Antioxidant and Antiadipogenic Activities of Galkeun-Tang, a Traditional Korean Herbal Formula

Soo-Jin Jeong, Sae-Rom Yoo, Ohn-Soon Kim, Chang-Seob Seo, and Hyeun-Kyoo Shin

Herbal Medicine Formulation Research Group, Herbal Medicine Research Division, Korea Institute of Oriental Medicine, Daejeon 305-811, Republic of Korea

Correspondence should be addressed to Hyeun-Kyoo Shin; hkshin@kiom.re.kr

Received 1 August 2014; Revised 18 November 2014; Accepted 23 November 2014; Published 10 December 2014

1. Introduction

Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and antioxidative defenses [1]. Disruption in normal redox signaling can mediate toxic effects and, thus, is closely associated with various human diseases [2]. Obesity is a metabolic disorder caused by excess fat accumulation in adipose tissue [3]. Recent papers have confirmed a critical role for oxidative stress in the pathogenesis of obesity and its associated diseases [4–6]. A change in protection against antioxidative mechanisms was observed in an obese rodent model and in obese human males [7, 8]. Thus, the development of antioxidants could be a valuable approach for treating and preventing obesity or its related diseases. Antioxidative activities of synthetic or natural products have been reported in obesity models. Natural products are considered particularly attractive antiobesity drug candidates because of their higher efficacies and fewer side effects.

Many traditional herbal medicines have attracted increasing attention for their complementary therapeutic effects with few or no side effects compared with Western medicines [9, 10]. Galkeun-tang (GKT; Galgen-tang in Chinese and Kakkon-to in Japanese), a traditional herbal formula, has been used widely for treatment of the common cold, flu, and fever. In recent research papers, several pharmacological activities of GKT have been reported including antiviral, anti-inflammatory, and immune-regulating activities [11–15]. However, the effect of GKT on obesity or obesity-related diseases has not been reported. The pathogenesis of obesity and its associated diseases is closely related to an inflammatory reaction caused by nutrient excess and imbalance that has been termed “metainflammation” [16]. Thus, we hypothesized that the previously reported anti-inflammatory activity of GKT may influence obesity or its related diseases.

We, therefore, investigated the antioxidant and antadiapogenic effects of GKT. We analyzed its scavenging activities on 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals in in vitro systems. The effect on low-density lipoprotein (LDL) oxidation was assessed by measuring production of malondialdehyde (MDA). In addition, its inhibitory effects on adipogenesis, the process by which preadipocytes become differentiated adipocytes [17], were determined by Oil Red O staining and assays for triglyceride content,
glycerol-3-phosphate dehydrogenase (GPDH) activity, and leptin production in differentiated 3T3-L1 adipocytes.

2. Materials and Methods

2.1. Plant Materials. The seven herbal medicines forming GKT were purchased from Omniherb (Yeongcheon, Korea) and HMAX (Jecheon, Korea). The origin of these herbal medicines was taxonomically confirmed by Professor Je Hyun Lee, Dongguk University, Gyeongju, Korea. A voucher specimen (2008-KE02-1) has been deposited at the Herbal Medicine Formulation Research Group, Korea Institute of Oriental Medicine.

2.2. Preparation of GKT Water Extract. A GKT decoction consisting of seven herbal medicines including Puerariae Radix, Cinnamomi Ramulus, Ephedrae Herba, Paoniae Radix, Glycyrrhizae Radix et Rhizoma, Zingiberis Rhizoma Crudus, and Zizyphri Fructus was mixed (Table 1; 3.5kg; 80) and extracted in a 10-fold mass of water at 100°C for 2h under pressure (1kgf/cm²) using an electric extractor (COSMOS-660; Kyungseo Machine Co., Incheon, Korea). The water extract was then filtered through a standard sieve (200 mesh) and evaporated to dryness and freeze-dried to give a powder. The yield of GKT water extract was 12.6% (441.6g).

