

Original Article

Anti-adipogenic and antioxidant effects of the traditional Korean herbal formula Samchulgeonbi-tang: an *in vitro* study

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Abstract: Aims: Here we report *in vitro* anti-adipogenic and antioxidant effects of Samchulgeonbi-tang (SCGBT), a traditional Korean herbal formula. Methods: 3T3-L1 preadipocytes were differentiated into adipocytes with or without SCGBT. After differentiation, we measured Oil Red O staining, glycerol-3-phosphate dehydrogenase (GPDH) activity and leptin production. In addition, its effect on scavenging activities of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radicals in *in vitro* systems. Results: In differentiated 3T3-L1 adipocytes, SCGBT significantly inhibited lipid accumulation and triglyceride production, and mediated inactivation of GPDH, a major enzyme in the process of adipogenesis. Consistent with this, SCGBT stimulation significantly decreased the amount of leptin in 3T3-L1 adipose cells. Furthermore, SCGBT enhanced the scavenging activities on ABTS and DPPH radicals. The generation of malondialdehyde (MDA) during low-density lipoprotein (LDL) oxidation was significantly reduced by SCGBT treatment. Of interest, SCGBT extract inhibited reactive oxygen species (ROS) generation in 3T3-L1 adipocytes. Conclusion: Overall, our findings suggest that SCGBT has the potential for anti-adipogenic activity and antioxidant properties.

Keywords: Anti-adipogenic, antioxidant, korean herbal formula, Samchulgeonbi-tang

Introduction

The World Health Organization (WHO) defines obesity as “abnormal or excessive fat accumulation that presents a risk to health” (<http://www.who.int/topics/obesity/en/>). Obesity can be caused by a combination of excessive food intake, inadequate physical activity, and genetic susceptibility. Obesity is a serious risk factor for a number of chronic diseases, especially cancer, cardiovascular disease, type 2 diabetes, and osteoarthritis [1], and has increased at an alarming rate all over the world. Obesity was classified as a disease by the annual meeting of the American Medical Association in 2013. Thus, many anti-obesity agents have been developed as drugs or food supplements for preventing and treating obesity.

Currently, orlistat (Xenical), lorcaserin (Belviq), and a combination of phentermine and topiramate (Qsymia) are available as anti-obesity medications [2]. However, several side effects

of these drugs have been of serious concern, including gastroesophageal reflux disease, depression, liver failure, increased heart rate, psychiatric and cognitive effects, and even lethal effects [3-6]. Recently, herbal medicines have been considered attractive candidates as anti-obesity medications because of their reduced side effects and enhanced drug efficacy [7-10]. The antioxidant activities of herbal medicines offer an additional advantage in the control of obesity and obesity related diseases [11].

Samchulgeonbi-tang (SCGBT; Shenzhujianpi-tang in Chinese and Samjutsukenhi-to in Japanese) is a traditional herbal formula that has been used to treat chronic gastritis, gastric ulcers, gastroptosis, indigestion, diarrhea, and emesis in Asian countries [12]. In the present study, we investigated the anti-obesity effects and antioxidant activity of SCGBT. We analyzed inhibitory effects of SCGBT on adipogenesis by Oil Red O staining and assays for triglyceride

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Table 1. Composition of SCGBT

Latin name	nomenclature author	Family name	Supplier	Source	Amount (g)
Ginseng Radix	<i>Panax ginseng</i> C. A. Meyer	Araliaceae	Omniherb	Geumsan, Korea	3.750
Atractylodis Rhizoma	<i>Atractylodes japonica</i> Koidzumi	Compositae	Omniherb	China	3.750
Hoelen	<i>Poria cocos</i> Wolf	Polyporaceae	Omniherb	Yeongcheon, Korea	3.750
Magnoliae Cortex	<i>Magnolia officinalis</i> Rehder & E.H.Wilson	Magnoliaceae	HMAX	China	3.750
Citri Unshii Pericarpium	<i>Citrus unshiu</i> Markovich	Rutaceae	Omniherb	Jeju, Korea	3.750
Crataegi Fructus	<i>Crataegus pinnatifida</i> Bunge	Rosaceae	Omniherb	Inje, Korea	3.750
Ponciri Fructus	<i>Poncirus trifoliata</i> Rafinesqu	Rutaceae	HMAX	China	3.000
Paeoniae Radix	<i>Paeonia lactiflora</i> Pallas	Paeoniaceae	Omniherb	Hwasun, Korea	3.000
Amomi Fructus	<i>Amomum villosum</i> Lour	Zingiberaceae	HMAX	China	1.875
Massa Medicata Fermentata	Triticum aestivum L		Omniherb	Muju, Korea	1.875
Hordei Fructus Germinatus	<i>Hordeum vulgare</i> Linne	Gramineae	Omniherb	Muju, Korea	1.875
Glycyrrhizae Radix et Rhizoma	<i>Glycyrrhiza uralensis</i> Fisch	Leguminosae	HMAX	China	1.875
Zingiberis Rhizoma Crudus	<i>Zingiber officinale</i> Roscoe	Zingiberaceae	Omniherb	Yeongcheon, Korea	3.750
Zizyphi Fructus	<i>Ziziphus jujuba</i> Mill	Rhamnaceae	Omniherb	Yeongcheon, Korea	3.750
Total amount					43.500

