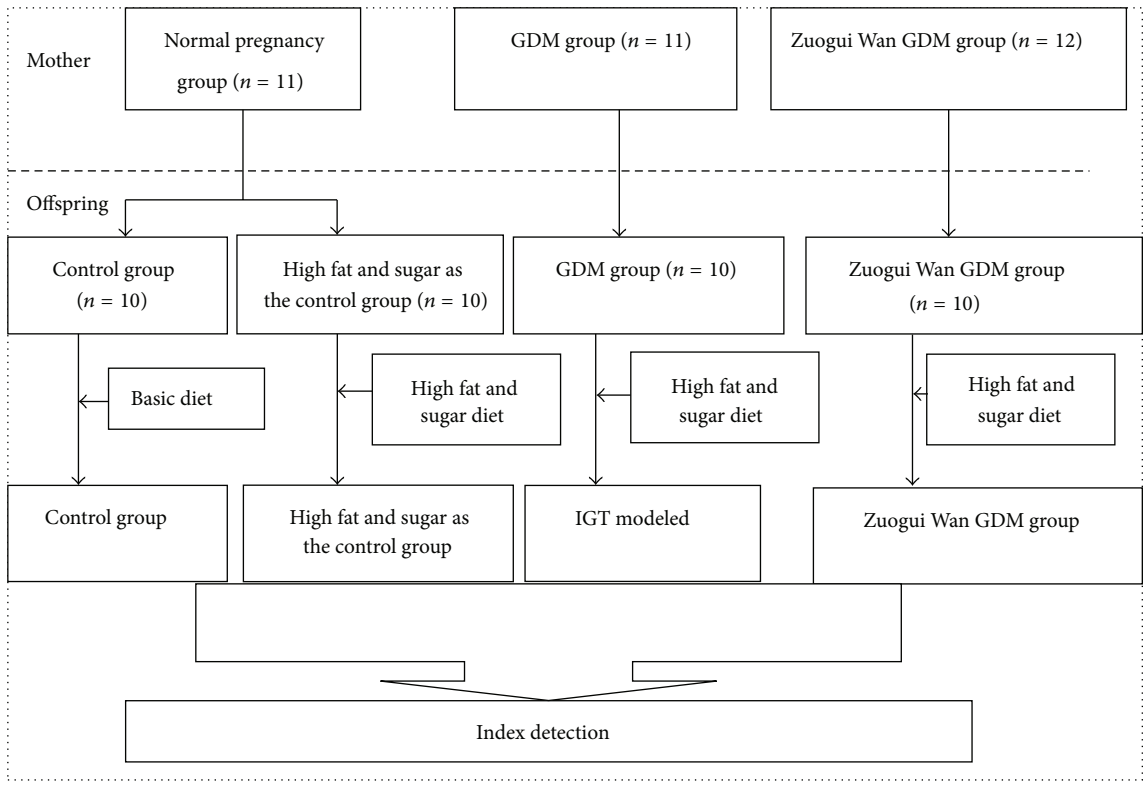

Figure 1: Grouping on mother rats and offspring rats.

the age of 4 weeks, each offspring group was treated with basic feed, ad libitum food and water. At the age of 5 weeks, each group was treated as follows: rats in GDM group ($n = 10$) were fed with high fat and sugar diet, ad libitum and water; rats in Zuogui Wan GDM group ($n = 10$) were fed with high fat and sugar diet, ad libitum food and water; rats in high fat and sugar as the control group ($n = 10$) were fed with high fat and sugar diet, ad libitum food and water; rats in control group ($n = 10$) were fed the basic diet, ad libitum food and water.

From week 8 on, fasting blood glucose and 2-hour plasma glucose (2hPG for short) were detected and recorded every week in each group. The means of 2hPG were higher than 7.8 mmol·L^{-1} and lower than 11.1 mmol·L^{-1} on the rats of GDM group on week 15, and IGT models were successful. One rat in control group was failed when taking blood.

3.3. Detection Index

3.3.1. Body Weight. Body weight was measured after the offspring were born (the total weight of the brood divided by the number), after 3 weeks of breastfeeding, and in week 6, week 8, week 10, week 12, week 14, and week 15 of all rats which formed new groups.

3.3.2. Fasting Blood Glucose and 2hPG. From week 8 on, fasting blood glucose and 2hPG were measured every week in each group.

3.3.3. Weight of Fat. In week 15, the IGT models were successful in GDM group, abdominal fat (which mainly contains the peritoneum fat, testis fat pad, and bilateral perirenal fat) was taken out, the total weight was checked by electronic balance, and the ratio of abdominal fat and body weight was calculated.

3.3.4. Total Cholesterol, Triglyceride, Low Density Lipoprotein, and High Density Lipoprotein. Blood was drawn from the abdominal artery from all rats. All the blood samples were centrifuged to get serum. Total cholesterol, triglyceride, low density lipoprotein, and high density lipoprotein were detected by using enzyme-conjugated colorimetric analysis method by automatic biochemistry analyzer according to the procedures of kits.

3.3.5. Insulin, Leptin, Adiponectin, and Insulin Resistance Index. Blood was drawn from the abdominal artery from all rats. All the blood samples were centrifuged to get serum. The insulin level was detected by using enzyme-immunoassay method according to the procedures of kits: insulin resistance index (HOMA-IR) = FINS $*$ FPG/22.5.

3.4. Data Processing. Data was processed in SPSS18.0 statistical software. Differences between groups were compared with one-way analysis of variance. $P < 0.05$ represented statistical significance in this study. The comparison results were shown by notations as follows: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

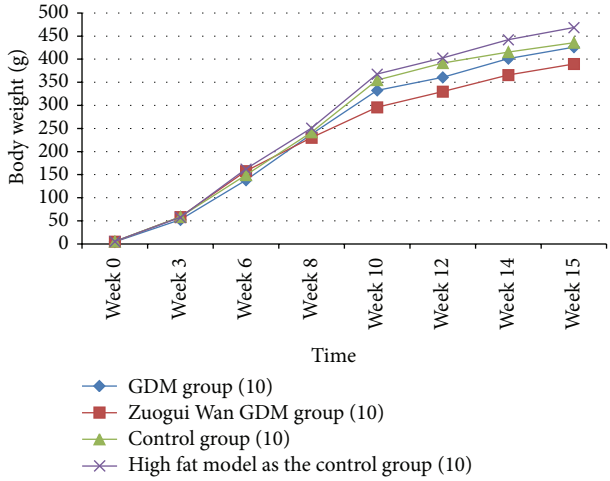

Figure 2: Change of body weight.

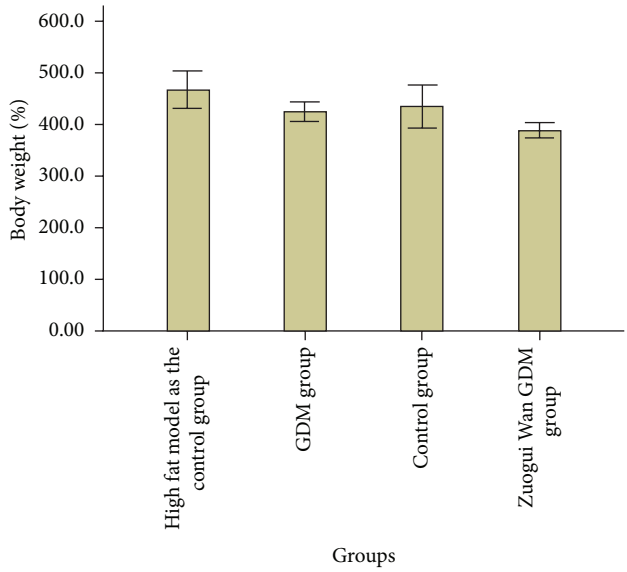

Figure 3: Body weight in week 15.

4. Results

4.1. Body Weight. As can be seen from Table 1 and Figure 2, compared with GDM group, body weight in week 0 was significantly higher ($P < 0.05$, $P < 0.01$, and $P < 0.01$) in Zuogui Wan gestational diabetes group, control group, and high fat model as the control group. In week 3, body weight was significantly higher ($P < 0.01$) in Zuogui Wan GDM group, control group, and high fat model as the control group compared with GDM group. In week 6, compared with GDM group, body weight was significantly higher ($P < 0.01$, $P < 0.001$) in Zuogui Wan GDM group and high fat model as the control group; the means of body weight in control group was higher than GDM group, but the difference was not significant. From week 8 to week 12, the means of body weight in GDM group was higher than that in Zuogui Wan GDM group, but the difference was not significant. From week 8 to week 10, body weight in control group and high fat model as the control group was higher than GDM group, but the difference was not significant. In week 12, compared with GDM group, body weight in high fat model as the control group was significantly higher ($P < 0.05$), body weight in control group was higher than that in GDM group, but the difference was not significant. In week 14 and week 15, compared with GDM group, body weight in Zuogui Wan GDM group was significantly lower ($P < 0.05$), body weight in high fat model as the control group was significantly higher ($P < 0.05$), and body weight in control group was higher, but the difference was not significant.

4.2. Fasting Blood Glucose and 2hPG. As can be seen from Table 2 and Figures 3 and 4, compared with GDM group, abdominal fat weight and abdominal fat weight/body weight in Zuogui Wan GDM group, control group, and high fat model as the control group were significantly lower ($P < 0.001$).

4.3. Fasting Blood Glucose and 2hPG. It is apparent in Table 3 and Figure 5 that, compared with GDM group, fasting blood glucose was significantly lower ($P < 0.001$, $P < 0.01$) in Zuogui Wan GDM group and control group. The means of fasting blood glucose in high fat model as the control group was lower than that in GDM group, but the difference was not significant. Compared with GDM group, 2hPG was significantly lower ($P < 0.001$) in Zuogui Wan GDM group, control group, and high fat model as the control group.

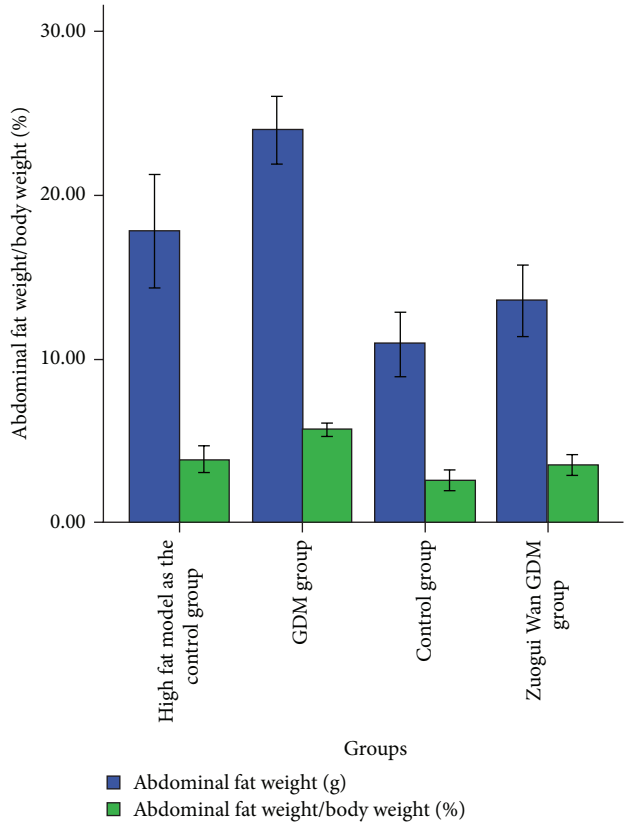

Figure 4: Abdominal fat weight and abdominal fat weight/body weight.

Table 1: Body weight (from week 0 to week 15, g).

Group	Week 0	Week 3	Week 6	Week 8	Week 10	Week 12	Week 14	Week 15
GDM group (10)	4.51 ± 0.54	52.27 ± 3.47	138.20 ± 15.43	237.80 ± 26.13	332.60 ± 32.75	360.80 ± 29.25	401.40 ± 26.62	426.20 ± 26.22
Zuogui Wan GDM group (10)	5.11 ± 0.50*	58.32 ± 4.31**	157.80 ± 15.68**	230.00 ± 34.41	295.90 ± 20.33	329.90 ± 18.38	365.8 ± 19.46*	389.80 ± 21.62*
Control group (10)	5.29 ± 0.80**	58.80 ± 3.96**	149.20 ± 10.09	241.91 ± 11.89	354.90 ± 58.67	391.80 ± 56.25	415.60 ± 57.14	435.90 ± 58.66
High fat model as the control group (10)	5.29 ± 0.80**	57.43 ± 2.69**	162.10 ± 12.18***	250.81 ± 24.58	367.60 ± 50.45	402.70 ± 48.59*	442.20 ± 49.39*	468.60 ± 50.45*

Note: all results are presented as mean ± SD. ∗ indicates a significant difference compared with GDM group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 2: Abdominal fat weight/body weight.

Group	Body weight (g)	Abdominal fat weight (g)	Abdominal fat weight/body weight (%)
GDM group (10)	426.20 ± 26.22	23.97 ± 2.88	5.62 ± 0.53
Zuogui Wan GDM group (10)	389.80 ± 21.62*	13.54 ± 3.06***	3.49 ± 0.85***
Control group (10)	435.90 ± 58.66	10.84 ± 2.75***	2.56 ± 0.85***
High fat model as the control group (10)	468.60 ± 50.45*	17.79 ± 4.85***	3.87 ± 1.15***

Note: all results are presented as mean ± SD. ∗ indicates a significant difference compared with GDM group. $^{*}P < 0.05$; $^{***}P < 0.001$.

Table 3: Fasting blood glucose and 2hPG (mmol·L^{-1}).

Group	Fasting blood glucose	2hPG
GDM group (10)	5.31 ± 0.28	9.01 ± 0.75
Zuogui Wan GDM group (10)	4.44 ± 0.47***	5.78 ± 0.60***
Control group (10)	4.77 ± 0.64**	6.50 ± 0.63***
High fat model as the control group (10)	5.02 ± 0.32	6.77 ± 0.70***

Note: all results are presented as mean ± SD. ∗ indicates a significant difference compared with GDM group. $^{**}P < 0.01$; $^{***}P < 0.001$.

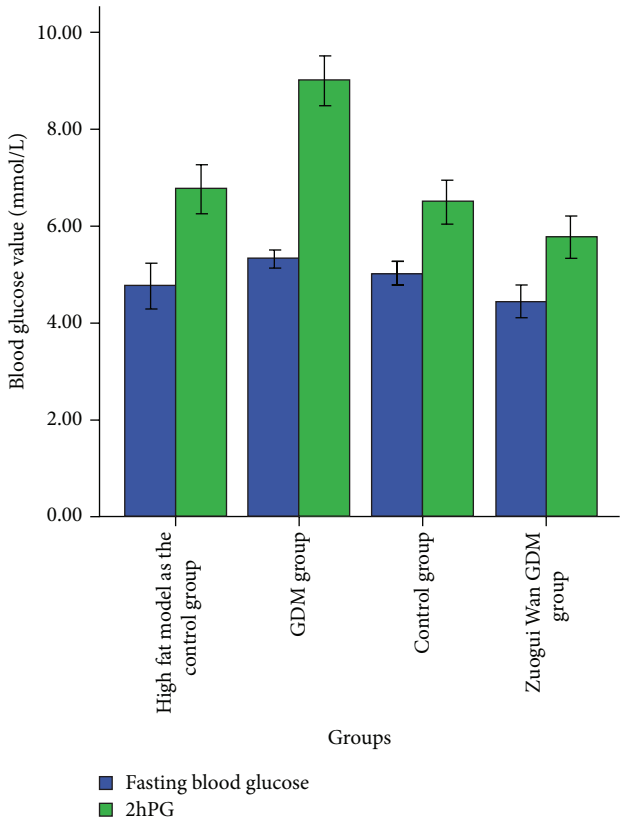

Figure 5: Fasting blood glucose and 2hPG.

4.4. Biochemical Index. As can be seen from Table 4, compared with GDM group, total cholesterol was significantly lower ($P < 0.001$, $P < 0.01$, and $P < 0.01$) in Zuogui Wan GDM group, control group, and high fat model as the control group. Low density lipoprotein (LDL) was significantly lower ($P < 0.001$, $P < 0.05$) in Zuogui Wan GDM group and control group compared with GDM group. The means of low density lipoprotein in high fat model as the control group was lower than that in GDM group, but the difference was not significant. The means of triglyceride in Zuogui Wan GDM group was lower than that in GDM group, but the difference was not significant. The means of triglyceride in high fat model as the control group was higher than that in GDM group, but the difference was not significant. The means of high density lipoprotein (HDL) in Zuogui Wan GDM group, control group, and high fat model as the control group was lower than that in GDM group, but the difference was not significant.