2.3. ABTS Radical Scavenging Activity. The free radical scavenging activity of GKT extract on ABTS was assessed by the method described by Re et al. [18] with slight modifications. ABTS radical cation was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate in the dark at room temperature for 16h. Absorbance of the reactant was then adjusted to 0.7 at a wavelength of 734 nm. A 100 μL aliquot of GKT solution at different concentrations (12.5–200 μg/mL) was mixed with 100 μL ABTS⁺ solution. The reaction mixture was incubated for 5 min in the dark at room temperature. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, CA). The radical scavenging capacity of the tested samples was calculated using the above formula:

\[
\text{Scavenging activity (}) \times 100.
\]

2.4. DPPH Radical Scavenging Activity. The free radical scavenging activity of GKT extract on DPPH was assessed using the method described by Moreno et al. [19]. In brief, a 100 μL aliquot of GKT sample solution at different concentrations (50–400 μg/mL) was mixed with 100 μL DPPH solution (0.15 mM in methanol). The reaction mixture was incubated for 30 min in the dark at room temperature. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, CA). The radical scavenging capacity of the tested samples was calculated using the above formula.

2.5. Oxidation of LDL by CuSO₄. LDL samples (500 μg protein/mL, Biomedical Technologies, Stoughton, MA) were prepared at 37°C in a medium containing 10 mM phosphate buffer (pH 7.4) and various concentrations (15.7–250 μg/mL) of GKT extract. After 5 min, oxidation was initiated by the addition of CuSO₄ (25 μM). After 6h of oxidation, lipid peroxidation (Section 2.6) and electrophoretic mobility (Section 2.7) of the LDL were measured as described below.

2.6. Determination of MDA Using TBARS Assay. Lipid peroxidation of LDL was estimated by the determination of the level of MDA using a TBARS assay kit (Bioassay Systems LLC, Hayward, CA) according to the manufacturer’s protocols. After oxidation, 50 μg of LDLs was mixed with 200 μL of thiobarbituric acid (TBA) and incubated at 100°C for 30 min. After completing the reaction, the absorbance at 535 nm was measured using a spectrophotometer.

2.7. Relative Electrophoretic Mobility (REM) Assay. The electrophoretic mobility of LDLs was measured using agarose gel (0.8% agarose in TAE buffer) electrophoresis and Coomassie Brilliant Blue R-250 staining. Electrophoresis was performed at 100 V for 30 min. REM was defined as the ratio of the distances migrated from the origin by oxidized LDL (oxLDL) versus native LDL.

2.8. Cell Culture and Differentiation. Mouse preadipocyte cell line 3T3-L1 was obtained from the American Type Culture Collection (ATCC, CL-173, Rockville, MD). The cells were cultured in DMEM (Gibco BRL, Carlsbad, CA) supplemented with 10% newborn calf serum (NCS, Gibco BRL, Carlsbad, CA) at 37°C. For adipocyte differentiation, the cells were stimulated with 3T3-L1 differentiation medium containing isobutylmethylxanthine, dexamethasone, and insulin.

### Table 1: Composition of GKT.

| Herbal medicine          | Scientific name          | Supplier       | Source         | Amount (g) |
|--------------------------|--------------------------|----------------|----------------|------------|
| Puerariae Radix          | Pueraria lobata          | Omniherb      | Jcheon, Korea  | 9.0        |
| Cinnamomi Ramulus        | Cinnamomi cassia         | HMAX          | China          | 6.0        |
| Ephedrae Herba           | Ephedra sinica           | HMAX          | China          | 6.0        |
| Paoniae Radix            | Paeonia lactiflora       | Omniherb      | Hwasun, Korea  | 6.0        |
| Glycyrrhizae Radix et Rhizoma | Glycyrrhiza uralensis | HMAX          | China          | 6.0        |
| Zingiberis Rhizoma Crudus | Zingiber officinale      | Omniherb      | Yeongcheon, Korea | 6.0       |
| Zizyphri Fructus         | Ziziphus jujuba          | Omniherb      | Yeongcheon, Korea | 5.0       |

Total amount 44.0
(MDI) (Zenbio Inc., Research Triangle Park, NC) for 48 h after reaching confluence. The medium was switched to DMEM containing 10% fetal bovine serum (FBS) and 1μg/mL insulin after 2 days and then changed to DMEM containing 10% FBS for an additional 4 days. Cells were treated with GKT extract (0, 25, 50, 100, 200, or 400 μg/mL) during 8 days of the differentiation period. GW9662 (Sigma, St. Louis, MO), a peroxisome proliferator-activated receptor-gamma (PPAR-γ) antagonist, was used as a positive control.