Table 2. Regression data, linear range, correlation coefficient, LOD and LOQ for marker compounds of SGCBT (n = 3)

Compound	Linear range (mg/mL)	Regression equation ^a	Correlation coefficient (R ²)	LOD ^b (mg/mL)	LOQ ^c (mg/mL)
Albiflorin	0.78-50.00	y = 11938.00x-45.60	1.0000	0.13	0.45
Paeoniflorin	0.78-100.00	y = 12182.00x-3175.60	0.9999	0.16	0.52
Liquiritin	0.39-50.00	y = 17784.00x-118.00	1.0000	0.07	0.24
Naringin	0.78-100.00	y = 17210.00x-470.60	1.0000	0.06	0.18
Hesperidin	0.78-100.00	y = 18422.00x-534.20	1.0000	0.06	0.19
Poncirin	0.78-100.00	y = 28646.00x-751.80	1.0000	0.05	0.15
Glycyrrhizin	0.78-50.00	y = 8246.90x-345.00	1.0000	0.20	0.65

^ay: peak area (mAU) of compounds; x: concentration (mg/mL) of compounds. ^bLOD = 3 × signal-to-noise ratio. ^cLOQ = 10 × signal-to-noise ratio.

content, glycerol-3-phosphate dehydrogenase (GPDH) activity, and leptin production in differentiated 3T3-L1 adipocytes. In addition, its effect on scavenging activities of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radicals in *in vitro* systems. Its effect on low-density lipoprotein (LDL) oxidation was assessed by measuring production of malondialdehyde (MDA). Furthermore, the effect of SCGCT on reactive oxygen species (ROS) generation in differentiated 3T3-L1 cells.

Materials and methods

Plant materials

The fourteen herbal medicines forming SCGBT were purchased from Omniherb (Yeongcheon, Korea) and HMAX (Jecheon, Korea). The origin of these herbal medicines was taxonomically

confirmed by Prof. Je Hyun Lee, Dongguk University, Gyeongju, Korea. A voucher specimen (2008-KE06-1-KE06-14) has been deposited at the K-herb Research Center, Korea Institute of Oriental Medicine.

Preparation of SCGBT extract

SCGBT decoction consisting of 14 herbal medicines including Ginseng Radix, Atractylodis Rhizoma, Poria Sclerotium, Magnoliae Cortex, Citri Unshius Pericarpium, Crataegi Fructus, Ponciri Fructus, Paeoniae Radix, Amomi Fructus, Massa Medicata Fermentata, Hordei Fructus Germinatus, Glycyrrhizae Radix et Rhizoma, Zingiberis Rhizoma Crudus, and Zizyphi Fructus was mixed (**Table 1**; 10.0 kg; 43.5 g × 230) and extracted in a 10-fold mass of water at 100°C for 2 h under pressure (1 kgf/cm²) using an electric extractor (COSMOS-

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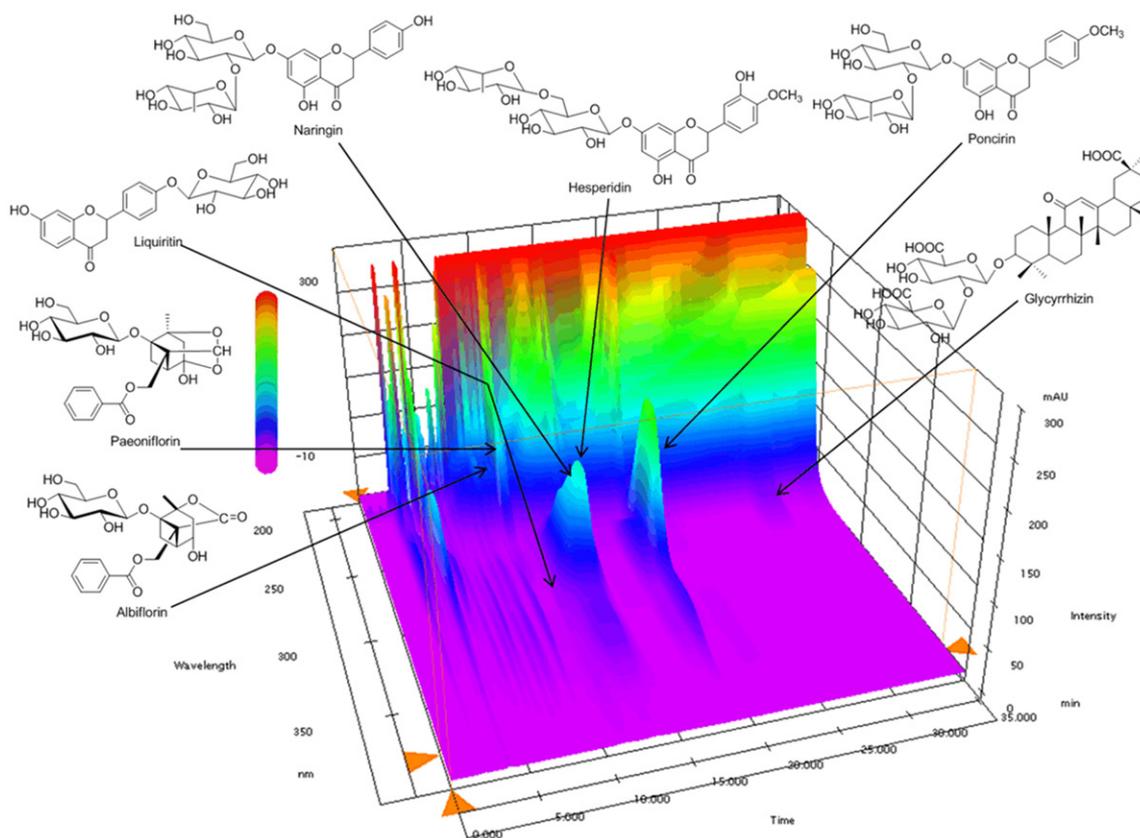