4.5. Insulin, Insulin Resistance Index, Leptin, and Adiponectin. As can be seen from Table 5, compared with GDM group, the insulin was significantly lower ($P < 0.001$, $P < 0.001$) in Zuogui Wan GDM group and control group. Insulin resistance index was significantly lower ($P < 0.001$, $P < 0.001$, and $P < 0.01$) in Zuogui Wan GDM group, control group, and high fat model as the control group than that in GDM group. Compared with GDM group, leptin was significantly lower ($P < 0.001$, $P < 0.001$) in Zuogui Wan GDM group and control group. Compared with GDM group, adiponectin was significantly higher ($P < 0.05$, $P < 0.001$, $P < 0.001$) in Zuogui Wan GDM group, control group, and high fat model as the control group.

5. Discussion

Impaired glucose tolerance is a kind of abnormal glucose metabolism status between diabetes and normal glucose tolerance. It is the precursor stage to diabetes with no obvious clinical symptoms. Currently, worldwide, the detection rate for IGT among all regions in both genders is higher than diabetes mellitus [7], according to the statistical materials in China, 8% who suffered from IGT will change to type two diabetes mellitus [8]; intervention work to prevent this is very important.

Our research group has conducted "TCM eugenics project" which has produced abundant results. For instance, it has been found that using traditional Chinese medicine prescriptions to tonify kidney in the embryonic stage can not only promote fetal intrauterine growth and cure intrauterine growth retardation but also improve the immune function of offspring in adult stage significantly [8]. Giving pregnant rats traditional Chinese medicine prescriptions can not only tonify kidney, but also prevent the occurrence of IGT in offspring in adult stage induced by a high fat and sugar diet [9].

Table 4: Biochemical Index (mmol·L^{-1}).

Group	Total cholesterol	Triglyceride	Low density lipoprotein	High density lipoprotein
GDM group (10)	2.86 ± 0.44	0.56 ± 0.09	0.62 ± 0.08	0.51 ± 0.08
Zuogui Wan GDM group (10)	2.15 ± 0.27***	0.46 ± 0.10	0.38 ± 0.08***	0.44 ± 0.10
Control group (9)	2.34 ± 0.43**	0.56 ± 0.11	0.51 ± 0.11*	0.47 ± 0.07
High fat model as the control group (10)	2.35 ± 0.16**	0.63 ± 0.20	0.55 ± 0.06	0.48 ± 0.05

Note: all results are presented as mean ± SD. ∗ indicates a significant difference compared with GDM group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 5: Insulin, insulin resistance index, leptin, and adiponectin.

Group	Insulin (mLU·L^{-1})	HOMA-IR (mmol∗mLU·L^{-2})	Leptin (ng·L^{-1})	Adiponectin (μg·mL^{-1})
GDM group (10)	10.27 ± 0.34	2.45 ± 0.16	5.61 ± 0.06	63.17 ± 1.51
Zuogui Wan GDM group (10)	9.00 ± 0.15***	1.77 ± 0.17***	4.57 ± 0.07***	68.40 ± 4.40*
Control group (9)	8.33 ± 0.49***	1.87 ± 0.14***	4.49 ± 0.12***	81.09 ± 3.27***
High fat model as the control group (10)	10.28 ± 0.30	2.18 ± 0.31**	5.88 ± 0.25	74.89 ± 5.10***

Note: all results are presented as mean ± SD. ∗ indicates a significant difference compared with GDM group. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

These results demonstrate that the "tonifying kidney" method and prescription from traditional Chinese medicine can improve the intrauterine growth environment, inherit excellent biological properties, enhance immunity, and delay or prevent the occurrence of disease in offspring.

Early experiments have demonstrated that giving Zuogui Wan to GDM rats has a therapeutic effect on GDM rats [10]. In this experiment, the birth weight of offspring rats in GDM group was significantly lower than that for other groups. This low weight stemmed from deficiencies during fetal growth. Mother rats from GDM group during pregnancy have obvious symptoms of "three high and one low," leading to loss of nutrients, so the birth weight of offspring rats was low. But changes occurred as the offspring aged. In the first 6 weeks, body weight in GDM group was lower than that in Zuogui Wan GDM group. But after week 6, GDM group showed an accelerated growth rate and began to catch up with other groups. In the 8th week, the body weight of offspring rats in GDM group had exceeded that in Zuogui Wan GDM group. This result supports Professor Barker's conclusion that "low birth weight children under 2 years old have slow weight gain, but the weight will grow rapidly in later 11 years, which is a fast growing compensatory acquired"; this compensatory production is a risk factor for hypertension, ischemic heart disease, and insulin resistance [11]. Because of administration of Zuogui Wan to their mothers, body weight in Zuogui Wan GDM group did not increase rapidly after week 6, but increased at a steady rate. The results of this study were also consistent with this conclusion from Barker's study.

In week 14, body weight of Zuogui Wan GDM group was significantly lower than that of GDM group. We believe this was because giving Zuogui Wan in pregnancy can strengthen fetal resistance and prevent obesity induced by high fat and sugar diet (especially reducing the abdominal fat weight, the ratio of the abdominal fat weight, and body weight). Insulin resistance is one of the pathological bases for IGT to occur. It has positive association with higher leptin [12] and negative association with adiponectin [13]. In this experiment, the levels of fasting plasma glucose, 2hPG, insulin, and leptin in Zuogui Wan GDM group were significantly lower than in GDM group, but the level of adiponectin was significantly higher than in GDM group. Therefore, it can be concluded that giving Zuogui Wan to GDM rats in embryonic period can reduce the level of fasting blood glucose, 2hPG, insulin, and leptin, fight against insulin resistance and leptin resistance, and also fight against the reduction of adiponectin level induced by high fat and sugar diet. Total cholesterol and LDL in Zuogui Wan GDM group were significantly lower than those in GDM group; triglycerides and HDL in Zuogui Wan GDM group were lower than in GDM group, but the difference was not statistically significant. To some extent, all of these result from treatment in utero.

As can be seen from the experiment result, giving Zuogui Wan to GDM rats in embryonic period can prevent IGT induced by high fat and sugar diet for adult rats. Traditional Chinese medicine believes that patients with diabetes often have some characteristics of "Yang is usually excessive, while Yin is frequently deficient," which was said by Danxi Zhu, a famous traditional Chinese medicine expert of Yuan Dynasty in China. Maternal gestation period needs blood filling; rats in GDM group must have characteristics of Yin which is often inadequate. The function of Zuogui Wan is nourishing Yin and tonifying the kidney. So it can supply adequate nutrients to the unborn baby rats. This is also consistent with the theory of "supplement the mother, the child will benefit," which was proposed in an old famous Chinese book named "Jiachuan Nvke Jingyan Zhaiqi."

In conclusion, the theory "kidney is responsible for reproduction, growth and reproduction," which has been proposed in Huandi Neijing for more than 2000 years, and whose availability has been proven in its practice, but this theory lacked evidence in modern medical support. This study provides an experimental basis from the perspective of modern medical evidence for the above theory. The result further sheds light on studies focusing on how to protect the offspring's IGT from GDM mothers.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by the National Science and Technology Cooperation Projects (no. 0102012DFA31330). The authors thank professor David Adelson (The University of Adelaide) for invaluable expertise and technical assistance with our experiment and modifying this paper.

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2015, Article ID 171742, 9 pages
http://dx.doi.org/10.1155/2015/171742

Review Article

Medicinal Plants Qua Glucagon-Like Peptide-1 Secretagogue via Intestinal Nutrient Sensors

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Received 25 August 2015; Accepted 24 November 2015

Academic Editor: Juntra Karbwang

Glucagon-like peptide-1 (GLP-1) participates in glucose homeostasis and feeding behavior. Because GLP-1 is rapidly inactivated by the enzymatic cleavage of dipeptidyl peptidase-4 (DPP4) long-acting GLP-1 analogues, for example, exenatide and DPP4 inhibitors, for example, liraglutide, have been developed as therapeutics for type 2 diabetes mellitus (T2DM). However, the inefficient clinical performance and the incidence of side effects reported on the existing therapeutics for T2DM have led to the development of a novel therapeutic strategy to stimulate endogenous GLP-1 secretion from enteroendocrine L cells. Since the GLP-1 secretion of enteroendocrine L cells depends on the luminal nutrient constituents, the intestinal nutrient sensors involved in GLP-1 secretion have been investigated. In particular, nutrient sensors for tastants, cannabinoids, and bile acids are able to recognize the nonnutritional chemical compounds, which are abundant in medicinal plants. These GLP-1 secretagogues derived from medicinal plants are easy to find in our surroundings, and their effectiveness has been demonstrated through traditional remedies. The finding of GLP-1 secretagogues is directly linked to understanding of the role of intestinal nutrient sensors and their recognizable nutrients. Concurrently, this study demonstrates the possibility of developing novel therapeutics for metabolic disorders such as T2DM and obesity using nutrients that are readily accessible in our surroundings.

1. Treatment of Type 2 Diabetes Mellitus

Diabetes is a metabolic disease characterized by high blood glucose levels caused by insufficient insulin production or insulin resistance. While type 1 diabetes mellitus (T1DM) is considered an autoimmune disease caused by pancreatic β cell destruction, type 2 diabetes mellitus (T2DM) is caused by lifestyle factors, such as age and obesity, and by insulin resistance, where the body cells fail to respond to insulin, and accounts for 90–95% of all diabetes cases [1].

Accumulating evidence implicates that chronic hyperglycemia caused by diabetes results in tissue damage and furthermore increases the risk of micro- and macrovascular diseases, sclerosis of the arteries, cardiovascular disease, diabetic kidney disease, and retinal disease [2]. According to the "Diabetes Fact Sheet in Korea 2013" published by the Korean Diabetes Association, 44.4% of diabetes patients have obesity, 54.6% have hypertension, and 80% have dyslipidemia.

Various therapeutic strategies for T2DM had been developed including stimulating insulin secretion (sulfonylurea), decreasing glucose release from the liver (biguanides), reducing carbohydrate absorption in the gastrointestinal (GI) tract (α-glycosidase inhibitor), and enhancing the susceptibility of insulin receptor (thiazolidinediones) [3, 4]. However, side effects and gradually declining efficacy are limitations of these strategies (Table 1).

T2DM therapeutics that regulates blood glucose and weight gain while restoring and enhancing β cell function is a potentially ideal strategy. In the last decade, incretin-based therapy has been spotlighted as a substitute strategy to circumvent the limitations of existing therapies.

2. GLP-1 Secretagogue for Type 2 Diabetes Treatment

Incretin is a gut hormone secreted from the small intestine during a meal and stimulates insulin secretion from the

Table 1: Existing therapeutics for T2DM.

Therapeutics	Limitations	Ref.
Non-incretin-based therapeutics for T2DM		
Sulfonylurea	Transiently stimulating excessive insulin secretion that causes hypoglycemia and hyperinsulinemia and eventually results in obesity and overweight.	[16, 17]
Biguanides	Causes gastrointestinal side effects, such as diarrhea and abdominal cramping. Rarely causes lactic acidosis.	[17]
Meglitinides	Transiently stimulating excessive insulin secretion that causes hypoglycemia and results in overweight.	[17]
α-glycosidase inhibitor	Causes indigested carbohydrates in gut lumen that may result in flatulence and diarrhea.	[18]
Thiazolidinediones	Causes significant water retention that causes edema and heart failure.	[19]
Incretin-based therapeutics for T2DM		
GLP-1 agonists	Inefficient to patients with severe β cell dysfunction or insulin resistance; low blood glucose reducing effect compared to the existing therapeutics; cause nausea and vomiting.	[20]
DPP-4 inhibitors	Stimulate unnecessary ductal cells that increase risk of pancreatitis; cause nausea; increase the risk of heart failure.	[21]

T2DM, type 2 diabetes mellitus; DPP-4, dipeptidyl peptidase-4.

pancreatic β cells. Glucagon-like peptide-1 (GLP-1) is a typical incretin hormone and is produced by differential posttranslational processing of proglucagon in the gut and brain [5]. GLP-1 secreted from enteroendocrine L cells plays various physiological roles in glucose homeostasis and feeding behavior. GLP-1 slows gastric emptying, inhibits gut motility, and suppresses appetite while it increases β cells proliferation, enhances β cells function, and stimulates pancreatic insulin secretion [5, 6]. The insulin stimulating effect of GLP-1 is tightly regulated by the blood glucose concentration. The insulin stimulating effect is abrogated when the blood glucose concentration is under 4.5 mmol/L and the half maximal effective concentration of GLP-1 is about 10 pmol/L [7]. Therefore, GLP-1 receptor agonists minimize the risk of hypoglycemia, reduce food intake, and lead to weight loss [8]. Indeed, lowered plasma active GLP-1 levels observed in T2DM patients support the GLP-1 receptor agonism as an appropriate therapeutic strategy [9]. Direct administration of GLP-1 restored initial insulin dyscrinism and showed a rapid blood glucose lowering effect [9]. However, direct administration of active GLP-1 has not been applied in clinical medicine because of its short half-life (<2 min) due to the enzymatic cleavage of dipeptidyl peptidase-4 (DPP4).

On the basis of the efforts to improve the GLP-1 activity (both the benefits and the limitations), incretin-based therapies, such as DPP4 inhibitors and stable GLP-1 analogs, have been developed in recent years. These incretin-based therapies show fewer side effects related to hypoglycemia and weight gain than existing non-incretin-based medications and even regenerate pancreatic β cells [8]. Nevertheless, inadequate effectiveness and causing pancreatitis are reported to be the limitations of the incretin-based therapies [10] (Table 1). GLP-1 analogues, such as exenatide and liraglutide, have similar effects to GLP-1 due to their binding to the GLP-1 receptor with greatly extended durability [11, 12]. However, patients prescribed GLP-1 analogues have suffered needle phobia and side effects such as nausea, vomiting, and anorexia [13, 14]. DPP4 is a ubiquitous enzyme involved in the enzymatic cleavage of more than 20 different endogenous peptides in humans [15]. Thus, unclear side effects due to inhibiting endogenous peptides are a potential risk of DPP4 inhibitor prescription.

A possible strategy to improve the limitations of existing therapeutics for T2DM is directly stimulating GLP-1 secreting cells. This strategy is based on the activation of cell surface receptors and their cellular signal transduction pathway involved in GLP-1 secretion. These receptors for the GLP-1 secretagogues are found on the surface of enteroendocrine L cells and are able to respond to the luminal nutrient composition.

3. Nutrient Sensing in the Gut

The GI tract is a significant sensor for the ingested nutrients throughout its length [33]. Ingested foodstuffs are digested into small nutritional compounds and then absorbed by the cells located in the surface of the gut lumen. Distinguishing the nutrients is critical for determining the nutritive and toxic qualities of ingested material [34, 35]. In particular, enteroendocrine cells which secrete gut-peptides, such as GLP-1, glucose-dependent insulinotropic peptide (GIP), peptide YY (PYY), and cholecystokinin (CCK), respond to the luminal nutrients, such as tastants, fatty acids, amino acids, cannabinoids, and bile acids (Figure 1) [36–38]. In this review, nutrient sensors expressed in enteroendocrine L cells, GLP-1 secreting cells existing throughout the intestine, are discussed.

3.1. Sweet Taste Receptors. Natural sugars, such as mono-, di-, and oligosaccharides, are fundamental energy sources to most organisms. The sweet taste perception that occurs in the oral cavity is able to recognize the natural sugars and facilitates their ingestion into the GI tract. The sugar sensing event

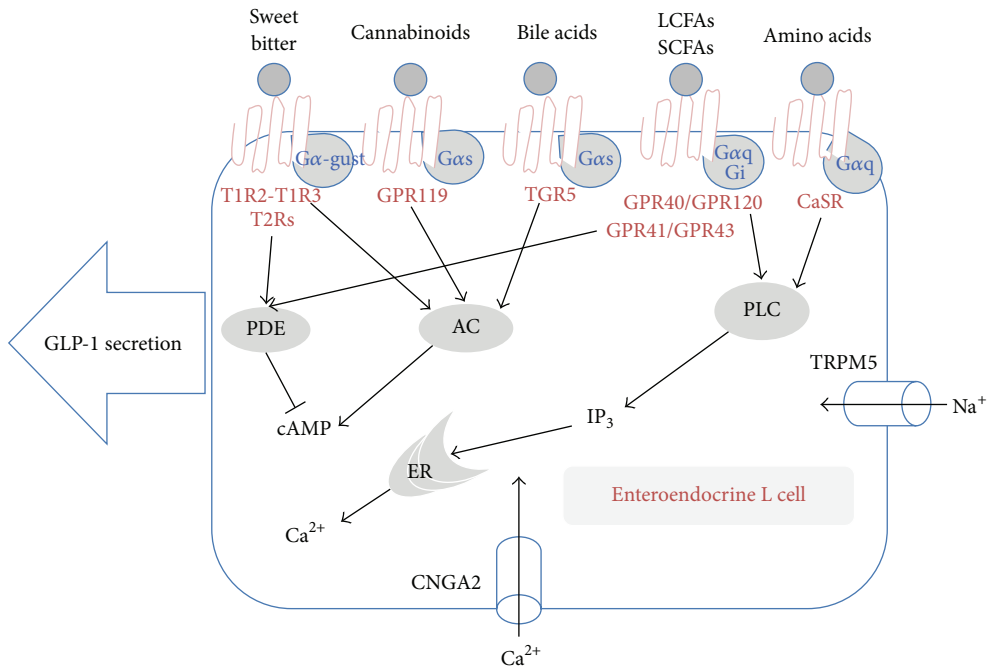

FIGURE 1: Nutrient sensors in the enteroendocrine L cells. Nutrient sensors existing in the endocrine cells and their possible signal transduction pathways. # LCFAs, long-chain fatty acids; SCFAs, short-chain fatty acids; PDE, phosphodiesterase; AC, adenylyl cyclase; PLC, phospholipase C; IP_3, inositol 1,4,5-triphosphate; ER, endoplasmic reticulum.

in the tongue is known to be due to a heterodimer of T1R2 and T1R3, which are the type 1 taste receptors [39]. Consequent activation of a taste specific G protein, gustducin, transmits the signal cascade through the transduction elements, such as phospholipase C (PLC), inositol 1,4,5-triphosphate (IP_3), diacylglycerol (DAG), adenylyl cyclase (AC), and the Ca^{2+}-sensitive transient receptor potential channel M5 (TRPM5) [34, 40]. In particular, the increase of intracellular second messengers, Ca^{2+} and cAMP, due to activation of adenylyl cyclase and IP_3, respectively, is involved in the sweet taste perception [41, 42].