2.1.1.2. Differentiation of 3T3-L1 Cells. Undifferentiated 3T3-L1 cells were treated with various concentrations of GKT for 24 h. To obtain differentiated adipocytes, 3T3-L1 preadipocytes were differentiated for 8 days in the presence of GKT. The cells were incubated with GKT extract at 37°C under 5% CO₂. Cell counting kit-8 (CCK-) 8 solution (Dojindo, Kumamoto, Japan) was added and incubated for 4 h. After incubation, the absorbance was read at 450 nm using a microplate reader (Benchmark Plus, Bio-Rad, Hercules, CA).

2.1.1.3. Oil Red O Staining. 3T3-L1 preadipocytes were differentiated into adipocytes by adding isobutylmethylxanthine, dexamethasone, and insulin (MDI) for 8 days. The cells were treated with or without GKT (200 μg/mL) or GW9662 (20 μM) during the differentiation period. The differentiated 3T3-L1 cells were fixed with 10% formalin for 15 min at room temperature and washed with 70% ethanol and phosphate-buffered saline (PBS). The cells were stained with Oil Red O (Sigma, St. Louis, MO) for 5 min and then washed with PBS. Cell images were collected using an Olympus CKX41 inverted microscope (Olympus, Tokyo, Japan). Stained oil droplets were dissolved in isopropyl alcohol and measured by reading the absorbance at 520 nm.

2.1.1.4. Triglyceride Quantification Assay. 3T3-L1 preadipocytes were differentiated into adipocytes by adding MDI for 8 days. The cells were treated with or without GKT (25, 50, 100, 200, or 400 μg/mL) or GW9662 (20 μM) during the differentiation period. The triglyceride content of cells was enzymatically determined using a commercial kit (R & D Systems, Minneapolis, MN) according to the manufacturer’s instructions. In brief, the culture supernatant was collected from the differentiated 3T3-L1 with or without GKT treatment. An equal ratio of the supernatants (50 μL) and assay diluent RDW (50 μL) was added to wells of a 96-well plate and incubated for 2 h at room temperature. After washing 5 times with 400 μL of wash buffer, 100 μL of mouse leptin conjugate was added to each well and incubated for 2 h at room temperature. After washing 5 times, 100 μL of substrate solution was added to each well and incubated for 30 min at room temperature in the dark. Finally, 100 μL of stop solution was added to each well and the absorbance was measured at 450 nm.

2.2. Chemicals and Reagents for HPLC Analysis. Cinnamaldehyde, cinnamic acid, glycyrrhizin, liquiritin, paeoniflorin, and puerarin (all purity ≥ 98.0%) were purchased from Wako Fine Chemicals (Osaka, Japan). The HPLC-grade reagents methanol, acetonitrile, and water were obtained from J. T. Baker (Phillipsburg, NJ). Glacial acetic acid was obtained from Junsei Chemical Co. (Tokyo, Japan).

2.2.1. Preparation of Standard and Sample Solutions for HPLC Analysis. Methanol standard stock solutions were made containing each of cinnamic acid, glycyrrhizin, liquiritin, paeoniflorin, and puerarin at 1.0 mg/mL. Cinnamaldehyde was dissolved in methanol at a concentration of 1.05 mg/mL. The stock solutions were stored below 4°C.

For HPLC analysis, lyophilized GKT extract was weighed (200 mg) into a 20 mL flask and distilled water was added to the volumetric mark, and then the mixture was passed through a 0.2 μm syringe filter before injection into the HPLC system.

2.2.2. HPLC Analysis of GKT. HPLC was performed on a Shimadzu LC-20A HPLC system (Shimadzu Co., Kyoto, Japan) consisting of a solvent delivery unit, an on-line degasser, a column oven, an autosampler, and a photodiode array (PDA) detector. The data processor employed LCsolution software (Version 1.24). The analytical column used was a Gemini C₁₈ (250 × 4.6 mm; particle size 5 μm, Phenomenex, Torrance, CA) maintained at 40°C. The mobile consisted of solvent A (1.0%, v/v, acetic acid in H₂O) and solvent B (1.0%, v/v, acetic acid in acetonitrile). The gradient flow was as follows: 5–70% B for 0–40 min, 70–100% B for 40–45 min, 100% B for 45–50 min, and 100–5% B for 55 min. The flow rate was 1.0 mL/min and the injection volume was 10 μL. The quantitative analysis of the six compounds was carried out at 230 nm (paeoniflorin), 254 nm (glycyrrhizin and puerarin), and 280 nm (cinnamaldehyde, cinnamic acid, and liquiritin).