Figure 1. Three-dimensional chromatogram of SCGBT by HPLC-PDA.

660; Kyungseo Machine Co., Incheon, Korea). The water extract was then filtered through a standard sieve (no. 270, 53 μm ; Chung Gye Sang Gong Sa, Seoul, Korea) and the solution was evaporated to dryness and freeze dried to give a powder. The yield of SCGBT water extract was 24.5% (2.45 kg).

Chemicals and reagents for HPLC analysis

Paeoniflorin, liquiritin, naringin, glycyrrhizin (all of purity $\geq 98.0\%$), and hesperidin (purity $\geq 92.0\%$) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Albiflorin and poncirin (both of purity $\geq 98.0\%$) were obtained from ChromaDex (Irvine, CA, USA). The HPLC-grade reagents methanol, acetonitrile, and water were obtained from J.T. Baker (Phillipsburg, NJ, USA). Glacial acetic acid was obtained from Merck KGaA (Darmstadt, Germany).

Preparation of standard and sample solutions for HPLC analysis

Standard stock solutions of each of the seven compounds albiflorin, paeoniflorin, liquiritin,

naringin, hesperidin, poncirin, and glycyrrhizin were dissolved in methanol at 1.0 mg/mL. For HPLC analysis, 200 mg of lyophilized SCGBT extract was dissolved in 20 mL of distilled water, and then the solution was filtered through a SmartPor GHP 0.2 μm syringe filter (Woongki Science, Seoul, Korea) before injection into the HPLC system.

HPLC analysis of SCGBT

Analysis of the levels of the above seven compounds in the SCGBT sample was performed using 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 PDA detector. The data processor employed LC solution software (Version 1.24). The analytical column used was a Gemini C₁₈ (250 \times 4.6 mm; particle size 5 μm , Phenomenex, Torrance, CA, USA) maintained at 40°C. The mobile phases consisted of 1.0% (v/v) acetic acid in water (A) and 1.0% (v/v) acetic acid in acetonitrile (B). The gradient flow was as follows: 15-65% B for 0-35 min, 65%-100% B for 35-45 min, 100% B for

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Table 3. Contents of six compound in the SCGBT by HPLC (n = 3)

Compound	Mean (mg/g)	SD	RSD (%)	Source
Albiflorin	1.39	0.01	0.64	Paeoniae Radix
Paeoniflorin	5.55	0.06	1.02	Paeoniae Radix
Liquiritin	1.61	0.00	0.27	Glycyrrhizae Radix et Rhizoma
Naringin	4.98	0.00	0.01	Ponciri Fructus
Hesperidin	6.76	0.01	0.13	Citri Pericarpium
Poncirin	5.44	0.01	0.17	Ponciri Fructus
Glycyrrhizin	2.78	0.01	0.29	Glycyrrhizae Radix et Rhizoma

45-50 min, and 100%-15% B for 55 min. The flow rate was 1.0 mL/min and the injection volume was 10 μ L. The quantitative analysis of the seven compounds was carried out at 230 nm (albiflorin and paeoniflorin), 254 nm (glycyrrhizin), and 280 nm (liquiritin, naringin, hesperidin, and poncirin).

Cell culture and differentiation

Mouse preadipocyte cell line 3T3-L1 was obtained from the American Type Culture Collection (ATCC, CL-173, Rockville, MD, USA). The cells were cultured in Dulbecco's minimal essential medium (DMEM; Gibco BRL, Carlsbad, CA, USA) supplemented with 10% newborn calf serum (NCS, Gibco BRL, Carlsbad, CA, USA) at 37°C. For adipocyte differentiation, the cells were stimulated with 3T3-L1 differentiation medium containing isobutylmethylxanthine, dexamethasone, and insulin (MDI) (Zenbio Inc., Research Triangle Park, NC, USA) for 48 h after reaching confluence. The medium was switched to DMEM containing 10% fetal bovine serum (FBS) and 1 μ g/mL insulin after 2 days, and then changed to DMEM containing 10% FBS for an additional 4 days. SCGBT extract was added to the cells for 8 days of the differentiation period. GW9662 (Sigma, St. Louis, MO, USA), a peroxisome proliferator-activated receptor (PPAR)- γ antagonist, was used as the positive control in this study.