In enteroendocrine L cells, sweet taste receptor-mediated GLP-1 secretion appears to be due to activation of Gα-gustducin. In human enteroendocrine NCI-H716 cells, glucose and sucrose only activate Gα-gustducin while they inactivate G$\alpha i_{1,2}$ and/or do not affect Gαs [43]. Moreover, abolished increase in the plasma GLP-1 and plasma insulin levels after oral glucose administration to Gα-gustducin null mice supports the involvement of Gα-gustducin in glucose sensing [43].

Gα-gustducin is a Gα-transducin-like G protein α-subunit expressed in 25~30% of taste receptor cells and is expected to activate PDE to decrease cAMP [41, 44]. Only 15% of Gα-gustducin-positive cells are coexpressed with GLP-1 in the mouse jejunum [33]. Exact understanding of the role of Gα-gustducin is challenging because of the following issues: (1) the low coexpression ratio of Gα-gustducin with taste cells and GLP-1-positive cells; (2) frequently found coexpression of Gα-gustducin with the other G protein α-subunits, such as G$\alpha i_{1,2}$, Gα_{15}, Gα_{16}, G$\alpha_{q/11}$, Gαs, and Gα-transducin; and (3) the ability of Gα-gustducin to activate different molecules, which have the opposite function on the intracellular cAMP levels [34]. One possible hypothesis is that Gα_{15} (mouse) and Gα_{16} (human), which are able to couple with promiscuous GPCR, participate in the sweet taste perception [45].

Sweet taste receptors are expressed in the enteroendocrine L cells with its signal transduction elements, which are also found in the lingual sweet taste receptors [43]. The GLP-1 secretion stimulated by artificial sweeteners supports the existence of sweet taste perception in the enteroendocrine L cells. *In vitro* studies using human and mouse enteroendocrine cells demonstrated that sucralose stimulated GLP-1 and GIP secretion [43, 46]. However, *in vivo* studies using healthy human subjects demonstrated that intragastric infusion of artificial sweeteners, for example, sucralose, aspartame, or acesulfame K, or fructose does not stimulate GLP-1, PYY, or GIP release while only glucose stimulated GLP-1 and PYY release [47, 48]. Furthermore, a human study using a sweet taste receptor inhibitor demonstrated that the glucose has a GLP-1 and PYY secreting effect via the sweet taste receptor while blocking the sweet taste receptor did not affect the GLP-1 secreting effect of the liquid meal [49].

These results showing secretion of GLP-1 and PYY depends on the structural analogy to glucose lead us to deduce that medicinal plants, which abundantly contain glycosidic compounds, have a possibility of stimulating GLP-1 secretion through the activation of sweet taste receptors in the enteroendocrine L cells.

Ginsenosides, triterpenoid saponins that are found abundantly in *Panax ginseng*, have been classified into dammarane type and oleanane type according to their carbon skeletons of aglycone. Several ginsenosides, such as Rb1, Re, Rb2, C-K, and Rg3, were reported to have antidiabetic and antiobesity effects [22, 24, 25, 32, 50].

Long-term intraperitoneal injection of Rb1 (10 mg/kg) showed drastically decreased food intake, body weight, and body fat mass and also attenuated basal hyperglycemia in obese rats [22]. In this study, Rb1 increased c-Fos expression, a marker of neuronal activity, in the cells of nucleus of the solitary tract (NTS), ventromedial hypothalamic nucleus (VMN), and arcuate nucleus of the hypothalamus (ARC), all of which are brain parts known to be involved in the feeding behavior [22].

Intraperitoneal injection of Re (20 mg/kg) for 12 days reduced fasting blood glucose levels to 180 ± 10.8 mg/dL compared to the saline treated group (235 ± 13.4 mg/dL) in ob/ob mice [24].

Oral treatment of Rg3 (25 mg/kg) showed 9% of blood glucose reduction efficacy compared to the control mice group during the OGTT [25]. This glucose lowering effect of Rg3 seems to be due to its insulin secreting effect on the pancreatic β cell.

The ginsenosides Rb1, Rb2, Re, and Rg3 have glucose residues attached on the four-carbon ring structure dammarane backbone and thus have a possibility of stimulating GLP-1 secretion via the sweet taste receptor signal transduction pathway in the enteroendocrine L cells.

T1R3 and gustducin also contribute to glucose absorption from the enterocytes and affect the GLP-1 secreting ability of the enteroendocrine cells through the regulation of Na^+-dependent glucose cotransporter 1 (SGLT1) expression [46]. Therefore, the role of sweet taste receptors in glucose homeostasis should be considered with the role of the enterocytes participating in glucose absorption.

3.2. Bitter Taste Receptors. Bitter tastants have not been considered as a fundamental nutrient but are abundantly found in medicinal plants, vegetables, alcohol beverages, and coffee. The bitter tastants can be recognized by the type 2 taste receptors (T2Rs) expressed in the tongue and small intestine. These bitter taste receptors are GPCR coupled with taste specific G protein gustducin identical to the sweet taste receptors [34].

The downstream signal transduction of the bitter perception triggered by the activation of gustducin closely resembles the sweet taste perception in the gut [51]. However, binding of the bitter tastant to its receptor results in a decrease of intracellular cAMP levels due to the activation of phosphodiesterase (PDE). In the bitter perception, the activation of PDE is due to the activation of Gα-gustducin rather than G$\alpha i_{1,2}$ [38]. It is still controversial as to why Gα-gustducin activates different intracellular molecules, AC and PDE, by the sweet and bitter stimuli, respectively. Moreover, the roles of both increased and decreased intracellular cAMP levels by each tastant binding have not been elucidated.

There are 25 deorphanized T2Rs expressed in human [52, 53]. These 25 T2Rs are also expressed in nonchemosensory cells, such as human airway smooth muscle cells and human enteroendocrine NCI-H716 cells, as well as the human tongue [38, 54, 55]. But the order of the mRNA expression levels of 25 T2Rs between these cells is quite different. This indicates that the bitter tastant recognized in the tongue might not be recognized as a bitter tastant in the gut. Indeed, denatonium benzoate, which is known to be the most bitter tastant in humans, shows a smaller GLP-1 secreting effect than quinine, another bitter tastant in human enteroendocrine NCI-H716 cells [38]. The different expression orders of T2Rs between the tongue and enteroendocrine cells may enable the bitter tastants to be novel GLP-1 secretagogues without inducing nausea and a repellent sensation from the tongue.

Quinine is a crystalline alkaloid that naturally occurs in the bark of the cinchona tree. It has been used as an antimalarial drug and a bitter flavor component of tonic water [56, 57].

Quinine exerts a GLP-1 secreting effect on human enteroendocrine NCI-H716 cells as well as the bitter taste receptor agonist denatonium [38]. A transcriptomic study demonstrated that several bitter taste receptors and GPCR pathway elements including PDE and IP_3 receptor were upregulated in response to quinine treatment in the human enteroendocrine NCI-H716 cells [26].

In Korean traditional medicine, a disease known as "Sogal" shares the same pathological physiology with diabetes mellitus. According to the "Donguibogam: Principles and Practice of Eastern Medicine," registered in UNESCO Memory of the World, bitter tasting medicinal plants are effective for the treatment of "Sogal."

Gentiana scabra fulfills the categories of bitter tasting medicinal plants in the "Donguibogam" and, thus, *Gentiana scabra* extract is frequently prescribed for treatment of "Sogal" in Korean traditional medicine. Indeed, *Gentiana scabra* root extract exerts a GLP-1 secreting effect on the human enteroendocrine cells through the G protein $\beta\gamma$-subunit-mediated pathway, and as a result GLP-1 and insulin are released and attenuate hyperglycemia in a type 2 diabetic mouse model [30]. Another study demonstrated that an ethyl acetate fraction of *Gentiana scabra* root extract exerts the strongest GLP-1 secreting effects among evaluated fractions and upregulated the PDE, PLC, and IP_3 receptors and DAG mRNA expression [58]. A mass spectrometry analysis revealed several bitter iridoid compounds contained in the *Gentiana scabra* extract and, among them, loganic acid exerts a GLP-1 secreting effect on the human enteroendocrine NCI-H716 cells [30]. *Gentiana scabra* extract (100 mg/kg) decreased glucose levels of db/db mice during the OGTT. Plasma GLP-1 and plasma insulin levels also drastically increased 10 min after the oral administration of *Gentiana scabra* extract along with the glucose gavage [30].

Anemarrhena asphodeloides, *Bupleurum falcatum*, and *Citrus aurantium* also fulfill the categories of bitter tasting medicinal plants and have been reported to stimulate GLP-1 secretion [27–29]. In particular, a hexane fraction of the *Bupleurum falcatum* extract (100 mg/kg) significantly decreased blood glucose levels of db/db mice during the OGTT [28].

However, it is not clear whether these medicinal plant extracts activate the bitter taste receptor, due to a lack of functional studies on receptor activation.

Since quinine is used as a bitter flavor in tonic water and *Gentiana scabra* is categorized as a bitter herbal medicine, they clearly activate bitter taste receptors. Moreover, the fact that most medicinal plants have a bitter taste implies that

the bitter taste receptor agonists are abundantly present in medicinal plants. However, as described above, not every bitter taste recognized at the tongue is also bitter to the gut. Therefore, it is essential to elucidate which T2Rs are involved in the GLP-1 secreting event of the bitter taste medicinal plants (or their active compound) in the enteroendocrine L cells.

3.3. Cannabinoid Receptor. Cannabinoids are psychoactive compounds derived from the body of humans and animals, including endocannabinoid, such as 2-oleoylglycerol (2-OG), or plants, including phytocannabinoids, such as tetrahydrocannabinol (THC). While the cannabinoid receptor type 1 (CB1) and type 2 (CB2) are predominantly expressed in the central nervous system (CNS) and are involved in the hunger signal, GPR119 is predominantly expressed in the pancreas and GI tract and is involved in anorexigenic hormone release such as GLP-1 [59].

GPR119 is known to function as a cannabinoid receptor in human and rodent enteroendocrine K and L cells and pancreatic islets [60–62]. Therefore, GPR119 is expected to play a critical role in glucose homeostasis and incretin secretion. Indeed, a GPR119 null mouse showed reduced GLP-1 secretion in response to oral administration of glucose [63].

The cellular mechanism for GPR119 has not been extensively revealed, but it is believed to couple with Gαs subunit and to elevate intracellular cAMP levels. GPR119 is assumed to associate with dietary fat-induced satiety because its agonists including 2-OG naturally occur from the digestion of triacylglycerol [40].

On the strength of studies using the human enteroendocrine cell lines and the rodent model, a number of pharmaceutical companies have tried to develop synthetic GPR119 agonists such as PSN-821 (phase 2), MBX-2982 (phase 2), and GSK1292263 (phase 2) for therapeutic purposes (the information of each clinical trial can be accessed through the https://clinicaltrials.gov/ website).

However, the effect of the synthetic GPR119 agonist in human trials is still controversial. AR231453 showed no GLP-1 secreting effect in a human primary enteroendocrine cell culture and JNJ38431055 and GSK1292263 showed limited therapeutic effects on T2DM patients [64, 65], while they stimulated incretin secretion in enteroendocrine cells and a rodent model [63, 66, 67]. These crucial differences between the human clinical trial/human primary cell culture and the rodent model/cell line have presented severe limitations in drug developmental studies.

The agonist for GPR119 is an attractive therapeutic target due to its presence in both the enteroendocrine cells and pancreatic β cells. Therefore, the finding of phytocannabinoids (plant derived), which affect only GPR119 but not CB1 or CB2, could be a splendid strategy to treat T2DM. Since the GPR119 agonists are able to regulate both food intake and glucose homeostasis, they have a possibility of serving as efficient therapeutics for metabolic disorders such as obesity and T2DM.

Gordonoside F is a steroid glycoside isolated from African cactiform *Hoodia gordonii*, which has been used for Xhomani Bushmen as an anorexiant during hunting trips [31]. In a recent study using the GPR119 knock-out mice, oral administration of a *H. gordonii* extract (1000 mg/kg) and its active compound Gordonoside F (200 mg/kg) stimulated glucose-dependent GLP-1 and insulin release, showed a blood glucose lowering effect during an oral glucose tolerance test (OGTT), and decreased the cumulative food intake GPR119-dependently [31].

3.4. Bile Acid Receptor. Bile acids are steroid acids responsible for biliary lipid secretion, cholesterol elimination, and facilitating the absorption of fat-soluble vitamins and digested dietary lipids into the small intestine [68]. Bile acids are primarily synthesized in the liver and metabolized by gut microflora from the colon. Humans primarily synthesize cholic acid (CA) and chenodeoxycholic acid (CDCA) from the liver, and the CA and CDCA are changed into various secondary bile acids such as deoxycholic acid (DCA), lithocholic acid (LCA), or ursodeoxycholic acid (UDCA).

Bile acids have been reported to bind to the farnesoid X receptor (FXR; also known as bile acid receptor, BAR) and TGR5 (also known as G protein-coupled bile acid receptor 1, GPBAR1). FXR, a nuclear receptor in the liver and intestine, is involved in triglyceride metabolism, glucose metabolism, and liver growth [69].

TGR5, a membrane type G protein-coupled bile acid receptor, is likely involved in metabolic events in the body. Similar to GPR119, TGR5 couples with Gαs protein and thus elevates the intracellular cAMP levels by binding bile acids [70]. TGR5 expression was found in mouse enteroendocrine STC-1 cells and human enteroendocrine NCI-H716 cells. In the STC-1 cells, LCA and DCA treatment stimulated GLP-1 secretion through TGR5 [71].

In human enteroendocrine NCI-H716 cells, the ginsenoside C-K has been reported to stimulate GLP-1 secretion TGR5 dependently [32]. A notable point about ginsenoside C-K is that it is naturally produced by other ginsenosides, such as Rb1, Rb2, Rc, and Rd, by the metabolic process of the gut microflora [32, 72].

The structural alteration of the phytochemicals by the gut microflora confers the chance to change the binding motif to the expected nutrient sensor. This point provides extended understanding to the investigation of medicinal plant as GLP-1 secretagogues. Perhaps variables such as the gut microflora metabolism that occur *in vivo* cause different results between the cell line/animal studies and human trials.

4. Conclusion

As has been reviewed, medicinal plants have an enormous possibility of being developed as GLP-1 secretagogues. They contain abundant bitter tastants, which can act as bitter taste receptor agonists, unexplored phytocannabinoids, which are possible GPR119 agonists, and glycosidic compounds, which are possible sweet taste receptor agonists. Moreover, the structural alteration of the aglycone structure by the gut microflora provides various binding motifs to the exiting and/or deorphanized intestinal nutrient sensors.

Table 2: Medicinal plants derived GLP-1 secretagogues via intestinal nutrient sensors.

