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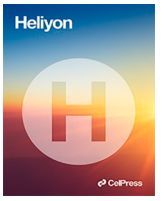

Research article

Sipyimigwanjung-tang, a traditional herbal medication, alleviates weight gain in a high-fat diet-induced obese mice model

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ABSTRACT

Obesity leads to the development of metabolic syndrome and comorbidities. Overweight and obesity continue to be a relentless global issue. Sipyimigwanjung-tang (SGT), a traditional herbal medication, was first mentioned in *Dongui Sasang Shinpyun* and has been used to treat edema, meteorism, and jaundice, which are common findings associated with obesity. The main physiological feature of obesity is expanded adipose tissue, which causes several impairments in liver metabolism. Therefore, this study aimed to investigate the anti-obesity effects of SGT in the epididymal white adipose tissue (eWAT) and livers of high-fat diet (HFD)-induced obese mice. SGT significantly blocked HFD-induced weight gain in C57BL/6N mice. In addition, SGT effectively reduced the increased weight and adipocyte size in eWAT of HFD-induced obese C57BL/6N mice. Moreover, SGT significantly decreased the elevated gene expression of *Peroxisome proliferator-activated receptor γ*, *CCAAT/enhancer-binding protein α*, and *Sterol regulatory element-binding protein 1* in the eWAT of HFD-induced obese mice. Furthermore, SGT significantly decreased lipid accumulation in the livers of HFD-induced obese mice and differentiated 3T3-L1 adipocytes. Hence, the present study provides substantial evidence that SGT has potential therapeutic effects on obesity.

1. Introduction

According to recent statistics, overweight/obesity continues its relentless global rise, surpassing ~30% of the world population [1]. Obesity causes the development of metabolic syndrome and comorbidities, including nonalcoholic fatty liver disease, type 2 diabetes, hyperlipidemia, hypertension, cardiovascular disease, chronic kidney disease, osteoarthritis, and malignancies, resulting in elevated mortality in obese individuals [2]. For long-term use (>12 weeks), only five anti-obesity drugs, including orlistat, lorcaserin, phentermine/topiramate, naltrexone/bupropion, and liraglutide, have currently been approved by the US FDA [3]. However, given the

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List of abbreviations

SGT	Sipyimigwanjung-tang
HFD	high-fat diet
WAT	white adipose tissue
eWAT	epididymal white adipose tissue
PPAR	Peroxisome proliferator-activated receptor
C/EBP	CCAAT/enhancer-binding protein
SREBP	Sterol regulatory element binding protein

huge costs and high disease burden of obesity, present pharmacological therapies do not effectively resolve the clinical heterogeneity, containing side effects and contraindications that can affect the treatment outcomes for obese patients [4]. Therefore, it is necessary to explore novel pharmacological agents to treat obesity.

Adipogenesis is mainly controlled by two families of transcription factors, peroxisome proliferator-activated receptor (PPAR) and CCAAT/enhancer-binding protein (C/EBP) [5]. PPARs are ligand-activated transcription factors affiliated with the nuclear hormone receptor family [6] and have emerged as critical regulators of lipid and carbohydrate metabolism [7]. Decades of evidence have firmly revealed the role of PPARγ in adipocyte differentiation; the bulk of transcriptional inhibitors and activators known to control adipogenesis act via modulation of PPARγ expression and/or activity [8]. C/EBP family is associated with a wide variety of cellular processes, e.g., cell growth, immune response, metabolism, and differentiation, and the first discovered member among them was named C/EBPα, which is highly expressed in adipose tissue, liver, and the myeloid lineage [9]. A review of C/EBPs revealed that the absence of C/EBPα leads to non-expressed adipose-specific genes and non-detected triacylglycerol accumulation in 3T3-L1 cells [10]. In addition, a previous study demonstrated a relationship between PPARγ and C/EBPα, showing that PPARγ-lacking cells express remarkably decreased levels of C/EBPα [11]. Additionally, it was suggested that mutual regulation of PPARγ and sterol regulatory element binding protein (SREBP)1c may take place to modulate adipogenesis and lipid metabolism via a feedback loop [12], and there is also a strong correlation between SREBP1c and C/EBPα [13]. SREBP1 plays a critical regulatory role in enhancing the expression of master adipogenic genes and is greatly induced during adipocyte differentiation [14]. Overall, these findings indicate that PPARγ, C/EBPα, and SREBP1 are required for adipocyte differentiation.

Sipyimigwanjung-tang (SGT), a traditional herbal medication, was first mentioned in *Dongui Sasang Shinpyun* and it is the prescription for curing stomach and intestines diseases, edema, meteorism (known as tympanites), and jaundice [15]. Edema is a common finding in obesity [16], and previous studies proved a relationship between obesity and jaundice, showing that overweight patients had a 50% higher risk of obstructive jaundice than patients with normal weights [17]. SGT comprises 12 medicinal herbs that have many biological activities in obesity-related conditions [18–28]; however, the anti-obesity effects of SGT have not been explored.

Based on the above findings, we investigated the anti-obesity effects of SGT via inhibition of key adipogenic transcription factors in high fat-diet (HFD)-induced obese mice and 3T3-L1 adipocytes.