Cytotoxicity assay

To test cytotoxicity against differentiated adipocyte cells, 3T3-L1 preadipocytes were differentiated for 8 days in the presence or absence of SCGBT. CCK-8 solution (Dojindo, Kumamoto, Japan) was added and incubated for 4 h. After the incubation, the absorbance was read at

450 nm using a microplate reader (Benchmark Plus, Bio-Rad, MN, USA).

Triglyceride quantification assay

The contents of triglyceride were enzymatically measured using a commercial kit (BioVision Inc, Milpitas, CA, USA). Briefly, the 3T3-L1 adipocytes treated with SCGBT were homogenized in 5% NP-40 assay buffer, and the sample slowly heated to solubilize all triglycerides. The samples were mixed with lipase and triglyceride reaction mixture.

After 1 h of incubation, the sample absorbance was measured at 570 nm.

Glycerol-3-phosphate dehydrogenase (GPDH) activity assay

After the induction of adipocyte differentiation and treatment with SCGBT, 3T3-L1 cells were washed twice with PBS. GPDH activity was determined using a commercial kit (Takara, Tokyo, Japan) that monitored the dihydroxyacetone phosphate (DHAP)-dependent oxidation of NADH at 340 nm, and results were expressed as unit/mg of protein.

Leptin immunoassay

Leptin levels were assayed using a mouse leptin immunoassay kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. In brief, the culture supernatant was collected from the differentiated 3T3-L1 adipocytes treated with or without SCGBT. An equal ratio of the supernatants (50 μ L) and assay diluent RD1W (50 μ L) was added to wells of a 96-well plate and incubated for 2 h at room temperature. After washing 5 times with 400 μ L of wash buffer, 100 μ L of mouse leptin conjugate was added to each well and incubated for 2 h at room temperature. After washing 5 times, 100 μ L of substrate solution was added to each well and incubated for 30 min at room temperature in the dark. Finally, 100 μ L of stop solution was added to each well and the absorbance was measured at 450 nm.

Detection of ROS generation

ROS generation from 3T3-L1 cells were assessed using Total ROS Detection kit (Enzo Life Sciences, Plymouth Meeting, PA). Cells

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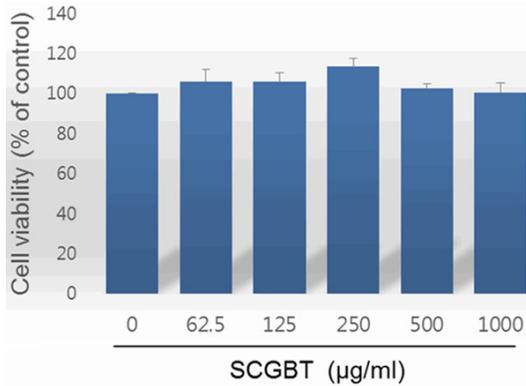


Figure 2. Cytotoxic effects of SCGBT extract in 3T3-L1 adipocytes. Adipocyte differentiation was induced by adding isobutylmethylxanthine, dexamethasone and insulin (MDI) into 3T3-L1 preadipocytes for 8 days. The cells were exposed to various concentrations of SCGBT (0, 62.5, 125, 250, 500, or 1000 µg/ml) during the differentiation period. Cell viability was determined using CCK-8 assay kit by measuring the absorbance at 450 nm. Data are presented as mean ± SEM.

were fixed in 4% paraformaldehyde and 100% acetone, and stained with Oxidative Stress Detection Reagent (standard green fluorescence). Cells were also immunostained with anti-perilipin antibody (Cell Signaling Tech., Danvers, MA) and TRITC-conjugated anti-rabbit IgG antibody (Abcam, Cambridge, UK). The cells were mounted with mounting medium containing DAPI and visualized by Olympus FLUOVIEW FV10i confocal microscope (Olympus, Tokyo, Japan).

ABTS radical scavenging activity

Free radical scavenging activity by the SCGBT extract on ABTS was assessed by the method described by Re *et al.* [13] with slight modifications. ABTS radical cation was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate, and stored in the dark at room temperature for 16 h. Absorbance of the reactant was later adjusted to 0.7 at a wavelength of 734 nm. A 100 µL aliquot of sample 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 734 nm with a spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, CA). The radical scav-

enging capacity of the tested samples was calculated using the following Equation:

$$\text{Scavenging activity (\%)} = (1 - \text{absorbance of SCGBT} / \text{absorbance of control}) \times 100$$

DPPH radical scavenging activity

Free radical scavenging activity by SCGBT on DPPH extract was assessed using the method described by Moreno *et al.* [14]. In brief, a 100 µL aliquot of 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.

Oxidation of LDL by CuSO₄

LDL samples (500 µg protein/mL, Biomedical Technologies, Stoughton, MA, USA) 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 SCGBT extract. After 5 min, the oxidation was initiated by the addition of CuSO₄ (25 µM). After 6 h oxidation, lipid peroxidation and the electrophoretic mobility of the LDL were measured as described below.

Determination of TBARS

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.