Origin	Testing agents	Effects	Model	Ref.
Sweet taste receptor				
Panax ginseng	Rb1	Decrease food intake, body weight, and body fat mass; decrease fasting blood glucose; decrease blood glucose during the IPGTT; increase plasma insulin	HFD obese rats	[22]
		GLP-1 secretion	Human L cell line	[23]
	Rb2	GLP-1 secretion	Human L cell line	[23]
	Re	Reduces fasting blood glucose	*ob*/*ob* mice	[24]
	Rg3	Insulin secretion Decrease blood glucose during the OGTT	Rodent β cell line ICR mice	[25]
Bitter taste receptor				
Plasmodium falciparum	Quinine	GLP-1 secretion	Human L cell line	[26]
Anemarrhena asphodeloides	EA fr.	GLP-1 secretion	Human L cell line	[27]
Bupleurum falcatum	HX fr.	GLP-1 secretion Decrease blood glucose during the OGTT	Human L cell line *db*/*db* mice	[28]
Citrus aurantium	HX fr.	GLP-1 secretion	Human L cell line	[29]
Gentiana scabra	Extract Loganic acid	GLP-1 secretion	Human L cell line	[30]
	Extract	Increase plasma GLP-1 and plasma insulin; decrease blood glucose during the OGTT	*db*/*db* mice	
Cannabinoid receptor				
Hoodia extract	Extract Gordonoside F	Decreases blood glucose during the OGTT; increases plasma GLP-1 and insulin; decreases cumulative food intake	C57BL/6 mice	[31]
		Glucose stimulated insulin secretion	Isolated rat islets	
Bile acid receptor				
Panax ginseng	Compound K	GLP-1 secretion	Human L cell line	[32]

HFD, high-fat diet; IPGTT, intraperitoneal glucose tolerance test; OGTT, oral glucose tolerance test; EA fr., ethyl acetate fraction; HX fr., *n*-hexane fraction.

The existence of GPR119 in both enteroendocrine cells and also pancreatic β cells encourages the development of novel efficient therapeutics for metabolic disorders including obesity and T2DM.

GLP-1 secretagogues stimulate endogenous GLP-1 secretion from enteroendocrine cells. GLP-1 secretagogues do not share the limitations with existing therapeutics for T2DM. Moreover, GLP-1 secretagogues may directly affect obese patients via suppression of food intake.

Considering the tremendous number of phytochemicals and the potential of deorphanized intestinal nutrient sensors, GLP-1 secretagogues from medicinal plants should be actively investigated (Table 2).

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), which is funded by the Ministry of Education, Science and Technology (20131A1A2004960).

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2015, Article ID 474032, 9 pages
http://dx.doi.org/10.1155/2015/474032

Research Article

The Combination of Resveratrol and Quercetin Attenuates Metabolic Syndrome in Rats by Modifying the Serum Fatty Acid Composition and by Upregulating SIRT 1 and SIRT 2 Expression in White Adipose Tissue

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Received 19 August 2015; Accepted 18 October 2015

Academic Editor: Jae-Young Um

Resveratrol (RSV) and quercetin (QRC) modify energy metabolism and reduce cardiovascular risk factors included in the metabolic syndrome (MetS). These natural compounds upregulate and activate sirtuins (SIRTs), a family of NAD-dependent histone deacetylases. We analyzed the effect of two doses of a commercial combination of RSV and QRC on serum fatty acid composition and their regulation of SIRTs 1–3 and PPAR-γ expression in white adipose tissue. MetS was induced in Wistar rats by adding 30% sucrose to drinking water for five months. Rats were divided into control and two groups receiving the two different doses of RSV and QRC in drinking water daily for 4 weeks following the 5 months of sucrose treatment. Commercial kits were used to determine serum parameters and the expressions of SIRTs in WAT were analysed by western blot. In MetS rats body mass, central adiposity, insulin, triglycerides, non-HDL-C, leptin, adiponectin, monounsaturated fatty acids (MUFAs), and nonesterified fatty acids (NEFAs) were increased, while polyunsaturated fatty acids (PUFAs) and HDL-C were decreased. SIRT 1 and SIRT 2 were downregulated, while PPAR-γ was increased. RSV + QRC administration improved the serum health parameters modified by MetS and upregulate SIRT 1 and SIRT 2 expression in white abdominal tissue in MetS animals.

1. Introduction

Metabolic Syndrome (MetS) is a complex and heterogeneous disease which is actually considered as an epidemic. MetS groups several cardiometabolic risk factors including abdominal obesity, hyperglycemia, dyslipidemia, insulin resistance, inflammation, and high blood pressure which predispose to the development of type-2 diabetes and cardiovascular diseases [1].

Several factors are involved in the development of MetS which are linked to adipose tissue dysfunction, one of them being the circulating free fatty acids (FFA). In a MetS model developed by our group, we have found alterations in serum lipid composition, that is, high levels of nonesterified fatty acids (NEFAs) and of monounsaturated fatty acids (MUFAs) which have been proposed as contributors of the acquisition of insulin resistance and hypertension [2–4]. Moreover, FFA and their derivatives trigger physiological responses such as adipogenesis and adipokine secretion [5].

Flavonoid intake is positively associated with a decrease in the incidence of metabolic and obesity-related disorders. Resveratrol (RSV) (3,4′,5-trihydroxystilbene)

is a phytoalexin found in the skin and seeds of grapes and red wine. RSV may protect against diet-induced obesity and metabolic diseases such as hepatic steatosis and insulin resistance [6]. Quercetin (QRC) (3,5,7,3′,4′-pentahydroxyflavone) is a polyphenolic flavonoid compound present in onions, broccoli, tomatoes, apples, and berries and it possesses antioxidant, anti-inflammatory, and antiatherogenic properties including hepatoprotection [7]. RSV and QRC could be promising therapeutic agents acting as sirtuin activators. They have shown benefic effects for the treatment of metabolic diseases such as obesity and MetS.

The sirtuin (SIRT) family of NAD^+-dependent protein deacetylases and ADP-ribosyltransferases has emerged as an exciting target for cardiovascular disease management since they can impact the cardiovascular system both directly and indirectly by modulating whole body metabolism [8]. Mammals contain seven sirtuins (SIRTs 1–7) that are localized in distinct subcellular compartments. SIRT 1, SIRT 6, and SIRT 7 are found in the nucleus; SIRT 2 is primarily cytosolic; and SIRTs 3–5 are found in mitochondria [9]. In addition to the differences in subcellular localization, the sirtuins are also expressed in varying amounts in different tissues.

QRC is more effective in reducing adipogenesis in preadipocytes, whereas RSV is more effective in inhibiting lipid metabolism in mature adipocytes. Other studies suggest the synergistic effect of both natural compounds to treat metabolic disorders [10, 11]. Moreover, these compounds are now available in tablets on the market.

Thus, the goal of this study was to examine the effect of the commercial mixture of RSV and QRC on the serum FA profile and on the SIRTs 1–3 expression in white adipose tissue (WAT).

2. Materials and Methods

2.1. Animals. All experiments were conducted in accordance with the Institutional Ethical Guidelines.

Weanling male Wistar rats aged 25 days and weighing 50± 4 g, $n = 12$ per group were separated into two groups: group 1, control rats (C), given tap water for drinking, and group 2, MetS rats, receiving 30% sugar in their drinking water during 5 months.

One-third of each group of rats (control or MetS) received orally in drinking water or sucrose solution a mixture of RSV and QRC daily for 4 weeks (provided by ResVitalé which contains 20 mg of QRC per 1,050 mg of RSV) in one of the following doses: (1) RSV + QRC 10 mg/kg/day–0.19 mg/Kg/day (RSV 10 + QRC 0.19); and (2) RSV + QRC 50 mg/kg/day–0.95 mg/Kg/day (RSV 50 + QRC 0.95). Groups without RSV + QRC treatment only received the vehicle in which the natural compounds were dissolved. The mixture of RSV and QRC was previously dissolved in 1 mL ethanolic solution (20%).

All animals were fed Purina 5001 rat chow (Richmond, IN) *ad libitum*, which provides 14.63 KJ/g with 23% protein, 12% fat, and 65% carbohydrate, and were kept under controlled temperature and a 12 : 12-hour light-dark cycle.

Systolic arterial blood pressure was measured in conscious animals using the tail cuff method as described previously [12].

2.2. Blood Samples. At the end of experimental period and after an overnight fasting (12 h), the animals were killed by decapitation and blood was collected. Serum was isolated by centrifugation and stored at −70°C until needed. Serum insulin, adiponectin, and leptin were determined using commercial radioimmunoassay (RIA) kits specific for rat (Linco Research Inc., Missouri, USA); the sensitivity was of 0.1 ng/mL; and intra- and interassay coefficients of variation were 5%, 10%, and 10%, respectively. Glucose concentration was assayed using an enzymatic Kit SERA-PAK^R Plus (Bayer Corporation, Sées, France).

Total cholesterol (TC) and plasma triglyceride concentrations were measured using commercial enzymatic assays (RANDOX Laboratories, UK). The high-density lipoprotein (HDL) cholesterol content was determined in the bottom fraction obtained after ultracentrifugation of plasma at density of 1.063 g/mL for 2.5 h at 100,000 rpm (Beckman optima TLX) [13, 14]. The non-HDL-C is defined as the difference between the values of TC and HDL-C and includes LDL-C, IDL, and VLDL. Recently non-HDL-C has become a commonly used marker for a blood lipid pattern associated with increased risk of heart disease.

The homeostasis model assessment of insulin resistance (HOMA-IR) was used as the physiological index of insulin resistance. The HOMA-IR was calculated from the fasting glucose and insulin concentrations by the following formula: (insulin (μU/mL) × glucose (in mmol/L)/22.5) [14].

2.3. WAT Homogenate. Abdominal WAT was removed and weighed. The samples were immediately frozen in liquid nitrogen and stored at −70°C for later analysis. Frozen WAT samples were homogenized (25% w/v) in a lysis buffer pH = 8 (25 mM HEPES, 100 mM NaCl, 15 mM imidazole, 10% glycerol, and 1% Triton X-100) and protease inhibitor cocktail [14]. The WAT homogenate was centrifuged at 19,954 g for 10 min at 4°C; the supernatant was separated and stored at −70°C. The protein concentration of each sample was measured using the Bradford method [15].

2.4. SIRT 1, SIRT 2, SIRT 3, and PPAR-γ Expression. Protein expression was examined by Western blot analysis. A total of 50 μg protein was separated by SDS-PAGE (12% polyacrylamide gel) and transferred to a PVDF membrane. The blots were blocked for 3 hours at room temperature with Tris buffer solution (TBS) containing 5% nonfat dry milk and 0.05% Tween 20. The membranes were incubated overnight at 4°C with rabbit primary polyclonal antibodies (SIRT 1, Santa Cruz Biotechnology, Santa Cruz, CA; SIRT 2, SIRT 3, and PPAR-γ, Abcam) at a final dilution of 1 : 1000. Then, the membranes were incubated for 2 h at room temperature with a secondary antibody (goat anti-rabbit horseradish peroxidase conjugated, dilution 1 : 10,000, Santa Cruz Biotechnology, Santa Cruz, CA). After incubation, the blots were visualized using a chemiluminescence kit (Immobilon Western, Millipore, MA, USA). Blots were stripped and reincubated with monoclonal

Table 1: The effects of RSV + QRC administration on body characteristics and biochemical parameters from control and MetS rats.

	Control			MetS		
	Without treatment	RSV 10 + QRC 0.19 mg/kg/day	RSV 50 + QRC 0.95 mg/kg/day	Without treatment	RSV 10 + QRC 0.19 mg/kg/day	RSV 50 + QRC 0.95 mg/kg/day
Body weight (g)	502.0 ± 19.5	463.8 ± 20.8	514.2 ± 20.9	570.4 ± 13.4[a]	490.8 ± 11.6[c]	489.8 ± 10.5[c]
Central adiposity (g)	6.0 ± 0.7	4.5 ± 0.6	6.3 ± 0.9	12 ± 0.6[a]	11.5 ± 0.9	8.9 ± 1.1[c]
Blood pressure (mm Hg)	103.3 ± 1.0	101.2 ± 2.7	108.4 ± 3.5	140.5 ± 1.0[a]	122.7 ± 3.8[c]	115.5 ± 2.9[c]
Glucose (mg/dL)	119.9 ± 12.2	118.8 ± 13.7	87.9 ± 5.9	121.8 ± 20.3	85.3 ± 9.5	90.7 ± 7.7
Insulin (ng/mL)	0.26 ± 0.02	0.25 ± 0.05	0.18 ± 0.04	0.47 ± 0.04[a]	0.29 ± 0.05[c]	0.23 ± 0.02[c]
HOMA-IR	1.3 ± 0.2	0.61 ± 0.03	0.9 ± 0.12	2.1 ± 0.3[b]	0.9 ± 0.1[c]	0.81 ± 0.1[c]
Leptin (ng/mL)	2.3 ± 0.3	3.3 ± 0.3	2.6 ± 0.1	4.2 ± 0.3[a]	5.2 ± 0.3[d,e]	3.8 ± 0.3[e]
Adiponectin (μg/mL)	3.8 ± 0.2	4.2 ± 0.3	3.7 ± 0.3	6.7 ± 0.3[a]	6.1 ± 0.5[e]	5.8 ± 0.2[e]

Values are mean ± SEM. HOMA-IR: homeostatic model assessment of insulin resistance; $n = 12$; [a]$P < 0.01$ MetS without treatment versus control without treatment; [b]$P < 0.05$ MetS without treatment versus control without treatment; [c]$P < 0.01$ against same group without treatment; [d]$P < 0.01$ versus same group with different doses; [e]$P < 0.01$ against control with same dose.

α-actin antibody as control. Images from films were digitally acquired by GS-800 densitometer with the Quantity One software (Bio-Rad). The values of each band density are expressed as arbitrary units (AU).

2.5. Total Fatty Acid and Nonesterified Fatty Acids Lipid Extraction. Fatty acids (FA) and NEFAs were extracted and identified by gas liquid chromatography, from serum (100 μL) and from the administrated RSV + QRC commercial mixture (50 μg), using the method described previously [4].

2.6. Statistical Analysis. Results were expressed as mean ± standard error of the mean (SEM). For multiple comparisons, we applied one-way analysis of variances (ANOVA) using the SigmaPlot 11 program. Differences were considered significant when the P value was <0.05.

3. Results

Table 1 summarizes the characteristics of the groups of rats used. Experimental animals developed MetS characterized by hypertension, central adiposity, hyperinsulinemia, and insulin resistance (HOMA-IR). Leptin and adiponectin concentrations were significantly higher in the MetS than in control rats. In MetS rats, the treatment with RSV + QRC (both doses) prevented the increase in body weight and significantly decreased the central adiposity; however, leptin concentrations remained high when compared to controls. A tendency towards lower values was observed with the high dose in adiponectin concentrations. Systolic arterial pressure diminished in the MetS RSV-QRC-treated group in a dose-dependent manner.

RSV + QRC significantly reduced insulin concentration in MetS rats and restored HOMA-IR. No differences were found between both doses. Fasting serum glucose levels were not significantly different among the groups. RSV + QRC supplementation did not alter significantly any parameters in control group (Table 1).

Table 2 shows the lipidic profile of both MetS and control animals. MetS showed dyslipidemia (high levels of triglycerides and non-HDL-C and low levels of HDL-C). RSV + QRC significantly reduced the concentration of triglycerides. The high dose was able to diminish the amount of non-HDL-C in experimental group. Although a statistical significance was only present with the low dose in HDL-C, a clear tendency towards increased values was found with the high dose in the MetS group.

In the control group, only the non-HDL-C was significantly decreased with RSV + QRC administration (Table 2). No changes were observed in TC content among the groups.

The FA composition (%) of serum from control and MetS rats is shown in Table 3. Seric concentrations of palmitoleic acid, oleic acid, and MUFA were significantly increased, while stearic and PUFA decreased in MetS rats in comparison to control rats. The treatment with RSV + QRC restored the levels of oleic acid (by the highest RSV + QRC dose) and PUFA (with both doses). In the control group, the treatment with RSV + QRC increased the levels of arachidonic and PUFA in a dose-dependent way.

Table 4 shows the results corresponding to the NEFA present in serum from the six groups studied. NEFAs such as MUFA and palmitoleic and oleic acids were significantly increased in MetS when compared to control animals. SFA, PUFA, stearic, linoleic, and arachidonic levels are diminished in MetS in comparison to those in the controls. In MetS rats, both doses of RSV + QRC increased stearic acid and the highest dose of RSV + QRC significantly diminished palmitoleic acid content. In the control group, the treatment with the natural compounds had no effect.

Additionally, we analyzed the FA composition of the RSV + QRC administrated and we found that linoleic, oleic, and palmitic acids were the most abundant FA (37.8 ± 2.1%, 22.1 ± 1.0%, and 21.6 ± 1.1%, resp.). Stearic (11.9 ± 0.9%), palmitoleic (3.8 ± 0.6%) and arachidonic (2.8 ± 0.3%) acids also were present (data not shown).

To address the effect of RSV + QRC administration on the expression of SIRTs in WAT, we performed immunoblotting analyses.