2.2.3. Statistical Analysis. All data were presented as mean ± standard error of the mean (S.E.M.). Group differences were assessed by one-way ANOVA and post hoc Tukey’s multiple comparison test using Graphpad InStat ver.3.10 (Graphpad Software, Inc., San Diego, CA). Significance of differences from the normal control was taken as P < 0.05.

3. Results

3.1. HPLC Analysis of GKT. All calibration curves were obtained by assessment of peak areas from standard
solutions in the concentration ranges: puerarin, cinnamic acid, and glycyrrhizin, 2.34–300.00 µg/mL; paeoniflorin, liquiritin, and cinnamaldehyde, 0.78–100.00 µg/mL, 1.56–200.00 µg/mL, and 1.64–105.00 µg/mL, respectively. The retention times were 13.81, 15.72, 17.65, 25.77, 28.08, and 33.55 min for puerarin, paeoniflorin, liquiritin, cinnamic acid, cinnamaldehyde, and glycyrrhizin, respectively. The calibration curves, correlation coefficients ($R^2$), limit of detection (LOD), and limit of quantification (LOQ) of the six marker compounds are summarized in Table 2. Using optimized chromatography conditions, a three-dimensional chromatogram was obtained using the HPLC-PDA detector (Figure 1). The concentrations of the six marker compounds were 2.01–12.17 mg/g and are summarized in Table 3.

### 3.2. Antioxidant Activity of GKT
To evaluate the antioxidant activity of GKT, we tested its scavenging activities on ABTS and DPPH radicals. The ABTS radical scavenging activity of GKT is presented in Table 4. The extracts of GKT showed a dose-dependent radical scavenging activity. The concentration of GKT required for 50% inhibition (IC$_{50}$) of ABTS radicals was 51.16 µg/mL, while the IC$_{50}$ value of ascorbic acid, as a positive control, was 3.22 µg/mL. The antioxidant activities obtained for GKT by the DPPH method are shown in Table 5. Similar to the ABTS assay, GKT reduced DPPH radical formation in a concentration-dependent manner. The IC$_{50}$ of GKT against DPPH radicals was 242.96 µg/mL, while the IC$_{50}$ value of ascorbic acid was 10.43 µg/mL.

### 3.3. Effect of GKT on Cu$^{2+}$-Mediated Oxidation of LDL
The generation of MDA equivalents during LDL oxidation was estimated by the TBARS assay. As shown in Figure 2(a), when LDL was incubated with CuSO$_4$ for 6 h, a significant amount of MDA was produced. The prevention of MDA formation by GKT was evaluated in Figure 2(b). The results indicated that GKT significantly inhibited the generation of MDA, with an IC$_{50}$ value of 32.14 µg/mL. The antioxidant effect of GKT on LDL oxidation is summarized in Table 6.
Figure 1: Three-dimensional chromatogram of GKT by HPLC-PDA.

Figure 2: Inhibitory effects of GKT on Cu\textsuperscript{2+}-induced LDL oxidation. The indicated concentrations of GKT and LDLs were incubated with CuSO\textsubscript{4} for 6 h at 37°C. The TBARS level (a) and electrophoretic mobility (b) of LDLs were measured using a TBARS assay kit and agarose gel electrophoresis, respectively. The data are presented as the mean values of three experiments ± S.E.M. ** *P < 0.01 compared with the oxLDL group.
increase in TBARS was detected. In contrast, GKT significantly reduced the amount of TBARS formed in a dose-dependent manner (IC$_{50}$: 53.23 μg/mL). The alteration of mobility in agarose gel electrophoresis reflects the increase in the negative charge of LDL particles which occurs during oxidation [20]. When the oxidation was carried out in the presence of GKT, the increase in electrophoretic mobility of oxLDL was significantly reduced (Figure 2(b)). These data suggest that GKT has an inhibitory effect on LDL oxidation.