Relative electrophoretic mobility (REM) assay

The electrophoretic mobility of LDLs was measured using agarose gel (0.8% agarose in Tris acetate EDTA 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 oxLDL versus native LDL.

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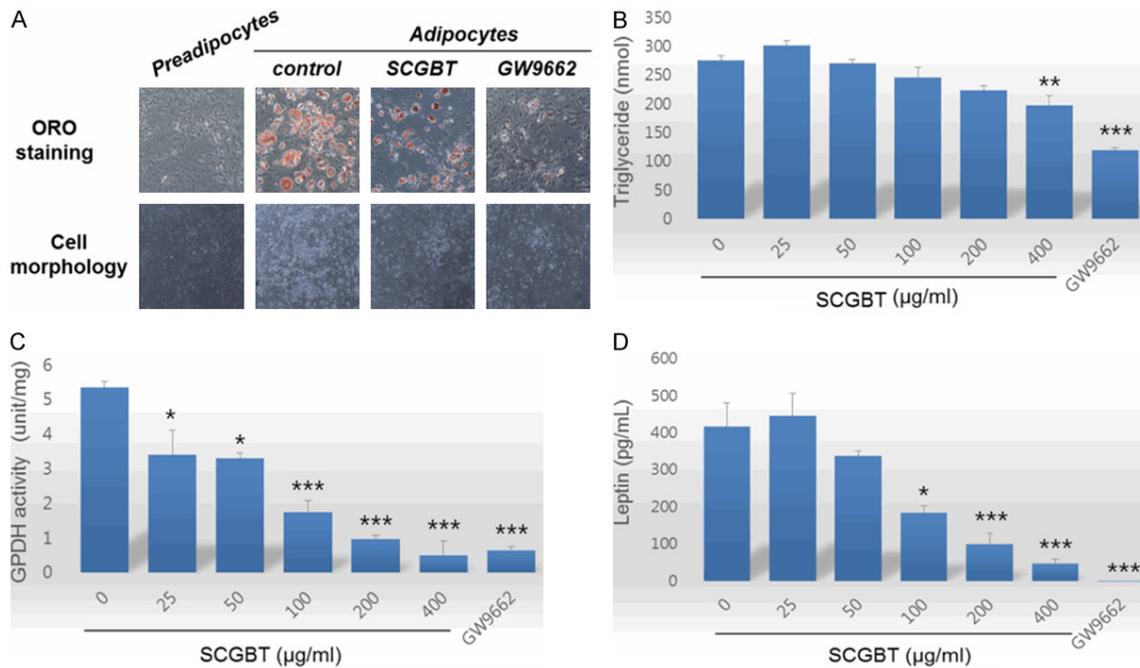


Figure 3. Inhibitory effects of SCGBT extract on adipogenesis in 3T3-L1 adipocytes. 3T3-L1 preadipocytes were differentiated into adipocytes by adding isobutylmethylxanthine, dexamethasone, and insulin (MDI) for 8 days. A. The cells were treated with or without SCGBT (200 $\mu\text{g/ml}$) or GW9662 (20 μM) during the differentiation period. Lipid accumulation in the cells was analyzed by Oil Red O staining. The cells stained with Oil Red O were visualized using an Olympus CKX41 inverted microscope at $\times 200$ magnification. B. The contents of triglyceride were enzymatically measured at 570 nm using a commercial kit (BioVision Inc, Milpitas, CA, USA). C. GPDH activity of the cells was assessed by determination of the decrease in NADH at 340 nm using a Takara glycerol-3-phosphate dehydrogenase (GPDH) activity assay kit. D. Culture supernatant was collected from the SCGBT-treated cells. Leptin production was determined by ELISA using a mouse leptin immunoassay kit (R&D Systems) and calculating absorbance at 540 nm subtracted from that at 450 nm. Data are represented as the mean \pm SEM. *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$ compared with the differentiated control.

Statistical analysis

All data are presented as mean \pm standard error of the mean (SEM). Group differences were assessed by one-way ANOVA and post-hoc Tukey Kramer's multiple comparison test using Graphpad InStat ver.3.10 (Graphpad software, Inc. San Diego, CA, USA). The significance of the differences from the normal control was taken as $P < 0.05$.

Results

HPLC analysis of SCGBT

All calibration curves were obtained by assessment of peak areas from standard solutions in the following concentration ranges: albiflorin and glycyrrhizin, 0.78-50.00 $\mu\text{g/mL}$; paeoniflorin, naringin, hesperidin, and poncirin, 0.78-100.00 $\mu\text{g/mL}$; liquiritin, 0.39-50.00 $\mu\text{g/mL}$. The retention times of the albiflorin, paeoniflorin, liquiritin, naringin, hesperidin, poncirin, and

glycyrrhizin were 8.77, 9.66, 11.49, 13.47, 13.98, 19.13, and 30.30 min, respectively. The calibration curves, correlation coefficient (R^2), limit of detection (LOD) and limit of quantification (LOQ) of the seven marker compounds are summarized in **Table 2**. Using optimized chromatography conditions, a three-dimensional chromatogram was obtained using HPLC-PDA detector (**Figure 1**). The concentrations of six marker compounds were 1.39-6.76 mg/g and are summarized in **Table 3**.