The data in Figures 1, 3, and 4 show SIRT 1 (62 KDa), SIRT 2 (43 KDa), and SIRT 3 (28 KDa) levels, respectively,

Table 2: The effects of RSV + QRC administration on serum triglycerides, total cholesterol (TC), HDL-C, and non-HDL-C levels from control and MetS rats.

	Control			MetS		
	Without treatment	RSV 10 + QRC 0.19 mg/kg/day	RSV 50 + QRC 0.95 mg/kg/day	Without treatment	RSV 10 + QRC 0.19 mg/kg/day	RSV 50 + QRC 0.95 mg/kg/day
Triglycerides (mg/dL)	77.8 ± 7.9	71.4 ± 7.4	57.8 ± 9.2	133.7 ± 6.3[a]	103.2 ± 9.7[e]	90.5 ± 5.4[c,e]
TC (mg/dL)	57.6 ± 5.6	55.5 ± 3.4	45.7 ± 1.7	52.3 ± 3.5	56.6 ± 5.7	38.2 ± 4.7
HDL-C (mg/dL)	28.2 ± 2.5	27.1 ± 1.8	28.6 ± 1.6	17.6 ± 1.8[a]	29.1 ± 4.2[c,d]	20.3 ± 2.4
non-HDL-C (mg/dL)	22.8 ± 2.1	29.4 ± 2.7	17.1 ± 0.4[d]	35.2 ± 3.02[a]	27.5 ± 2.2	17.9 ± 2.8[c,d]

Values are mean ± SEM. $n = 12$; [a]$P < 0.01$ MetS without treatment versus control without treatment; [c]$P < 0.01$ against same group without treatment; [d]$P < 0.01$ versus same group with different doses; [e]$P < 0.01$ against control with same dose.

Table 3: Effect of RSV + QRC administration on seric fatty acid (FA) composition from control and MetS rats.

	Control			MetS		
FA %	Without treatment	RSV 10 + QRC 0.19 mg/kg/day	RSV 50 + QRC 0.95 mg/kg/day	Without treatment	RSV 10 + QRC 0.19 mg/kg/day	RSV 50 + QRC 0.95 mg/kg/day
Palmitic acid	32.7 ± 0.9	31.7 ± 0.6	32.1 ± 1.2	33.5 ± 0.6	32.7 ± 1.1	32.2 ± 1.2
Palmitoleic acid	4.3 ± 0.7	5.6 ± 1.1	4.5 ± 0.8	6.7 ± 0.3[a]	6.2 ± 0.5	7.3 ± 0.6
Stearic acid	22.2 ± 0.9	22.8 ± 0.9	22.6 ± 1.1	19.4 ± 1.1	19.5 ± 1.0	19.8 ± 0.5
Oleic acid	14.3 ± 1.1	13.5 ± 0.6	13.3 ± 0.7	21.9 ± 1.3[a]	22.1 ± 0.4	17.7 ± 1.1[c]
Linoleic acid	13.6 ± 0.8	12.6 ± 0.8	14.1 ± 0.9	10.5 ± 1.2	11.3 ± 0.7	10.3 ± 0.5
γ-linoleic acid	0.3 ± 0.03	0.9 ± 0.4	0.4 ± 0.1	0.8 ± 0.3	0.5 ± 0.1	0.3 ± 0.1
Dihomo-γ-linoleic acid	1.2 ± 0.5	0.6 ± 0.1	0.6 ± 0.2	0.7 ± 0.4	0.4 ± 0.05	0.8 ± 0.4
Arachidonic acid	5.5 ± 1.1	10.7 ± 1.1[c]	12.1 ± 0.9[c]	6.7 ± 0.9	7.9 ± 0.6	7.3 ± 0.4
SFA	59.9 ± 1.4	55.9 ± 2.0	54.7 ± 1.7	54.0 ± 1.5	52.3 ± 1.5	52.7 ± 1.7
MUFA	17.8 ± 1.0	19.0 ± 1.4	17.8 ± 0.8	28.6 ± 1.6[a]	27.6 ± 0.5	25.6 ± 1.4
PUFA	20.3 ± 1.6	23.5 ± 1.2[c]	27.2 ± 1.6[c,d]	15.3 ± 0.8[a]	20.1 ± 1.2[c]	19.2 ± 0.5[c]

SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, and PUFA: polyunsaturated fatty acid. Data are mean ± SEM. $n = 12$; [a]$P < 0.01$ MetS without treatment versus control without treatment; [c]$P < 0.01$ against same group without treatment; [d]$P < 0.05$ versus same group with different dose.

Table 4: Effect of RSV + QRC administration on seric nonesterified fatty acids (NEFAs) composition from control and MetS rats.

	Control			MetS		
NEFAs %	Without treatment	RSV 10 + QRC 0.19 mg/kg/day	RSV 50 + QRC 0.95 mg/kg/day	Without treatment	RSV 10 + QRC 0.19 mg/kg/day	RSV 50 + QRC 0.95 mg/kg/day
Palmitic acid	34.9 ± 1.3	32.5 ± 1.4	33.6 ± 0.9	33.6 ± 0.8	32.8 ± 0.9	33.6 ± 0.7
Palmitoleic acid	6.2 ± 0.9	5.3 ± 0.8	5.1 ± 0.9	13.1 ± 0.8[a]	11.6 ± 0.5	10.9 ± 0.5[c]
Stearic acid	22.2 ± 0.5	24.1 ± 0.7	23.1 ± 0.8	16.2 ± 0.5[a]	19.2 ± 1.1[c]	19.8 ± 0.5[c]
Oleic acid	17.8 ± 1.0	19.4 ± 1.0	18.6 ± 0.9	22.5 ± 0.9[a]	23.0 ± 0.5	21.9 ± 0.5
Linoleic acid	13.7 ± 1.4	14.3 ± 0.6	13.9 ± 0.9	8.8 ± 0.3[b]	8.1 ± 0.4	9.0 ± 0.4
γ-linoleic acid	1.3 ± 0.2	1.4 ± 0.3	1.4 ± 0.3	1.6 ± 0.3	1.1 ± 0.2	1.2 ± 0.3
Dihomo-γ-linoleic acid	0.7 ± 0.08	0.8 ± 0.1	0.6 ± 0.07	0.5 ± 0.1	0.4 ± 0.05	0.6 ± 0.07
Arachidonic acid	3.1 ± 0.5	2.4 ± 0.2	3.9 ± 0.4	2.1 ± 0.3[b]	3.1 ± 0.7	2.5 ± 0.3
SFA	57.2 ± 1.6	56.7 ± 1.6	56.7 ± 0.9	49.8 ± 0.9[a]	52.0 ± 1.05	53.4 ± 1.1
MUFA	22.6 ± 1.4	24.6 ± 1.1	23.7 ± 0.6	36.2 ± 0.9[a]	34.3 ± 0.6	32.4 ± 0.8
PUFA	17.6 ± 0.9	18.7 ± 0.6	19.8 ± 1.3	13.5 ± 0.7[b]	13.7 ± 1.1	14.1 ± 1.1

SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, and PUFA: Polyunsaturated fatty acid. Data are mean ± SEM. $n = 12$; [a]$P < 0.01$ MetS without treatment versus control without treatment; [b]$P < 0.05$ MetS without treatment versus control without treatment; [c]$P < 0.01$ against same group without treatment.

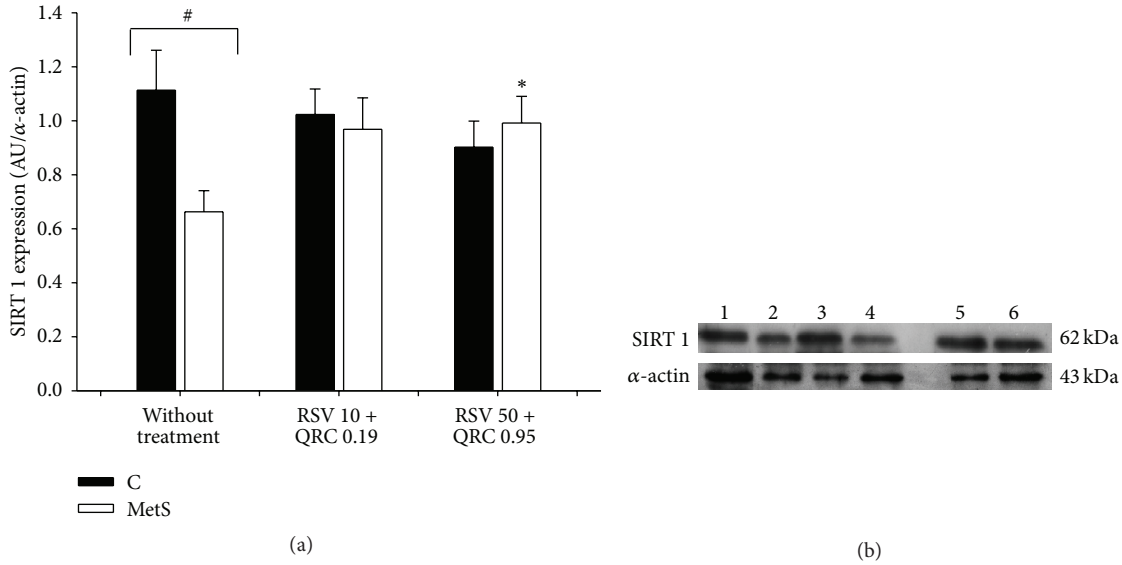

Figure 1: RSV plus QRC leads to SIRT 1 expression in WAT from MetS rats. (a) Protein expression, data represent mean ± SEM ($n = 6$ per group). $^{\#}P < 0.05$; $^{*}P < 0.05$ against MetS without treatment. (b) Representative Western blot analysis. Line 1: control without treatment; line 2: control treated with RSV 10 + QRC 0.19; line 3: control treated with RSV 50 + QRC 0.95; line 4, MetS without treatment; line 5: MetS treated with RSV 10 + QRC 0.19; line 6: MetS treated with RSV 50 + QRC 0.95.

of control and MetS rats treated with RSV + QRC. The expression of SIRT 1 and SIRT 2 in MetS rats was reduced when compared to control rats. When assessing the effect of RSV + QRC treatment in MetS group, we observed a clear tendency towards increased values of SIRT 1 expression with the lowest dose and a significant increase with RSV 50 + QRC 0.95 (Figures 1(a) and 1(b)). There was a significant increase in SIRT 2 expression with both doses of RSV + QRC (Figures 3(a) and 3(b)). There was no significant change in SIRT 1 and SIRT 2 expression in control rats treated with RSV + QRC.

SIRT 3 expression in WAT was similar in control and MetS rats (Figures 4(a) and 4(b)). Although SIRT 3 expression levels were not significantly modified, a tendency towards reduced values was observed in MetS animals treated with the highest dose. In control rats, the opposite effect was observed and the level of expression of the protein was significantly higher than in MetS rats.

We also evaluated the effect of RSV + QRC on PPAR-γ expression, a target of SIRT 1 (Figures 2(a) and 2(b)). As expected, the levels of PPAR-γ were significantly increased in MetS rats in comparison to control animals. The administration of both of the doses of RSV + QRC did not significantly modify PPAR-γ expression. In contrast, the highest dose of RSV + QRC significantly increased PPAR-γ expression in the control group.

4. Discussion

MetS is actually considered as an epidemic and is a complex and heterogeneous disease. Therapeutic tools used to control MetS include lifestyle changes (increases in physical activity and caloric restriction), pharmacological agents, and natural compounds. Although several studies have shown that the RSV and/or QRC that are present in plants and fruits have beneficial effects on metabolic disorders by regulating sirtuin expression and activity, the effect of these compounds on changes in serum FFA still remain unclear. In the present work, we analyzed the effects of two doses of a combination of RSV and QRC on body fat, serum parameters, SIRTs 1–3, and PPAR-γ expression in a rat model of MetS.

MetS rats exhibited increased body weight, central adiposity, hypertension, insulin resistance, and elevated circulating levels of adiponectin and leptin (Table 1). These results are in accordance with our previous report [14]. Several studies have demonstrated beneficial effects of RSV and QRC reducing body fat and improving insulin sensitivity [16]. In MetS rats, both of the doses of RSV + QRC treatment tested in this paper were equally efficient in reducing body weight, blood pressure, and insulin levels without having an effect on the concentration of adiponectin and leptin.

In the MetS group, RSV + QRC treatment attenuated the increase in blood pressure in a dose-dependent manner (14% and 22% by RSV 10 + QRC 0.19 and RSV 50 + QRC 0.95, resp.) (Table 1). The antihypertensive effect of RSV and QRC may be due to activation of several mechanisms which have already been described and that include increased NO availability caused by the elevation of NOS activity and by a decrease in oxidative stress and inflammation [17–19].

Our MetS model had high circulating levels of leptin and adiponectin compared to control animals, suggesting the presence of resistance to these adipokines as previously reported (Table 1) [14]. A possible mechanism by which polyphenols might act is by regulating adipokine levels and their intracellular signaling mechanisms [10, 18, 20]. In the present study, the supplementation with RSV + QRC had no effect upon the leptin concentration and only caused a slight decrease in adiponectin levels (13% by RSV 50 + QRC 0.95). This discrepancy with other reports might be

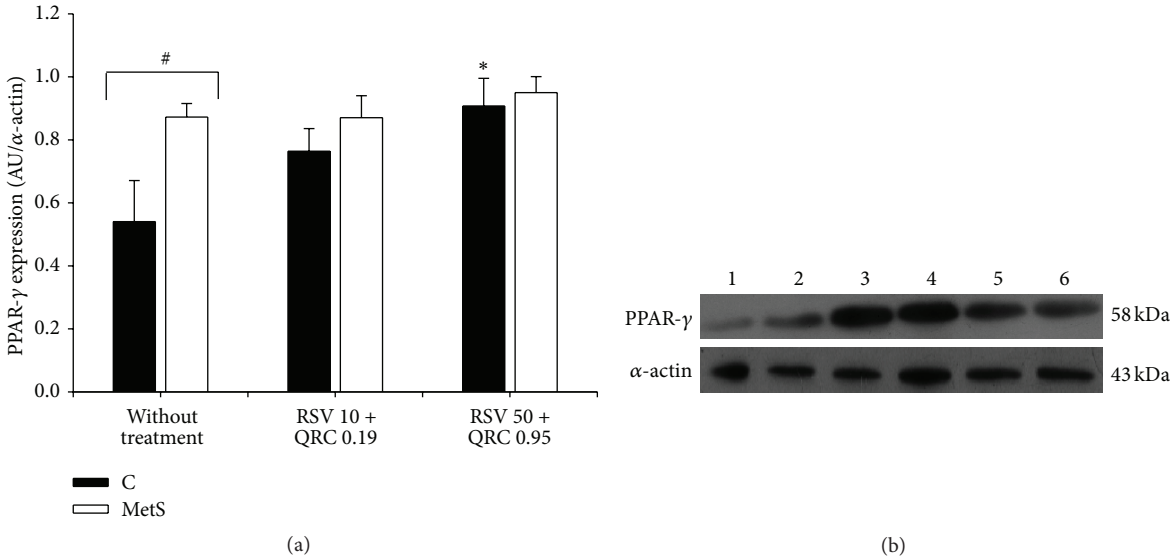

Figure 2: Effect of RSV + QRC administration on PPAR-γ expression in WAT from control and MetS rats. (a) Protein expression, data represent mean ± SEM ($n = 6$ per group). $^{\#}P < 0.05$; $^{*}P < 0.05$ against control without treatment. (b) Representative Western blot analysis. Line 1: control without treatment; line 2: control treated with RSV 10 + QRC 0.19; line 3: control treated with RSV 50 + QRC 0.95; line 4, MetS without treatment; line 5: MetS treated with RSV 10 + QRC 0.19; line 6: MetS treated with RSV 50 + QRC 0.95.