2. Material and methods

2.1. Preparation of SGT

The composition of SGT is shown in Table 1. All 12 herbs (Nanum Pharmaceutical Company (Seoul, Republic of Korea)) were extracted in water at 99 °C for 3 h. The filtered extract was freeze-dried and the yield was calculated as 29.8%. The powder was dissolved in phosphate-buffered saline (PBS) for subsequent experimentation.

Table 1
Composition of SGT.

Scientific name	Pharmacognostic name	Amount
Cynanchum Wilfordii Hemsley	Cynanchi Wilfordii Radix	4g
Polygonum multiflorum Thunberg	Polygoni Multiflori Radix	4g
Alpinia officinarum Hance	Alpinia Officinarum Rhizoma	4g
Alpinia oxyphylla Miquel	Alpiniae Oxyphyllae Fructus	4g
Citrus unshiu Markovich	Citri Unshius Pericarpium	4g
Citrus reticulate Blanco	Citri Unshius Pericarpium Immaturus	4g
Poncirus trifoliate Rafinesque	Ponciri Fructus Immaturus	4g
Cyperus rotundus L.	Cyperi Rhizoma	4g
Zingiber officinale Roscoe	Zingiberis Rhizoma	4g
Aucklandia lappa Decne	Aucklandiae Radix	4g
Magnolia officinalis Rehder et Wilson	Magnoliae Cortex	4g
Areca catechu L.	Arecae Pericarpium	4g

2.2. High performance liquid chromatography (HPLC) analysis

Standards of hesperidin, naringin, 6-gingerol, honokiol, and magnolol were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). The aqueous extract of SGT and the five standards were dissolved in methanol. Thereafter, HPLC was performed using an LC-20A system (Shimadzu, Kyoto, Japan) with an automatic liquid sampler (SIL-20A), binary pump (LC-20AD), and wavelength detector (SPD-20A). The extract and five standards were analyzed under the following conditions: column, Spursil 5 μm C18-EP (4.6 × 250 mm, 5 μm; DiKMA, California, USA); mobile phase, distilled water with 0.1% acetic acid (solvent system A), and acetonitrile with 0.1% acetic acid (solvent system B) in a gradient mode (B from 10 to 90 % in 50 min); sample injection volume, 10 μL; flow rate, 1.0 mL/min; column temperature, ambient, UV wavelength, 210 nm.

2.3. Cell culture and differentiation of 3T3-L1 preadipocytes

3T3-L1 preadipocytes were cultured in DMEM with 10% bovine serum (Thermo Fisher Scientific, MA, USA), 1% penicillin (Thermo Fisher Scientific), 1 g/L HEPES, and 1.5 g/L $NaHCO_3$, and maintained in a humidified atmosphere of 5% CO_2 at 37 °C. To induce adipocyte differentiation, cells were seeded at a 2×10^5 into 6-well plates. After cells reached confluence, they were differentiated with culture medium containing 10% fetal bovine serum (FBS), 1 μg/mL insulin, 0.5 mM 3-isobutyl-1-methylxanthine, and 1 μM dexamethasone. After two days, fresh culture medium containing 10% FBS and 1 μg/mL insulin was added and left for two days, and then fresh culture medium containing 10% FBS was changed every two days.

2.4. Cell viability measurement

3T3-L1 preadipocytes (1×10^4 cells/well) were seeded in 96-well plates overnight and treated with 0–1000 μg/mL SGT for 48 h. The 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution was treated and the cells were incubated at 37 °C for 2–4 h. After removal of the supernatant, 50 μL of dimethyl sulfoxide was added and the MTT-formazan product was estimated using an Epoch® microvolume spectrophotometer (Bio Tek Instruments Inc., Winooski, VT, USA).

2.5. Oil Red O staining

3T3-L1 adipocytes were fixed with 10% formaldehyde in PBS at 25 °C for 1 h. After fixation, cells were washed three times with distilled water and then stained with 3 mg Oil red O power/1 mL 60% isopropanol at 25 °C for 2 h. Cells were rinsed three times with distilled water and photographed with an Olympus SZX10 microscope (Tokyo, Japan). The intracellular lipid content was measured by eluting with isopropanol using an Epoch® microvolume spectrophotometer at 520 nm.

2.6. Experimental animals

8-week-old male C57BL/6 N mice (18–20 g) were purchased from Daehan Biolink (Daejeon, Republic of Korea) and maintained under modified conditions (22 ± 2 °C, 55 ± 9% humidity, 12 h light/dark cycle). The mice were acclimatized to their environment for one week. Thereafter, mice were weighed and divided into three groups (n = 6 per group) as follows: normal diet group (control), 45% HFD group, and HFD supplemented with 10% of SGT. All mice had ad libitum access to food and water. During the experiment, body weight and food intake were checked every week. After 11 weeks, the mice were anesthetized with Zoletil 50 (20 mg/kg) by intraperitoneal injection and then euthanized by cervical dislocation. Epididymal white adipose tissue (eWAT) and liver tissues were excised and immersed in liquid nitrogen.