SCGBT is not cytotoxic for 3T3-L1 adipocytes

To evaluate the possible cytotoxicity of SCGBT against 3T3-L1 cells, preadipocytes were differentiated into adipocytes by adding the differentiation stimulator MDI. During differentiation for 8 days, the cells were exposed to various concentrations of SCGBT. As shown in **Figure 2**, no significant cytotoxic effect was observed in SCGBT-treated adipocytes.

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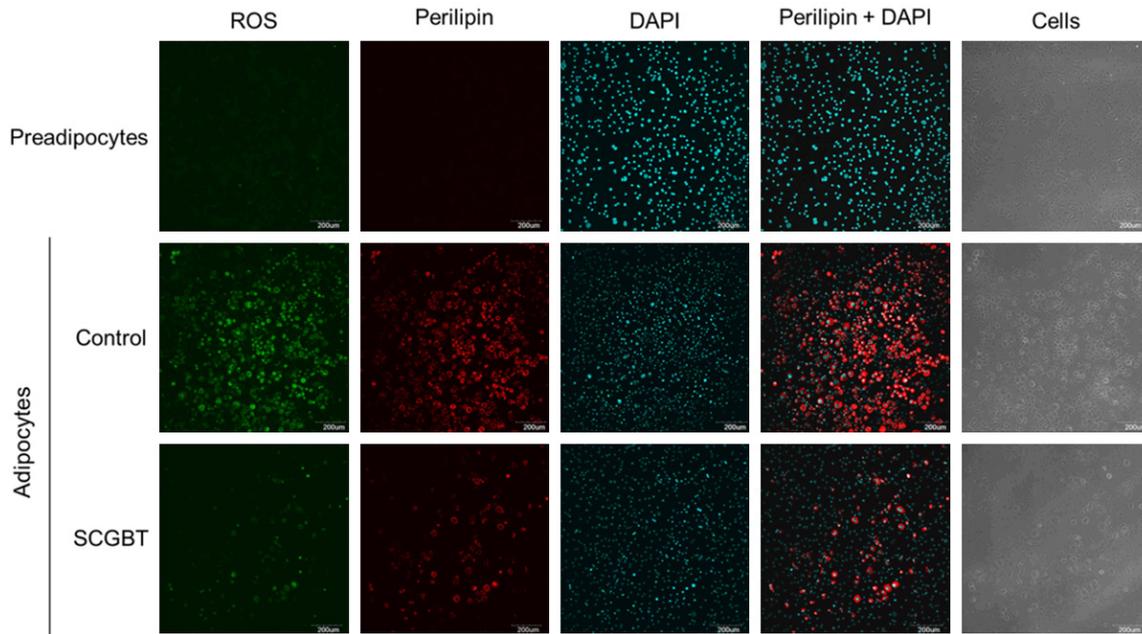


Figure 4. Inhibitory effects of SCGBT on ROS generation in 3T3-L1 adipocytes. 3T3-L1 preadipocytes were differentiated into adipocytes by adding isobutylmethylxanthine, dexamethasone, and insulin (MDI) for 8 days. The cells were treated with or without SCGBT (400 µg/ml) during the differentiation period. Cells were fixed in 4% paraformaldehyde and stained with Oxidative Stress Detection Reagent (green) and anti-perilipin antibody (red). The stained cells were mounted with medium containing DAPI (blue) and visualized by Olympus FLUOVIEW FV10i confocal microscope ($\times 100$).

SCGBT prevents adipogenesis in 3T3-L1 adipocytes

To analyze fat accumulation in the lipid droplets in differentiated 3T3-L1 adipocytes, we performed Oil Red O staining. As shown in **Figure 3A**, SCGBT significantly reduced the lipid droplets detectable with Oil Red O staining compared with the untreated differentiated cells. Consistent with the results of Oil Red O staining, the triglyceride content measured in differentiated 3T3-L1 adipocytes was significantly decreased by SCGBT treatment (**Figure 3B**). SCGBT also significantly induced inactivation of GPDH in a dose-dependent manner in these cells (**Figure 3C**). Furthermore, SCGBT exerted an inhibitory effect on leptin production compared with the untreated differentiated cells (**Figure 3D**). Similarly, GW9662, the positive control, dramatically inhibited adipogenesis in 3T3-L1 cells.

SCGBT inhibits ROS production in 3T3-L1 adipocytes

We next assessed the effect of SCGBT on ROS generation in adipocytes. As shown in **Figure 4**,

ROS generating cells (green) were markedly detected in adipocytes compared with preadipocytes. By contrast, SCGBT treatment reduced population of ROS-generating adipocytes. Furthermore, the immunostained cells with anti-perilipin (red), a lipid storage marker, were clearly detected in adipocytes and reduced in SCGBT-treated cells. Phase contrast pictures showed morphological difference between preadipocytes and adipocytes.