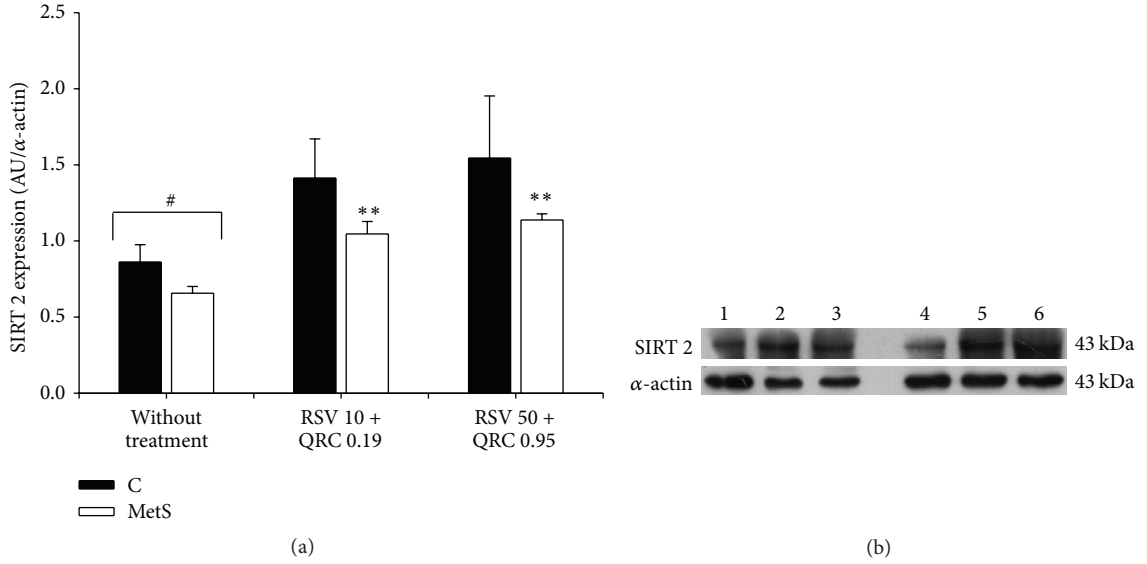

Figure 3: RSV plus QRC leads to SIRT 2 expression in WAT from MetS rats. (a) Protein expression, data represent mean ± SEM ($n = 6$ per group). $^{\#}P < 0.05$; $^{**}P < 0.01$ against MetS without treatment. (b) Representative Western blot analysis. Line 1: control without treatment; line 2: control treated with RSV 10 + QRC 0.19; line 3: control treated with RSV 50 + QRC 0.95; line 4, MetS without treatment; line 5: MetS treated with RSV 10 + QRC 0.19; line 6: MetS treated with RSV 50 + QRC 0.95.

due to the different administration periods used. Possibly, if we increased the duration of the polyphenol administration period, a difference in adipokine levels might become evident.

In the control rats, none of the parameters studied was affected by either dose of RSV + QRC (Table 1). These results are consistent with data from RSV studies conducted in lean metabolically normal rodents and in human subjects [21].

MetS rats developed dyslipidemia with decreased levels of HDL-C and increased levels of non-HDL-C and triglycerides when compared to control animals (Table 2). In the MetS group, the highest dose of RSV + QRC was effective in reducing triglycerides and non-HDL-C, while, in control animals, only the highest dose was able to reduce non-HDL-C concentration. Our results on the improvement of dyslipidemia with the RSV + QRC treatment are in accordance with those published by other authors who used natural compounds separately in other models of obesity or in isolated adipocytes [10, 18, 22]. The importance of testing these compounds in our model resides in the fact that our

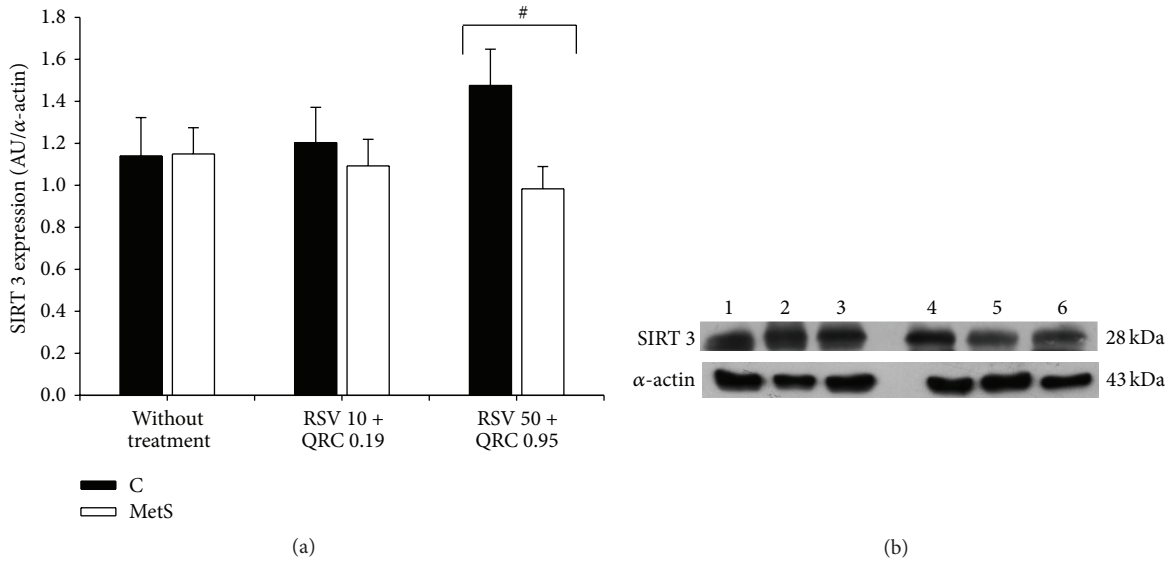

Figure 4: Effect of RSV + QRC administration on SIRT 3 expression in WAT from control and MetS rats. (a) Protein expression, data represent mean ± SEM ($n = 6$ per group). $^{\#}P < 0.05$. (b) Representative Western blot analysis. Line 1: control without treatment; line 2: control treated with RSV 10 + QRC 0.19; line 3: control treated with RSV 50 + QRC 0.95; line 4, MetS without treatment; line 5: MetS treated with RSV 10 + QRC 0.19; line 6: MetS treated with RSV 50 + QRC 0.95.

model resembles the appearance of MetS by the ingestion of high sucrose levels in a similar way as it happens in humans who consistently ingest sucrose in sweetened beverages.

Table 3 shows seric FA composition in both control and MetS rats. Circulating PUFA levels were decreased in MetS rats when compared to controls and these data positively correlate with the increase in central adiposity present in this group. Both doses of RSV + QRC increased PUFA concentrations in MetS rats in the same proportion (33%), while in control rats the increase in PUFA concentration seemed to be dose-dependent (15% and 35% by RSV 10 + QRC 0.19 and RSV 50 + QRC 0.95, resp.). Regarding this aspect, Rodriguez-Cruz et al. [23] reported that high levels of PUFAs are negative regulators of lipogenesis. PUFAs may function as activators/ligands of PPAR-γ limiting hyperplasia and hypertrophy of adipose tissue [24].

There are many evidences indicating that dietary MUFAs reduce key risk factors for MetS. However, as far as we know, there are very few studies that show the benefic effects of RSV + QRC on the seric profile of MetS rat models. In serum from MetS rats, MUFAs such as palmitoleic and oleic acids were increased when compared to those of control animals (55% and 50%, resp.) (Table 3). RSV 50 + QRC 0.95 significantly diminished oleic acid concentration without having an effect on palmitoleic levels. The decrease on circulating oleic acid by RSV + QRC may be related to the attenuation of high blood pressure [4].

There is a tendency to increase seric arachidonic acid concentration with the RSV + QRC treatment (with and without significant difference, resp.) in the control and MetS groups (Table 3). Some authors have reported that RSV and QRC may modulate arachidonic acid release and metabolism due to their anti-inflammatory activity [25–27]; however, future studies need to be undertaken to examine the effect of RSV + QRC on arachidonic acid metabolism and its contribution to diminished blood pressure and other parameters in our rat MetS model.

Circulating levels of NEFAs such as oleic and palmitoleic acid were higher in MetS when compared to their levels in control rats (Table 4). These fatty acids were more abundant than triglycerides, cholesterol esters, and phospholipids [28]. Thus, our results show that central adiposity leads to an important increase in NEFA and triglyceride production in MetS animals. High concentrations of NEFA are related to oxidative stress, hypertension, dyslipidemia, and insulin resistance [3, 4, 29]. The RSV + QRC treatment produced a slight decrease in oleic and palmitoleic levels (3% and 16%, resp.). Moreover, the decrease in fat accumulation was accompanied by a decrease in the concentration of free fatty acids with a concomitant decrease in triglyceride concentrations. Our results were consistent with other studies which indicated that RSV and QRC significantly suppressed the serum NEFA levels; however, in the present study we administer the combination of flavonoids and identified each NEFA, which had not been previously reported [19, 30, 31].

Several reports have shown that RSV and QRC are sirtuin activators; therefore, we investigated the effect of RSV + QRC on SIRTs 1–3 expression in WAT from control and MetS rats. Figure 1 shows that SIRT 1 was underexpressed in MetS rats and that the treatment with both doses of RSV + QRC restored SIRT 1 expression. Our data are in accordance with several animal studies that have provided strong evidence on the positive effect of RSV and QRC upregulating SIRT 1 in different models. SIRT 1 is an important regulator of hepatic glucose metabolism; it improves insulin signaling and promotes fatty acid metabolism [32, 33]. However, in the present work, SIRT 1 overexpression did not have a significant effect on adipokine secretion.

SIRT 1 regulates the pathway of cellular energy metabolism, controlling PGC1-α, p53, forkhead transcription factors (FOXO), p300, and PPAR-γ which plays an important role in the induction of cellular differentiation of adipocytes and in the regulation of lipid metabolism. Floyd et al. [34] reported that resveratrol modulates PPAR-γ protein levels and transcriptional activity in 3T3-L1 adipocytes. Moreover, SIRT 1 represses PPAR-γ in WAT by attaching to its cofactor's nuclear receptor corepressor (NCoR) [35]. In this study, when the expression of PPAR-γ was measured, its levels were higher in WAT from MetS rats than in control subjects (Figure 2). This result had been previously reported by our group [14]. The treatment with RSV + QRC had no effect on PPAR-γ expression on MetS group, while in the control group RSV 50 + QRC 0.95 significantly increased PPAR-γ levels. Although the treatment with these concentrations of polyphenols had no effect on the PPAR-γ expression in MetS animals, RSV + QRC could be regulating PPAR-γ activity. To further clarify this point, it would be important to evaluate the effect of RSV + QRC administration on gene expression of transcriptional targets of PPAR-γ and on other adipogenic markers such as C/EBPβ and SREBP1c. Our results agree with those of a previous study that reported that QRC did not modify PPAR-γ expression in 3T3-L1 preadipocytes [10].

SIRT 2 is reported to be the most abundant sirtuin in adipocytes, in white and brown adipose tissue [36]. As expected, SIRT 2 expression was decreased in WAT from MetS rats in comparison to control rats, which might promote fat accumulation (Figure 3). RSV + QRC induced SIRT 2 expression in a dose-dependent manner (59% and 73% by RSV10 + QRC 0.19 and RSV50 + QRC 0.95, resp.), while in the control group it had no effect. The effect of RSV + QRC on SIRT 2 expression agrees with the results previously reported by Gregory [37].

SIRT 3 is the major mitochondrial deacetylase regulating mitochondrial metabolism, adaptive thermogenesis, energy homeostasis, and apoptosis and it is decreased in obese mice [38, 39]. Moreover, SIRT 3 plays an important role in adaptive thermogenesis of brown adipose tissue regulating UCP-1, PGC-1α, cytochrome c oxidase, and ATP synthase expression. When we analyzed the expression of SIRT 3, we found that WAT from control and MetS rats expressed SIRT 3 in the same proportion and its expression was not significantly modified by the RSV + QRC treatment (Figure 4). However, there was a tendency to an increase in the SIRT 3 levels in control rats when compared to MetS animals treated with the highest dose of RSV + QRC (Figure 4). The precise reason for this discrepancy is unclear but it would be interesting to evaluate the effect of RSV + QRC on SIRT 3 activity.

In conclusion, our data suggest that RSV + QRC (particularly RSV50 + QRC 0.95) influences adipose tissue mass and function in a way that may positively interfere with the development of MetS. This effect may be associated with an increase in PUFA and with a decrease in circulating levels of MUFA and NEFA. Moreover, RSV + QRC upregulates SIRT 1 and SIRT 2 expression in WAT.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors thank Bertha Soto, Mario Pérez, Florencio Hernández, Edmundo Sánchez, and Oscar Grimaldo for the excellent technical assistance.

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2015, Article ID 352647, 8 pages
http://dx.doi.org/10.1155/2015/352647

Research Article

ACE Reduces Metabolic Abnormalities in a High-Fat Diet Mouse Model

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Received 16 March 2015; Accepted 27 July 2015

Academic Editor: Won-Seok Chung

The medicinal plants *Artemisia iwayomogi* (*A. iwayomogi*) and *Curcuma longa* (*C. longa*) radix have been used to treat metabolic abnormalities in traditional Korean medicine and traditional Chinese medicine (TKM and TCM). In this study we evaluated the effect of the water extract of a mixture of *A. iwayomogi* and *C. longa* (ACE) on high-fat diet-induced metabolic syndrome in a mouse model. Four groups of C57BL/6N male mice (except for the naive group) were fed a high-fat diet freely for 10 weeks. Among these, three groups (except the control group) were administered a high-fat diet supplemented with ACE (100 or 200 mg/kg) or curcumin (50 mg/kg). Body weight, accumulation of adipose tissues in abdomen and size of adipocytes, serum lipid profiles, hepatic steatosis, and oxidative stress markers were analyzed. ACE significantly reduced the body and peritoneal adipose tissue weights, serum lipid profiles (total cholesterol and triglycerides), glucose levels, hepatic lipid accumulation, and oxidative stress markers. ACE normalized lipid synthesis-associated gene expressions (peroxisome proliferator-activated receptor gamma, PPARγ; fatty acid synthase, FAS; sterol regulatory element-binding transcription factor-1c, SREBP-1c; and peroxisome proliferator-activated receptor alpha, PPARα). The results from this study suggest that ACE has the pharmaceutical potential reducing the metabolic abnormalities in an animal model.

1. Introduction

Metabolic syndrome has become a serious health issue worldwide as the prevalence of obesity increases [1]. A previous cohort study reported a strong relationship between metabolic syndrome and an increased risk of mortality [2]. Metabolic syndrome is defined as a cluster of metabolic abnormalities including obesity, hyperlipidemia, hyperglycemia, insulin resistance, and hypertension [3]. Generally, an individual is considered to have metabolic syndrome if he/she has central obesity plus any two of the above factors [1]. Improving these pathological conditions, especially obesity, is major therapeutic goals for treating metabolic syndrome.

Lifestyle modifications, including exercise and diet control, are recommended as a first-line treatment for managing metabolic syndrome [3]. However, drug therapy is now also considered an attractive method of treating metabolic syndrome. Various agents are currently used specifically for lowering lipid profiles or blood pressure, but novel therapies targeting multiple etiological factors are particularly desirable [4]. Natural remedies are currently attracting more attention as therapeutic or protective agents for treating metabolic syndrome [5].

In traditional Korean medicine (TKM) and traditional Chinese medicine (TCM), metabolic abnormalities are considered indicative of "*dampness/phlegm*" (濕痰) and "*blood stasis*" (瘀血) [6]. *Artemisia iwayomogi* (*A. iwayomogi*) is a representative herb treating dampness/phlegm in TKM and TCM. Previous studies have reported the therapeutic properties of *A. iwayomogi* on obesity, hyperlipidemia, and liver fibrosis [7, 8]. Conversely, *Curcuma longa* (*C. longa*) has been used to treat the pathological condition of blood stasis

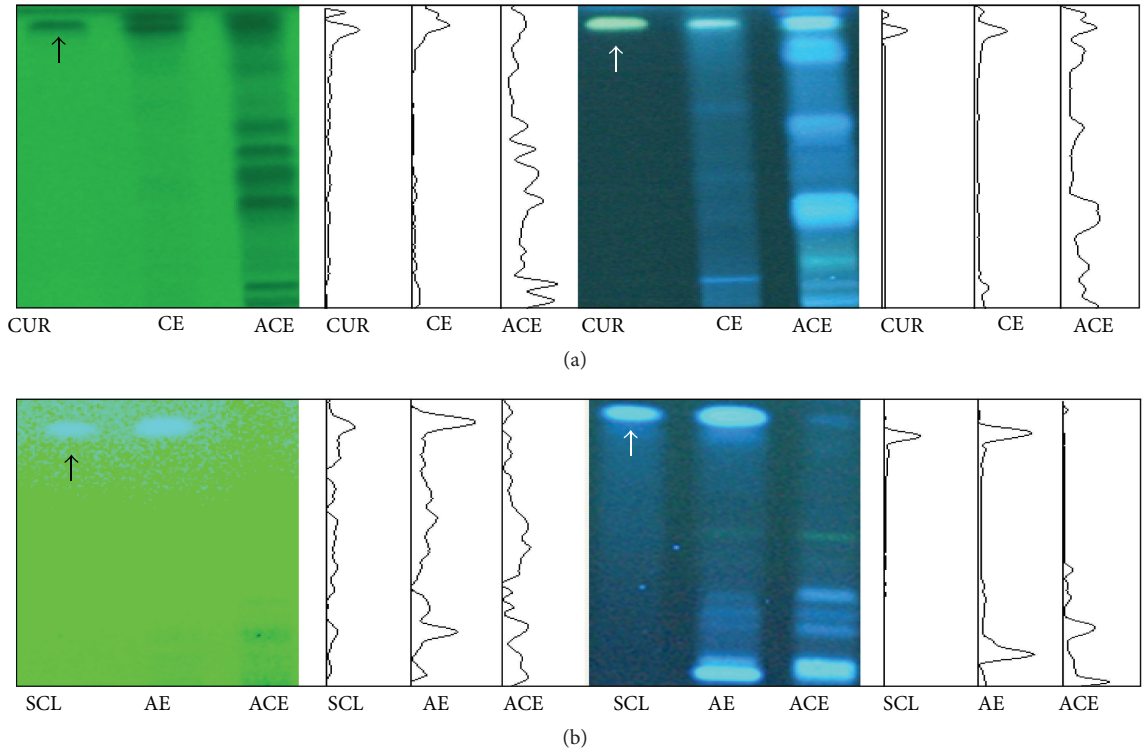

Figure 1: Fingerprint analysis of ACE using high-performance thin-layer chromatography (HP-TLC). ACE and its two major components were analyzed using HP-TLC and compared with reference compounds. Scopoletin (SCL, 0.1 μg/μL (a)), *Artemisia iwayomogi* (AI, 10 μg/μL (a)), curcumin (CUR, 0.1 μg/μL (b)), *Curcuma longa* radix (CL, 10 μg/μL (b)), and ACE (10 μg/μL ((a) and (b))) were applied to prewashed silica gel 60 F254 TLC plates and then separated in the mobile phase (chloroform : ethyl acetate : methanol : water = 17 : 46 : 25 : 12). The migrated components were visualized under UV light at 254 nm (left) or 366 nm (right).

and reportedly exerts a beneficial effect on hyperlipidemia [9, 10]. Based on the long-term clinical experience and prestudies for the synergic effects using various combinations of herbs, the formula, the mixture of *A. iwayomogi and C. longa*, was finalized. However, to date, no study has evidenced the combined effect of *A. iwayomogi* and *C. longa* (ACE) on metabolic syndrome.