3.4. Cytotoxic Effects of GKT in 3T3-L1 Cells. To evaluate the possible cytotoxicity of GKT against 3T3-L1 preadipocytes, the cells were treated with various concentrations of GKT for 24 h. As shown in Figure 3(a), GKT had no cytotoxicity against 3T3-L1 preadipocytes. Additionally, the cytotoxicity of GKT was assessed in the differentiated adipocytes. During differentiation for 8 days, the cells were exposed to various concentrations of GKT. No significant cytotoxic effect was observed in GKT-treated adipocytes (Figure 3(b)).

3.5. The Inhibitory Effects of GKT on Adipogenesis of 3T3-L1 Adipocytes. Triglyceride production is one of the important events which occurs during adipogenesis [21]. Oil Red O staining was carried out to examine lipid accumulation in the differentiated 3T3-L1 adipocytes. The number of lipid droplets detectable with Oil Red O staining was obviously increased in adipocytes compared with preadipocytes (Figure 4(a)). However, GKT treatment significantly reduced lipid accumulation compared with the untreated differentiated cells. In addition, the intracellular triglyceride content was measured in 3T3-L1 adipocytes treated with GKT. Consistent with the results of Oil Red O staining, triglyceride production was significantly increased in the differentiated adipocytes compared with preadipocytes, but GKT significantly inhibited triglyceride production compared with untreated differentiated cells (Figure 4(b)).

GPDH is an enzyme that generates glycerol-3-phosphate from dihydroxyacetone phosphate for lipid biosynthesis in adipocytes [22]. As shown in Figure 5(a), treatment with GKT at the concentration of 25–400 μg/mL significantly inhibited the activity of GPDH compared with untreated differentiated adipocytes. Consistent with this, GKT also had a suppressive effect on secretion of leptin, a key adipokine [23], compared with the untreated differentiated cells (Figure 5(b)). Similarly, treatment with GW9662, the positive control, dramatically inhibited adipogenesis in 3T3-L1 cells.

4. Discussion

In the present study, we demonstrate that a traditional herbal medicine GKT has antioxidant and antiadipogenesis properties. For quality control of the GKT extract, we performed a quantitative determination of the six main components in GKT using HPLC coupled with a PDA. The investigated components were as follows: puerarin from Puerariae Radix, cinnamaldehyde and cinnamic acid from Cinnamomi Ramulus, paeoniflorin from Paeoniae Radix, and liquiritin and glycyrrhizin from Glycyrrhizae Radix et Rhizoma. The optimized HPLC-PDA method was applied for simultaneous quantitation of the six components in GKT (Figure 1). Among these components, puerarin and glycyrrhizin, which are marker components of Puerariae Radix and Glycyrrhizae Radix et Rhizoma, were detected at 10.29 mg/g and 12.17 mg/g, respectively, as the major components of GKT (Table 3). The establishment of this HPLC-PDA method will be helpful in improving quality control of GKT.
Figure 4: Inhibitory effect of GKT extract on triglyceride accumulation in 3T3-L1 adipocytes. 3T3-L1 preadipocytes were differentiated into adipocytes by adding isobutylmethylxanthine, dexamethasone, and insulin (MDI) for 8 days. The cells were treated with or without GKT or GW9662 (20 μM) during the differentiation period. (a and b) Lipid accumulation in the cells was analyzed by Oil Red O staining. (a) The cells stained with Oil Red O were visualized using an Olympus CKX41 inverted microscope at ×200 magnification (left panel). Stained oil droplets were dissolved in isopropyl alcohol and measured by reading absorbance at 520 nm (right panel). (b) The content of triglyceride was enzymatically measured at 570 nm using a commercial kit (BioVision Inc., Milpitas, CA). Data are presented as the mean ± S.E.M. **P < 0.01 and ***P < 0.001 compared with the differentiated control.