SCGBT exerts antioxidant activities

To evaluate the antioxidant activity of SCGBT, we tested its scavenging activities on ABTS and DPPH radicals. SCGBT showed dose-dependent radical scavenging activity (**Figure 5A**). The scavenging activities of SCGBT were 13.02%, 25.15%, 41.12%, 64.88%, and 89.67% at 12.5, 25, 50, 100, and 200 µg/mL concentrations, respectively. The concentration required for 50% reduction (RC_{50}) of ABTS radicals was 71.23 µg/mL, whereas the RC_{50} value of ascorbic acid, the positive control, was 3.22 µg/mL. The antioxidant activities obtained for SCGBT using the DPPH method are shown in **Figure 5B**. Similarly to the ABTS assay,

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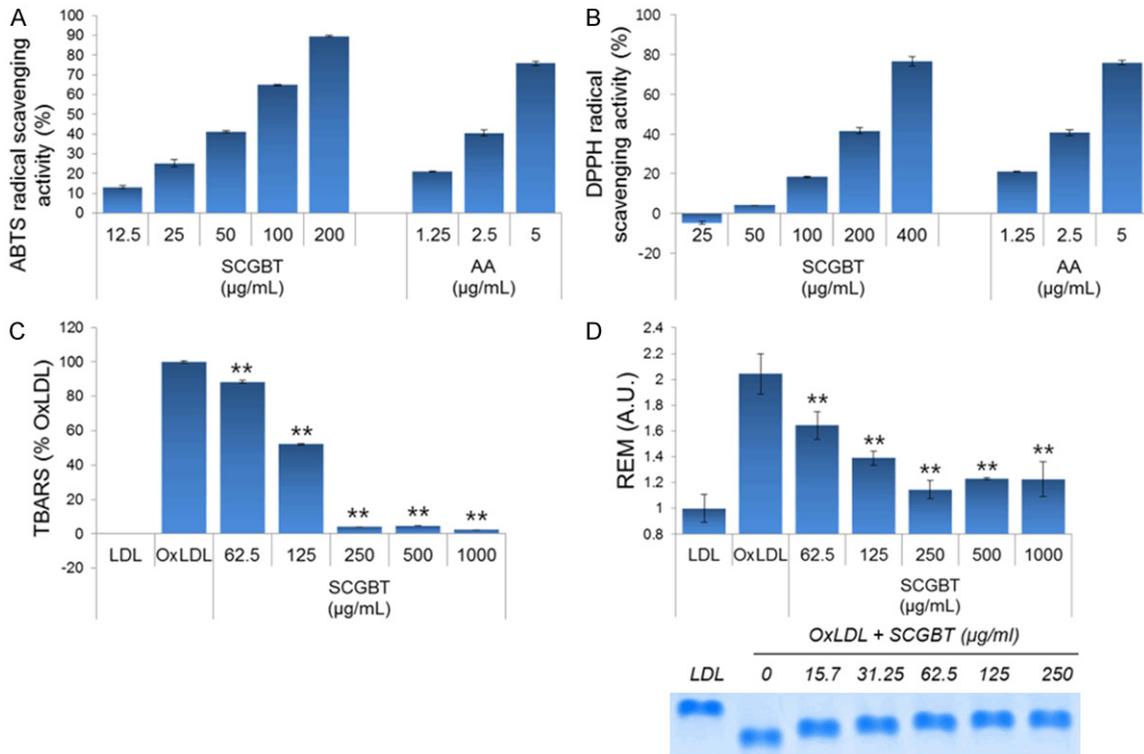


Figure 5. Antioxidant effects of SCGBT. Antioxidant activity of different concentrations of SCGBT and ascorbic acid (AA) assessed by ABTS (A) or DPPH (B) radical scavenging method. Inhibitory effects of SCGBT on Cu^{2+} -induced LDL oxidation were determined by TBARS (C) and REM (D) assays. Indicated concentrations of SCGBT and LDLs were incubated with CuSO_4 for 6 h at 37°C . The TBARS levels and electrophoretic mobility of LDLs were measured using the TBARS assay kit and agarose gel electrophoresis, respectively. Data are presented as mean \pm SEM. ** $P < 0.01$ compared with the OxLDL group.

SCGBT reduced the DPPH radical formation in a concentration-dependent manner. The RC_{50} of SCGBT and ascorbic acid on DPPH radicals was found to be $260.88 \mu\text{g/mL}$ and $10.43 \mu\text{g/mL}$, respectively. These results indicate that SCGBT has strong scavenging power for ABTS and DPPH radicals and should be explored as a potential antioxidant.

SCGBT prevents oxidation of LDL induced by CuSO_4

The effects of SCGBT on Cu^{2+} -mediated oxidation of LDL were determined by TBARS and REM assays. The generation of MDA equivalents during LDL oxidation was estimated by the TBARS assay. As shown in **Figure 5C**, when LDL was incubated with CuSO_4 for 6 h, a significant increase in TBARS was detected. In contrast, SCGBT significantly reduced the amount of TBARS formed in a dose-dependent manner (IC_{50} : $120.72 \mu\text{g/mL}$). The oxidative modification of the protein moiety in LDL during Cu^{2+} -

mediated oxidation was estimated by REM assay. When the oxidation was carried out in the presence of SCGBT, the increased electrophoretic mobility of oxLDL was significantly reduced (**Figure 5D**). These data suggest that SCGBT has an inhibitory effect on LDL oxidation.