Therefore, we evaluated the effect of ACE on major manifestations of metabolic syndrome including obesity, hyperlipidemia, and fatty liver using a high-fat diet mouse model.

2. Materials and Methods

2.1. Preparation and Fingerprinting of ACE. *A. iwayomogi* and *C. longa* were purchased from a traditional medicine store (Jeong-Seong Drugstore, Daejeon, Korea). To produce ACE, 250 g of each herb was mixed and boiled in 2 L of distilled water for 90 min using an automatic nonpressure pot (Dae-Woong, Seoul, Korea). After filtering using a 300-mesh filter and inspiration for 60 min, the extract was centrifuged for 15 min at 150 ×g. Finally, the supernatant was lyophilized using a vacuum freeze-drying system and stored at −20°C. The extraction yield was 12.59%.

To confirm the reproducibility of the extraction procedure, a high-performance thin-layer chromatography-(HP-TLC-) based fingerprint was produced using the CAMAG sample application technique (Muttenz, Switzerland, Figure 1) [11].

2.2. Chemicals and Reagents. The following reagents were purchased from Sigma Aldrich (St. Louis, MO, USA): 7-hydroxy-6-methoxycoumarin (scopoletin), curcumin, p-dimethylaminobenzaldehyde, 1,1,3,3-tetraethoxypropane (TEP), chloramines-T, 5,5-dithiobis-2-nitrobenzoic acid (DTNB), reduced glutathione, glutathione reductase (GSH-Rd), glutathione peroxidase (GSH-Px), β-nicotinamide adenine dinucleotide phosphate (β-NADP), and β-NADPH. Perchloric acid was obtained from GFS Chemical Co. (Columbus, OH, USA), thiobarbituric acid (TBA) from Lancaster Co. (Lancashire, England, UK), and hydrogen peroxide from Junsei Chemical Co., Ltd. (Tokyo, Japan).

2.3. Animals and Experimental Design. Fifty specific pathogen-free C57BL/6N male mice (6 weeks old, 22–24 g) were obtained from Koatech (Gyeonggi-do, Korea). The mice had free access to pelleted food (Koatech, Gyeonggi-do, Korea) and water and were housed in a room with a temperature maintained at 23 ± 2°C and under a 12:12-h light-dark cycle. After a one-week acclimatization period, mice were randomly divided into five groups: naive group (n = 10, AIN-76, Dyets Inc., Bethlehem, PA, USA), control group (n = 10, 60% high-fat diet, D124912, Research Diets, Inc., New Brunswick,

NJ, USA), ACE groups ($n = 10$, 60% high-fat diet with 100 or 200 mg/kg ACE), and curcumin group ($n = 10$, 60% high-fat diet with curcumin 50 mg/kg). ACE and curcumin were mixed into the high-fat diet and the quantity of drug was determined by calculating the daily feeding amount (approximately 3 g/day); 1.10 g or 2.20 g ACE; and 0.55 g curcumin per 1 kg high-fat diet.

This animal experiment was approved by the Institutional Animal Care and Use Committee of Daejeon University (DJUARB2012003) and was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (Bethesda, MD, USA).

2.4. Measurement of Food Intake and Body, Liver, and Adipose Tissue Weights. Food intake and body weight were monitored weekly. Mice were euthanized using ether on the final day of experiment after a 12-h fast and whole blood was collected *via* the abdominal aorta. The liver and adipose tissues (epididymal, retroperitoneal, and visceral) were removed, weighed, and frozen in liquid N2 or stored in RNAlater (Qiagen, Valencia, CA, USA).

2.5. Histopathological Analysis. For the histopathological evaluation, freshly isolated liver and white adipose tissues (epididymal and retroperitoneal) were fixed in 10% formalin for 24 h. Following sufficient rinsing in flowing water, tissues were processed in a paraffin automatic processor using a programmed cascade. The paraffin-embedded samples were dissected into 4-μm thick sections and stained with hematoxylin and eosin (H&E). After H&E staining of the liver and adipose tissues, representative histopathological features such as steatosis and adiposity were observed under a microscope. After one photograph per sample was obtained for the stained adipose tissue using an optical microscope operating at magnifications of ×200 and ×400, the size of 10 randomly selected adipocytes per photograph was measured using a computer image analysis program (NIH, USA) to obtain average values. For immunohistochemistry against 4-hydroxynonenal (4-HNE), sections were incubated with 4-HNE primary antibody (1 : 200; Abcam, Cambridge, UK) and biotinylated secondary antibody (Nichirei Biosciences, Tokyo, Japan), followed by avidin-biotin-peroxidase complex. The immunoreactive signal was developed using its substrates, AEC (Abcam). The slides were counterstained with Mayer's hematoxylin (Sigma Aldrich) and examined under an optical microscope (Leica Microsystems, Wetzlar, Germany).

2.6. Determination of Lipid Levels in Liver Tissue. Livers were homogenized in PBS and protein concentrations determined. Then, 300 μL of homogenate was extracted with 5 mL of chloroform/methanol (2 : 1) and 0.5 mL of 0.1% sulfuric acid [12]. An aliquot of the organic phase was collected, dried under nitrogen, and resuspended in 2% Triton X-100. Hepatic triglyceride content was determined using commercially available kits. Data were normalized for differences in protein concentration.

2.7. Serum Lipid Profiles and Glucose. Serum levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, and glucose were determined using an autoanalyzer (Chiron, Emeryville, CA, USA).

2.8. Determination of Reactive Oxygen Species (ROS) in Serum and Liver Tissues. The total amount of ROS in the serum or liver tissue samples was determined using a method described previously [13]. The amount of ROS was determined at 505 nm using a spectrophotometer (Molecular Devices Corp., Sunnyvale, CA, USA).

Radioimmune precipitation assay (RIPA) buffer-based liver tissue homogenates were centrifuged at 10,000 ×g for 15 min. The supernatants were transferred to clean tubes and stored at −70°C until required. The protein concentration was determined using the bicinchoninic acid (BCA) protein assay (Sigma Aldrich).

2.9. Determination of Lipid Peroxidation (Malondialdehyde, MDA) in Liver Tissue. Lipid peroxidation levels in the liver tissues were evaluated using the thiobarbituric acid reactive substances (TBARS) assay as described previously [14]. The absorbance was measured at 535 and 520 nm using a spectrophotometer (Cary 50; Varian, Palo Alto, CA, USA) and compared with the value from a freshly prepared 1.1.3.3-tetraethoxypropane (TEP) standard.

2.10. Determination of Protein Carbonyl Contents in Liver Tissue. Protein carbonyl contents in liver tissue were determined according to the manufacturer's protocol [15]. The absorbance at 370 nm was measured using a spectrophotometer (Molecular Devices Corp.).

2.11. Determination of Total Antioxidant Capacity (TAC) in Liver Tissue. TAC levels in the liver tissue were determined as previously described [16]. The absorbance was measured at 600 nm using a spectrophotometer (Molecular Device Corp). TAC was expressed as gallic acid equivalent antioxidant capacity (GEAC).

2.12. Determination of Total Glutathione (GSH) Content, GSH-Reductase (GSH-Rd), and GSH-Peroxidase (GSH-Px) in Liver Tissue. Total GSH content and GSH-Rd activity were determined as previously described [17, 18]. The absorbance was measured at 405 nm or 412 nm using a spectrophotometer (Molecular Device Corp.). GSH-Px activity was determined according to a previous method [19]. The final absorbance was measured at 340 nm using a UV-visible spectrophotometer (Varian, Agilent Technologies, Santa Clara, CA, USA).

2.13. Determination of Superoxide Dismutase (SOD) and Catalase in Liver Tissue. SOD activity was determined using an SOD assay kit according to the manufacturer's protocol (Dojindo Laboratories, Kumamoto, Japan). The standard concentration was serially diluted from 100 to 0.01 U/mL of bovine erythrocyte SOD (Sigma Aldrich).

Table 1: Food intake, body, liver, and adipose tissue weights.

Groups	Naive	Control	ACE 100	ACE 200	Curcumin 50
Food intake (g/day/mouse)	2.55 ± 0.54	2.61 ± 0.49	2.94 ± 0.58**	2.6 ± 0.6	2.65 ± 0.58
Body weight					
Initial day (g)	20.6 ± 0.6	21.1 ± 0.8	20.8 ± 0.97	21.0 ± 0.6	21.23 ± 0.68
Final day (g)	27.1 ± 1.3	40.7 ± 1.4$^{\#\#\#}$	36.7 ± 1.6***	37.7 ± 1.4***	38.0 ± 1.2***
Liver weight					
Absolute (g)	1.08 ± 0.12	1.68 ± 0.13$^{\#\#\#}$	1.54 ± 0.12*	1.51 ± 0.15**	1.59 ± 0.07
Relative (%)	3.85 ± 0.49	4.42 ± 0.34$^{\#\#}$	4.11 ± 0.30	3.84 ± 0.42**	4.01 ± 0.28*
Adipose tissue weight					
Epididymal (g)	0.80 ± 0.31	2.83 ± 0.23$^{\#\#\#}$	2.37 ± 0.49*	2.38 ± 0.55*	2.42 ± 0.24*
Retroperitoneal (g)	0.43 ± 0.23	1.07 ± 0.15$^{\#\#\#}$	1.03 ± 0.14	0.89 ± 0.18*	1.01 ± 0.13
Visceral (g)	0.47 ± 0.18	1.26 ± 0.31$^{\#\#\#}$	1.17 ± 0.17	1.18 ± 0.15	1.09 ± 0.18
Total (g)	1.80 ± 0.52	5.17 ± 0.41$^{\#\#\#}$	4.58 ± 0.56*	4.46 ± 0.62**	4.53 ± 0.34**

Data are expressed as mean ± standard deviation (SD). $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared with naive group. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with control group. ACE (water extract of *Artemisia iwayomogi* and *Curcuma longa*).

Catalase activity was determined as previously described [20]. The absorbance of the purple formaldehyde adduct was measured at 550 nm using a spectrophotometer (Molecular Devices Corp.).

2.14. Gene Expression Analysis Using Real-Time Polymerase Chain Reaction (qPCR). Total RNA was extracted from liver tissue samples with Trizol reagent (Molecular Research Center, Cincinnati, OH, USA). cDNA was synthesized from total RNA (2 μg) in a 20-μL reaction using a High-Capacity cDNA Reverse Transcription Kit (Ambion, Austin, TX, USA). Real-time polymerase chain reaction (qPCR) was performed using SYBRGreen PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and qPCR amplification was performed using a standard protocol with the IQ5 PCR Thermal Cycler (Bio-Rad, Hercules, CA, USA). The following primers (forward and reverse) were used: PPAR-γ (NM_011146), 5′-TGG GAG ATT CTC CTG TTG AC-3′, and 5′-AGG TGG AGA TGC AGG TTC TA-3′; SREBP-1c (NM_011480), 5′-GAG CGA GCG TTG AAC TGT A-3′, and 5′-ACT TCA ACG ATG GGG ACT TG-3′; FAS (NM_007988), 5′-TGT GAG TGG TTC AGA GGC AT-3′, and 5′-TTC TGT AGT GCC AGC AAG CT-3′; PPAR-α (NM_011144), 5′-CCT GAA CAT CGA GTG TCG AA-3′, and 5′-GTA CTG GCA TTT GTT CCG GT-3′; and β-actin (NM_007393), 5′-AGG CTG TGC TGT CCC TGT ATG-3′, and 5′-TGG CGT GAG GGA GAG CAT-3′.

2.15. Statistical Analyses. The results are expressed as mean ± standard deviation (SD). The statistical significance of differences between groups was analyzed using one-way analysis of variance (ANOVA), followed by Fisher's least-significant difference (LSD) test. In all analyses, $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Food Intake and Body, Liver, and Adipose Tissue Weights. Food intake did not differ significantly between the control and naive groups but food intake in the ACE 100 group was significantly increased compared with the control group ($P < 0.01$, Table 1).

After a 10-week high-fat diet, the average body weight in the control group was 1.5-fold heavier compared with the naive group. Administration of ACE significantly reduced the increase of body weight compared with the control group ($P < 0.001$ for both 100 and 200 mg/kg, Table 1).

The absolute and relative liver weights were significantly higher in the control group compared with the naive group. The increase in liver weight was significantly less in the ACE-treated groups than the control group ($P < 0.05$ or $P < 0.01$, Table 1).

The weights of epididymal, retroperitoneal, and visceral adipose tissues were significantly increased (3.5-, 2.5-, and 2.7-fold, resp.) in the control group compared with the naive group. These increases in regional adipose tissues were reduced significantly with ACE 100 mg/kg ($P < 0.05$ in epididymal and total tissues) and ACE 200 mg/kg ($P < 0.05$ in epididymal and retroperitoneal tissues; $P < 0.01$ in total tissue, Table 1). Curcumin had a similar effect on body, liver, and adipose tissue weights.

3.2. Histopathological Analysis of Adipose Tissue and Liver. Histological examination of epididymal and retroperitoneal adipose tissues revealed that the adipocyte size markedly increased by 2.5-fold in the control group and that ACE administration significantly reduced these increases ($P < 0.05$ or $P < 0.01$, Figure 2).

The high-fat diet induced lipid accumulation in hepatic tissue, as evidenced by multiple and large blanks of lipid droplets in the control group. However, administration of ACE ameliorated these histological alterations (Figure 3(a)). Curcumin exerted an effect similar to ACE.

3.3. Hepatic Cholesterol and Triglyceride Content. The high-fat diet considerably elevated hepatic cholesterol and triglyceride serum levels (1.3- and 1.9-fold, resp.). ACE administration significantly lowered hepatic cholesterol ($P < 0.05$

Table 2: Serum biochemistry parameters.

Groups	Naive	Control	ACE 100	ACE 200	Curcumin 50
TC (mg/dL)	116.9 ± 15.0	182.05 ± 33.86$^{\#\#\#}$	141.54 ± 6.47***	137.16 ± 17.03***	145.77 ± 14.24**
LDL-C (mg/dL)	37.3 ± 10.5	79.2 ± 38.6$^{\#\#\#}$	38.7 ± 10.1***	37.8 ± 10.7***	46.1 ± 29.8**
HDL-C (mg/dL)	53.5 ± 5.8	74.3 ± 8.8$^{\#\#\#}$	77.7 ± 8.6	80.00 ± 9.0	85.81 ± 6.7**
Triglyceride (mg/dL)	102.4 ± 14.1	159.12 ± 15.1$^{\#\#\#}$	125.56 ± 14.4***	115.5 ± 12.3***	112.9 ± 19.2***
Glucose (mg/dL)	198.3 ± 51.8	281.3 ± 24.9$^{\#\#}$	267.0 ± 39.4	233.3 ± 31.2*	225.6 ± 51.4**

Data are expressed as mean ± standard deviation (SD). $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared with naive group. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with control group. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ACE (water extract of *Artemisia iwayomogi* and *Curcuma longa*).