Figure 5: Inhibitory effects of GKT on adipogenesis-related factors in 3T3-L1 adipocytes. 3T3-L1 preadipocytes were differentiated into adipocytes by adding isobutylmethylxanthine, dexamethasone, and insulin (MDI) for 8 days. The cells were exposed to various concentrations of GKT during the differentiation period. (a) GPDH activity of the cells was assessed by measuring the decrease in NADH at 340 nm using the TAKARA GPDH activity assay kit. (b) Culture supernatant was collected from the GKT-treated cells. Leptin production was determined by ELISA at 540 nm subtracted from 450 nm using a mouse leptin immunoassay kit (R&D Systems). Data are presented as the mean ± S.E.M. ***P < 0.001, **P < 0.01, and *P < 0.05 compared with the differentiated control.
triglyceride production, GPDH activity, and leptin production in adipocytes without cytotoxic effects (Figures 3–5).

Oxidative stress plays an important role in the pathogenesis of various diseases including obesity. Thus, the "oxidative stress paradigm" is an appealing concept for developing novel therapeutics [24]. Excess oxidative stress in obese patients coincides with fat accumulation in adipocytes [4]. We induced the cellular differentiation of 3T3-L1 mouse patients coincides with fat accumulation in adipocytes [4]. We induced the cellular differentiation of 3T3-L1 mouse adipocytes into adipocytes. This cell line is useful for studying adipogenesis [25]. After adipocyte differentiation, a significant increase of intracellular lipid accumulation compared with untreated adipocytes was observed by Oil Red O staining. In contrast, GKT significantly inhibited the amount of lipid accumulation compared with untreated differentiated adipocytes (Figure 4(a)). Microscopy further confirmed the inhibitory effect of GKT on adipogenesis. GKT treatment markedly reduced the increase in the number and size of adipocytes, a hallmark of adipocyte endocrine function [26]. Lipid droplets consist of a core of lipid esters and a surface lined with a phospholipid monolayer, and, in adipocytes, the lipid ester core contains triglycerides [27–29]. Consistent with the results of Oil Red O staining, GKT significantly decreased the content of triglyceride in adipocytes (Figure 4(b)). Furthermore, we evaluated the GPDH activity in differentiated 3T3-L1 adipocytes. GPDH is activated strongly in mature adipocytes and plays a role in the triglyceride biosynthesis pathway [30, 31]. GPDH enzyme activity was significantly decreased in GKT-treated adipocytes (Figure 5(a)). The level of another key factor in adipogenesis, leptin, was measured in adipocytes differentiated in the absence or presence of GKT. Leptin is an adipokine exclusively produced by adipocytes in proportion to triglyceride production, GPDH activity, and leptin production in adipocytes without cytotoxic effects.

Acknowledgment

This work was supported by a Grant from the Korean Institute of Oriental Medicine (no. K14030).

References

[1] D. J. Betteridge, “What is oxidative stress?" Metabolism: Clinical and Experimental, vol. 49, no. 2, supplement 1, pp. 3–8, 2000.

[2] M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, and J. Telser, “Free radicals and antioxidants in normal physiological functions and human disease,” International Journal of Biochemistry and Cell Biology, vol. 39, no. 1, pp. 44–84, 2007.

[3] N. Rasouli, B. Molavi, S. C. Elbein, and P. A. Kern, “Ectopic fat accumulation and metabolic syndrome,” Diabetes, Obesity and Metabolism, vol. 9, no. 1, pp. 1–10, 2007.

[4] S. Furukawa, T. Fujita, M. Shimabukuro et al., “Increased oxidative stress in obesity and its impact on metabolic syndrome,” The Journal of Clinical Investigation, vol. 114, no. 12, pp. 1752–1761, 2004.

[5] C. K. Roberts, R. J. Barnard, R. K. Sindhu, M. Jurczak, A. Ehdaie, and N. D. Vaziri, “Oxidative stress and dysregulation of NAD(P)H oxidase and antioxidant enzymes in diet-induced metabolic syndrome,” Metabolism, vol. 55, no. 7, pp. 928–934, 2006.

[6] E. Tumova, W. Sun, P. H. Jones, M. Vrablik, C. M. Ballantyne, and R. C. Hoogeveen, “The impact of rapid weight loss on oxidative stress markers and the expression of the metabolic syndrome in obese individuals,” Journal of Obesity, vol. 2013, Article ID 729515, 10 pages, 2013.