Discussion

Many traditional herbal medicines have been used for prevention and treatment of a variety of diseases. Recently, interest in traditional herbal medicines has been considerably increased because they have fewer side effects and less toxicity compared with synthetic drugs [15, 16]. As mentioned above, SCGBT, which consists of 14 herbal components, is a traditional herbal medicine widely used for the treatment of stomach disorders [12]. Marker constituents of seven herbal components of this herbal medicine, including Ginseng Radix, Magnoliae Cortex, Citri Unshius Pericarpium,

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Ponciri Fructus Immaturus, Paeoniae Radix, Glycyrrhizae Radix et Rhizoma, and Zingiberis Rhizoma Crudus, are included in the Korean Pharmacopoeia (The Korean Pharmacopoeia, Ninth Edition) [17]. Therefore, we analyzed the levels of these seven compounds including hesperidin (Citri Unshius Pericarpium), naringin and poncirin (Ponciri Fructus Immaturus), albiflorin and paeoniflorin (Paeoniae Radix), and liquiritin and glycyrrhizin (Glycyrrhizae Radix et Rhizoma) using HPLC-PDA. An established HPLC-PDA method was applied for simultaneous analysis of the seven constituents in SCGBT. Among these components, hesperidin, paeoniflorin, and poncirin, which are marker components of Citri Unshius Pericarpium, Paeoniae Radix, and Ponciri Fructus Immaturus, were detected at 6.76 mg/g, 5.55 mg/g, and 5.44 mg/g, respectively, as the principal compounds in SCGBT (**Table 3**). This established HPLC-PDA method will be helpful for improvement in quality control of SCGBT.

The aim of this study was to determine the anti-adipogenic and antioxidative potential of SCGBT in 3T3-L1 adipocytes. To date, it has been reported that several marker compounds of SCGBT have anti-adipogenic, anti-obesity and/or antioxidative activities [18-22]. However, there is no evidence whether SCGBT itself possess anti-adipogenic and antioxidant potential. We conducted Oil Red O staining and a triglyceride assay to examine whether SCGBT extract could cause changes in triglyceride production. Our data revealed that the number of lipid droplets containing triglyceride visualized by Oil Red O staining was significantly increased in differentiated adipocytes. In contrast, SCGBT extract markedly suppressed formation of lipid droplets compared with control adipocytes (**Figure 3A**). Consistent with the results of Oil Red O staining, SCGBT treatment reduced the amount of triglyceride in adipocytes (**Figure 3B**). A significant inhibitory effect on triglyceride production was observed after treatment with 400 µg/ml SCGBT. Leptin is an adipokine exclusively released from adipocytes in proportion to triglyceride production [23]. In the present study, leptin production in adipocytes was significantly inhibited by SCGBT extract (**Figure 3D**). Furthermore, SCGBT treatment blocked the activation of GPDH, a key enzyme for lipid biosynthesis [24], compared with that in untreated adipocytes (**Figure 3C**). Overall, our data indi-

cate that SCGBT has potential as an anti-adipogenic agent.

Lipid accumulation in adipocytes is closely associated with oxidative stress [11]. Oxidative stress triggers the uncontrolled production of free radicals, causing various diseases including obesity [25]. Recently, Calzadilla *et al.* showed increased ROS production in 3T3-L1 differentiated cells compared with undifferentiated control [26]. Thus, we investigated if SCGBT can influence ROS generation of adipocytes. Consistent with Calzadilla's data, we found that adipocytes induced a significant ROS production (**Figure 4**). By contrast, SCGBT reduced ROS-generating adipocyte population. Our data also revealed that SCGBT treatment decreased cells reacted with anti-perilipin antibody in differentiated adipose cells. Perilipin is one of adipogenesis markers and plays a crucial role in triglyceride storage in lipid droplets [27]. Furthermore, we evaluated the antioxidant activity of SCGBT extract using *in vitro* ABTS and DPPH radical scavenging assays since antioxidants are widely used to protect cells against ROS-induced damage [28]. SCGBT significantly increased the ability to scavenge ABTS and DPPH radicals in a dose-dependent manner (**Figure 5A** and **5B**). Additionally, SCGBT reduced production of MDA and inhibited LDL oxidation mediated by CuSO₄ in a dose-dependent manner (**Figure 5C** and **5D**). These results suggest that SCGBT exerts antioxidant effects associated with anti-adipogenesis.

In summary, SCGBT extract has inhibitory effects on adipogenesis and antioxidant properties in 3T3-L1 cells, suggesting its potential as a valuable candidate for an anti-obesity medication that acts to target oxidative stress. Additional work will be required to better understand the molecular mechanisms responsible for the anti-adipogenic and antioxidant effects of SCGBT. In recent years, the antioxidant activities of natural products have been extensively investigated for controlling obesity [29-33]. In light of these results, herbal medicines can also be considered as promising antioxidants for treatment of obesity that have less toxicity and fewer side effects than synthetic drugs.

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Disclosure of conflict of interest

None.

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