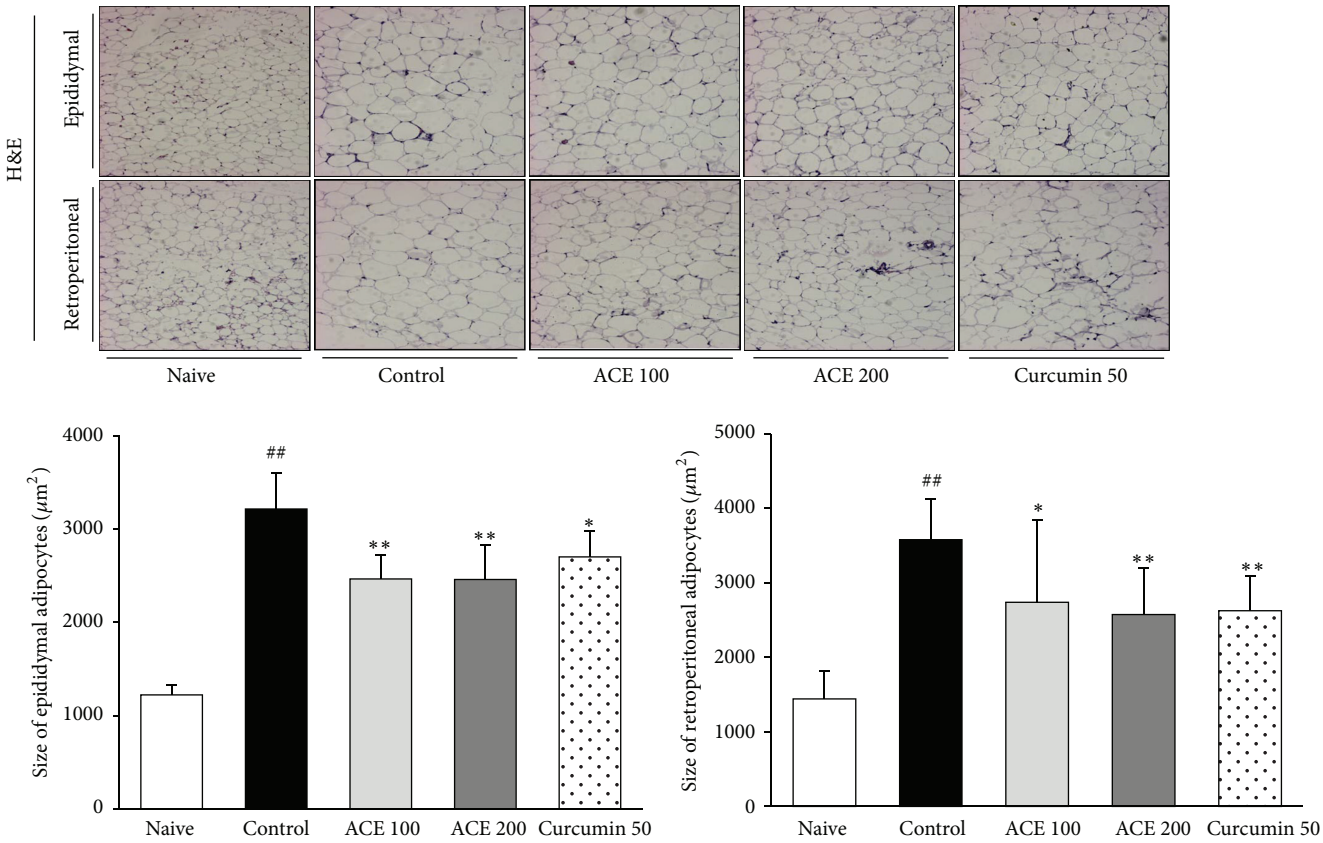

Figure 2: Histological findings of adipose tissues. Epididymal and retroperitoneal tissues were evaluated using hematoxylin and eosin (H&E) staining. All photographs are at ×200 magnification. Cell sizes of adipose tissues were quantified using computer image analysis. $^{\#\#}P < 0.01$ compared with naive group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control group.

for 200 mg/kg, Figure 3(b)) and triglyceride ($P < 0.05$ and $P < 0.01$ for 100 and 200 mg/kg, resp., Figure 3(c)) levels compared with the control group. The effects of curcumin were similar to ACE.

3.4. Serum Lipid Profiles and Glucose Levels. The high-fat diet significantly increased serum TC (1.6-fold), LDL-C (2.1-fold), HDL-C (1.4-fold), triglyceride (1.6-fold), and glucose (1.4-fold) levels. ACE treatment significantly ameliorated these alterations including TC, LDL-C, triglyceride, and glucose levels ($P < 0.05$ or $P < 0.01$ for 100 and 200 mg/kg). Serum HDL-C levels were not altered significantly in ACE-treated groups compared with the control group (Table 2). Curcumin exerted an effect similar to ACE on the profiles of all measured lipids and glucose levels.

3.5. Serum and Hepatic Levels of Oxidative Stress Biomarkers. The high-fat diet drastically increased the serum ROS (1.8-fold) and hepatic ROS levels (1.4-fold), malondialdehyde (MDA; 14.5-fold), and protein carbonyl (1.7-fold) compared with the naive group. However, ACE treatment significantly ameliorated the increase of serum ROS ($P < 0.05$ for both)

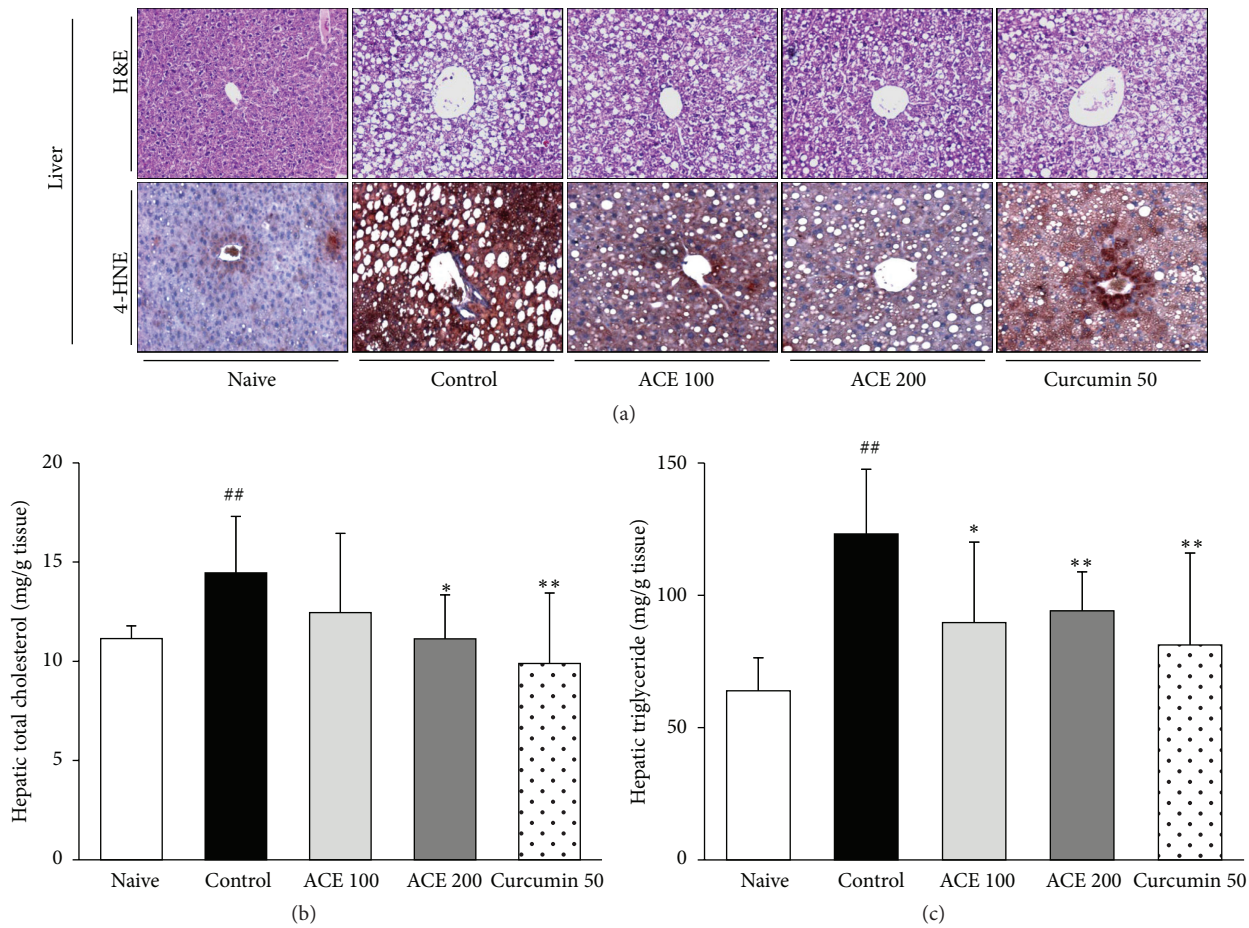

FIGURE 3: Histopathological findings and lipid profiles of liver tissue. (a) Hepatic tissues were evaluated using hematoxylin and eosin (H&E) staining (upper) and immunohistochemistry for 4-HNE (bottom). All photos are at ×200 magnification. Determination of hepatic cholesterol (b) and triglyceride (c) was performed. Data are expressed as mean ± standard deviation (SD). $^{\#\#}P < 0.01$ compared with naive group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control group.

and hepatic ROS levels ($P < 0.01$ for 200 mg/kg), MDA ($P < 0.001$ for both), and protein carbonyl ($P < 0.05$ for 100 mg/kg, Table 3).

The high-fat diet drastically altered the hepatic antioxidant biomarkers such as TAC, total GSH, GSH-Px, GSH-Rd, SOD, and catalase activity in the control group. In particular, total GSH, GSH-rd, and SOD activity were significantly depleted in the control group compared with the naive group, while ACE administration significantly ameliorated these depletions compared with the control group ($P < 0.05$ or $P < 0.01$). The hepatic TAC was not affected significantly by ACE treatment. Interestingly, the high-fat diet induced the GSH-Px increase and catalase activity in the control group, whereas ACE administration augmented their activity without statistical significance (Table 3). Curcumin exerted effects similar to ACE on oxidative stress biomarkers.

3.6. Gene Expression Analysis. The high-fat diet markedly upregulated the hepatic gene expressions of PPAR-γ, FAS, and SREBP-1c, while PPAR-α was downregulated. Administration of ACE significantly normalized the gene expression changes compared with the control group ($P < 0.05$ or $P < 0.01$, Figure 4). Curcumin exerted similar effects to ACE.

4. Discussion

Metabolic syndrome is the clustering of metabolic abnormalities, such as hyperlipidemia, glycemia, and hypertension in an individual. Diet-induced obesity is a critical etiological factor of theses metabolic abnormalities [21]. Abdominal obesity is a critical factor in the development or precession of various present-day disorders [22]. As expected, a 10-week high-fat diet led to obesity indicated by increased body and peritoneal adipose tissue weights, and ACE treatment significantly inhibited these pathological alterations. Furthermore, the high-fat diet significantly increased serum TC, LDL-C, and triglyceride levels as well as glucose levels, but ACE significantly attenuated these abnormalities in serum lipid profiles and glucose levels.

Hepatic steatosis is considered an important consequence of metabolic syndrome, leading to the subsequent development of necrosis, inflammation, cirrhosis, and hepatocellular

Table 3: Oxidative stress and antioxidant parameters.

Groups	Naive	Control	ACE 100	ACE 200	Curcumin 50
Serum					
ROS (U/mL)	772 ± 88	1,428 ± 402$^{\#\#}$	1,091 ± 211*	1,041 ± 368*	976 ± 358*
Liver					
ROS (U/mg protein)	127.9 ± 26.9	180.5 ± 57.8$^{\#\#}$	166.4 ± 54.1	96.6 ± 20.3**	109.3 ± 17.8**
MDA (μM/mg protein)	2.9 ± 1.7	42.1 ± 20.0$^{\#\#\#}$	10.0 ± 18.2***	5.13 ± 4.5***	2.8 ± 1.2***
Protein carbonyl (μM/mg protein)	50.5 ± 19.3	85.9 ± 21.5$^{\#\#}$	67.3 ± 8.9*	74.5 ± 10.8	64.4 ± 10.0*
TAC (μM/mg protein)	901 ± 138	648 ± 111$^{\#\#}$	521 ± 128	714 ± 263	550 ± 119
Total GSH (mM/mg protein)	2.31 ± 0.18	1.88 ± 0.17$^{\#\#\#}$	2.25 ± 0.33**	1.54 ± 0.44*	1.57 ± 0.29*
GSH-px (U/mg protein)	8.78 ± 2.71	10.88 ± 3.70	10.62 ± 3.21	14.75 ± 8.11	25.04 ± 7.80***
GSH-rd (U/mg protein)	7.7 ± 1.62	5.82 ± 0.43$^{\#\#}$	6.8 ± 1.20**	7.23 ± 1.93*	5.37 ± 0.87
SOD (U/mg protein)	181.1 ± 30.6	117.1 ± 16.4$^{\#\#}$	143.9 ± 24.8*	136.7 ± 14.6*	124.1 ± 35.5
Catalase (U/mg protein)	923 ± 99	1,168 ± 195$^{\#\#}$	1,421 ± 630	1,388 ± 455	1,060 ± 244

Data were expressed as mean ± standard deviation (SD). $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ compared with naive group. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with control group. ACE (water extract of *Artemisia iwayomogi* and *Curcuma longa*).

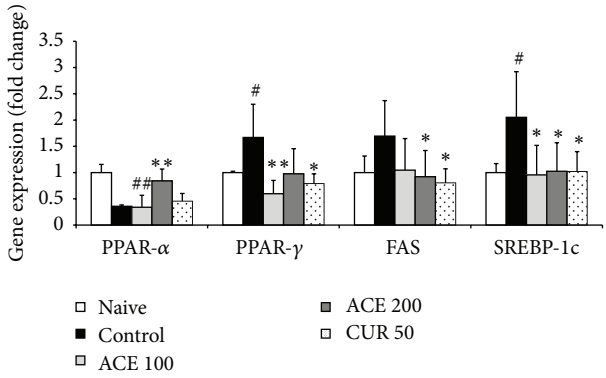

Figure 4: Gene expression levels in liver. Hepatic mRNA expression levels of FAS, PPAR-γ, SREBP-1c, and PPAR-α were determined using real-time polymerase chain reaction (qPCR). Data are expressed as average ± standard deviation (SD; fold change relative to naive group). $^{\#\#}P < 0.01$ compared with naive group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control group.

carcinoma [23]. In the present study, histological findings and hepatic lipid profiles revealed macrovesicular steatosis in liver tissues, which were reduced significantly with ACE treatment. Furthermore, PPARγ, FAS, and SREBP-1c are proteins involved in the production of lipids in hepatic tissues [24, 25] and PPARα is a key modulator of lipid lysis in the liver [26]. In the present study, the high-fat diet significantly induced upregulation of lipogenic genes (PPARγ, FAS, and SREBP-1c) and suppression of a lipolysis gene (PPARα). ACE ameliorated the altered gene expressions, which supports the antimetabolic abnormality effects of ACE.

Numerous experimental and clinical observations have suggested that oxidative stress is an essential pathogenic component of metabolic syndrome [27, 28]. Overconsumption of fat leads to excessive ROS production, which causes pathological changes in blood vessels, signaling pathways, and inflammation [27]. In the present study, a high-fat diet produced intense oxidative stress in the serum and liver, as evidenced by high levels of oxidants (ROS, MDA, and protein carbonyl) and low levels of antioxidants (GSH-system and SOD). ACE treatments significantly attenuated the altered oxidative stress markers.

The selection of ACE was based on the traditional oriental pharmacological theory of removing "*dampness/phlegm*" (濕痰) and "*blood stasis*" (瘀血) to treat metabolic syndrome. Many experimental studies have demonstrated antiobesity or antihyperlipidemic effects using the individual herbs *A. iwayomogi* or *C. longa* [8, 10]. One major component of *C. longa*, curcumin, a reference compound, was reported to have anti-insulin resistance and hyperlipidemia effects [9, 29] and exerted similar beneficial effects in the current study. We reported previously the antiatherosclerotic activity of ACE using an apoE knockout mouse model [11], but the present study is the first to demonstrate a pharmaceutical effect of ACE against metabolic syndrome. No adverse event was observed in any experimental groups of ACE and curcumin, respectively.

Together, our results conclusively show the pharmaceutical potential of ACE as an herb-derived remedy improving the metabolic abnormalities. Further studies are necessary to explore the underlying mechanisms of the activities and synergistic effects of ACE by comparing the two compositional herbs.

Conflict of Interests

The authors declare no conflict of interests.

Acknowledgment

This study was supported by a grant from the Traditional Korean Medicine R&D Project, Ministry of Health & Welfare, Republic of Korea (HI12C-1920-010014).

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