[7] I. D. Capel and H. M. Dorrell, “Abnormal antioxidant defence in some tissues of congenitally obese mice,” Biochemical Journal, vol. 219, no. 1, pp. 41–49, 1984.

[8] M. Ozata, M. Mengen, C. Oktenli et al., “Increased oxidative stress and hypozincemia in male obesity,” Clinical Biochemistry, vol. 35, no. 8, pp. 627–631, 2002.

[9] T. Xue and R. Roy, “Studying traditional Chinese medicine,” Science, vol. 300, no. 5620, pp. 740–741, 2003.

[10] D. Normile, “The new face of traditional Chinese medicine,” Science, vol. 299, no. 5604, pp. 188–190, 2003.

[11] M. Kurokawa, M. Tsurita, J. Brown, Y. Fukuda, and K. Shiraki, “Effect of interleukin-12 level augmented by Kokkon-to, a herbal medicine, on the early stage of influenza infection in mice,” Antiviral Research, vol. 56, no. 2, pp. 183–188, 2002.

[12] M. S. Wu, H. R. Yen, C. W. Chang et al., “Mechanism of action of the suppression of influenza virus replication by Ko-Ken Tang through inhibition of the phosphatidylinositol 3-kinase/Akt signaling pathway and viral RNP nuclear export,” Journal of Ethnopharmacology, vol. 134, no. 3, pp. 614–623, 2011.

[13] K. Nagasaka, M. Kurokawa, M. Imakita, K. Terasawa, and K. Shiraki, “Efficacy of Kokkon-to, a traditional herb medicine, in herpes simplex virus type 1 infection in mice,” Journal of Medical Virology, vol. 46, no. 1, pp. 28–34, 1995.

[14] K. Muraoka, S. Yoshida, K. Hasegawa et al., “A pharmacologic study on the mechanism of action of Kakkon-to: body temperature elevation and phagocytic activation of macrophages in dogs,” Journal of Alternative and Complementary Medicine, vol. 10, no. 5, pp. 841–849, 2004.

[15] H. Yano, S. Hiraki, and S. Hayasaka, “Effects of Kokkon-to and Sairei-to on experimental elevation of aqueous flare in pigmented rabbits,” Japanese Journal of Ophthalmology, vol. 43, no. 4, pp. 279–284, 1999.
[16] G. S. Hotamisligil, "Inflammation and metabolic disorders," *Nature*, vol. 444, no. 7121, pp. 860–867, 2006.

[17] M. I. Lefterova and M. A. Lazar, "New developments in adipogenesis," *Trends in Endocrinology & Metabolism*, vol. 20, no. 3, pp. 107–114, 2009.

[18] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans, "Antioxidant activity applying an improved ABTS radical cation decolorization assay," *Free Radical Biology and Medicine*, vol. 26, no. 9–10, pp. 1231–1237, 1999.

[19] M. I. N. Moreno, M. I. Isla, A. R. Sampietro, and M. A. Vattuone, "Comparison of the free radical-scavenging activity of propolis from several regions of Argentina," *Journal of Ethnopharmacology*, vol. 71, no. 1–2, pp. 109–114, 2000.

[20] D. L. Sparks and M. C. Phillips, "Quantitative measurement of lipoprotein surface charge by agarose gel electrophoresis," *Journal of Lipid Research*, vol. 33, no. 1, pp. 123–130, 1992.

[21] D. W. Haslam and W. P. T. James, "Obesity," *The Lancet*, vol. 366, no. 9492, pp. 1197–1209, 2005.

[22] L. P. Kozak and J. T. Jensen, "Genetic and developmental control of multiple forms of L-glycerol 3-phosphate dehydrogenase," *The Journal of Biological Chemistry*, vol. 249, no. 24, pp. 7775–7781, 1974.

[23] F. Zhang, M. B. Basinski, J. M. Beals et al., "Crystal structure of the obese protein leptin-E100," *Nature*, vol. 387, no. 6629, pp. 206–209, 1997.

[24] H. Sies, "Oxidative stress: oxidants and antioxidants," *Experimental Physiology*, vol. 82, no. 2, pp. 291–295, 1997.

[25] H. Green and M. Meuth, "An established pre-adipose cell line and its differentiation in culture," *Cell*, vol. 3, no. 2, pp. 127–133, 1974.

[26] T. Tsuda, F. Horio, K. Uchida, H. Aoki, and T. Osawa, "Dietary cyanidin 3-O-β-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice," *Journal of Nutrition*, vol. 133, no. 7, pp. 2125–2130, 2003.

[27] D. J. Murphy and J. Vance, "Mechanisms of lipid-body formation," *Trends in Biochemical Sciences*, vol. 24, no. 3, pp. 109–115, 1999.

[28] K. Tauchi-Sato, S. Ozeki, T. Houjou, R. Taguchi, and T. Fujimoto, "The surface of lipid droplets is a phospholipid monolayer with a unique fatty acid composition," *The Journal of Biological Chemistry*, vol. 277, no. 46, pp. 44507–44512, 2002.

[29] T. Fujimoto, Y. Ohsaki, J. Cheng, M. Suzuki, and Y. Shinohara, "Lipid droplets: a classic organelle with new outfits," *Histochemistry and Cell Biology*, vol. 130, no. 2, pp. 263–279, 2008.

[30] J. Pairault and H. Green, "A study of the adipose conversion of suspended 3T3 cells by using glycerophosphate dehydrogenase as differentiation marker," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 76, no. 10, pp. 5138–5142, 1979.

[31] L. S. Wise and H. Green, "Participation of one isozyme of cytosolic glycerophosphate dehydrogenase in the adipose conversion of 3T3 cells," *The Journal of Biological Chemistry*, vol. 254, no. 2, pp. 273–275, 1979.

[32] H. C. Chen and R. V. Farese Jr., "Determination of adipocyte size by computer image analysis," *Journal of Lipid Research*, vol. 43, no. 6, pp. 986–989, 2002.

[33] J.-H. Ju, H.-S. Yoon, H.-J. Park et al., "Anti-obesity and antioxidative effects of purple sweet potato extract in 3T3-L1 adipocytes in vitro," *Journal of Medicinal Food*, vol. 14, no. 10, pp. 1097–1106, 2011.

[34] S.-Y. Yu, Y.-J. Lee, J.-D. Kim et al., "Phenolic composition, antioxidant activity and anti-adipogenic effect of hot water extract from safflower (*Carthamus tinctorius L.*) seed," *Nutrients*, vol. 5, no. 12, pp. 4894–4907, 2013.

[35] Y.-J. Lee, K.-J. Kim, K.-J. Park, B.-R. Yoon, J.-H. Lim, and O.-H. Lee, "Buckwheat (*Fagopyrum esculentum M.*) sprout treated with methyl jasmonate (MeJA) improved anti-adipogenic activity associated with the oxidative stress system in 3T3-L1 adipocytes," *International Journal of Molecular Sciences*, vol. 14, no. 1, pp. 1428–1442, 2013.

[36] J. K. Prasain, N. Peng, R. Rajbhandari, and J. M. Wyss, “The Chinese Pueraria root extract (Pueraria lobata) ameliorates impaired glucose and lipid metabolism in obese mice”, *Phytomedicine*, vol. 20, no. 1, pp. 17–23, 2012.

[37] S. E. Jin, Y. K. Son, B.-S. Min, H. A. Jung, and J. S. Choi, "Anti-inflammatory and antioxidant activities of constituents isolated from Pueraria lobata roots," *Archives of Pharmacal Research*, vol. 35, no. 5, pp. 823–837, 2012.

[38] Y. Han, H. W. Jung, H. S. Bae, J.-S. Kang, and Y.-K. Park, “The extract of *Cinnamomum cassia* twigs inhibits adipocyte differentiation via activation of the insulin signaling pathway in 3T3-L1 preadipocytes,” *Pharmaceutical Biology*, vol. 51, no. 8, pp. 961–967, 2013.

[39] F. L. Song, R. Y. Gan, Y. Zhang, Q. Xiao, L. Kuang, and H. B. Li, “Total phenolic contents and antioxidant capacities of selected chinese medicinal plants,” *International Journal of Molecular Sciences*, vol. 11, no. 6, pp. 2362–2372, 2010.