2.7. Biochemical analysis

Blood was collected and immediately centrifuged (1000×*g* for 20 min at 4 °C) to obtain plasma. The plasma levels of triglycerides (TG) and total cholesterol (TC) were measured using commercial kits (Asan Pharmaceutical. Co., Ltd., Republic of Korea). All biochemical assays were performed following the manufacturer's instructions.

2.8. Histological examination

eWAT and liver tissue from mice were fixed in 10% formalin, embedded in paraffin, and cut into 5–10 μm sections. The sections

Table 2
Real-Time PCR primer sequences.

Gene	Forward (5′-3′)	Reverse (5′-3′)
Pparγ	ATCGAGTGCCGAGTCTGTGG	GCAAGGCACTTCTGAAACCG
Srebp1	ATCGCAAACAAGCTGACCTG	AGATCCAGGTTTGAGGTGGG
C/ebpα	GGAACTTGAAGCACAATCGATC	TGGTTTAGCATAGACGTGCACA
Gapdh	GACGGCCGCATCTTCTTGT	CACACCGACCTTCACCATTTT

were then stained with hematoxylin and eosin. Stained liver sections were examined for lipid droplets. Stained eWAT sections were used to estimate the adipocyte size. All observations were performed under an Olympus SZX10 microscope (Olympus).

2.9. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analysis

qRT-PCR analysis was performed as previously described [29]. Briefly, eWAT and liver tissues were homogenized, and total RNA was isolated. The total RNA was converted to cDNA and qPCR analysis was conducted using a StepOnePlus real-time PCR system (Applied Biosystems). *Gapdh* was used as an internal control. The sequences of mouse oligonucleotide primers are presented in Table 2.

2.10. Western blot analysis

Liver tissue was homogenized using PRO-PREPTM protein extraction solution (Intron Biotechnology, Seoul, Republic of Korea). Equal amounts (15–30 μg) of protein sample were separated on a sodium dodecyl sulfate-polyacrylamide gel and then transferred to a polyvinylidene fluoride membrane. Membranes were incubated with primary antibody PPARγ (sc-7273), C/EBPα (sc-9314), and SREBP1 (sc-13551) and incubated with horseradish peroxidase-conjugated secondary antibody for 2 h. The blots were washed three times using tris buffered saline with tween 20 and then visualized by enhanced chemiluminescence using AmershamTM Imager 680 (GE Healthcare Bio-Sciences AB, Sweden).

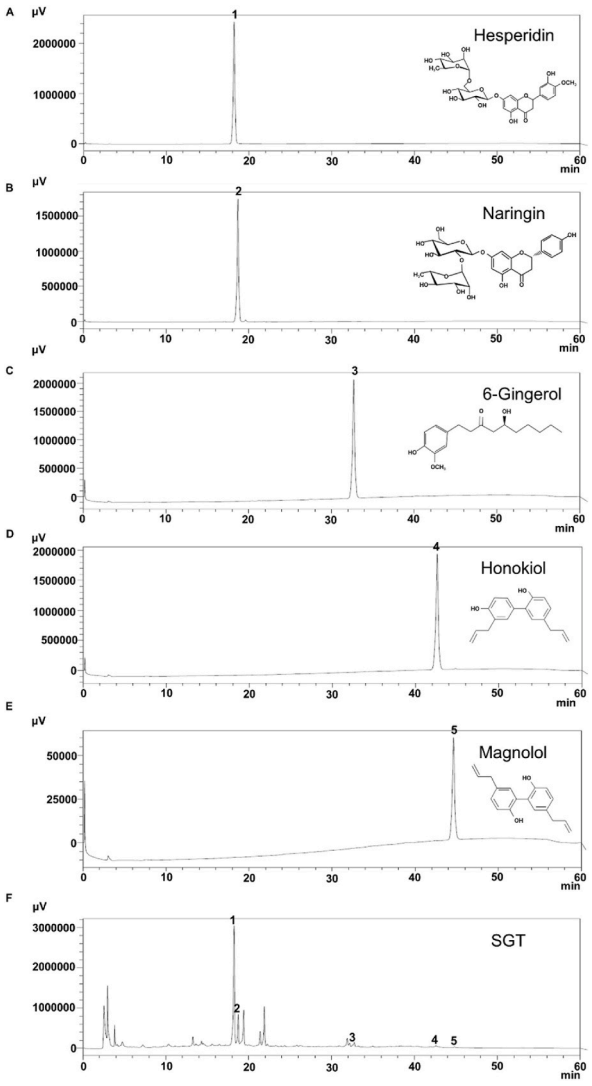

Fig. 1. Analysis of ingredients identified from SGT aqueous extract.
HPLC chromatograms of (A) hesperidin, (B) naringin, (C) 6-gingerol, (D) honokiol, (E) magnolol, and (F) SGT.

2.11. Statistical analysis

Results were presented as mean ± standard deviation of triplicate experiments. Statistical analysis was performed using SPSS version 19.0 (International Business Machines, Armonk, NY, USA). Statistical significance was determined using analysis of variance and Dunnett's post-hoc test. Statistical significance was set at $p < 0.05$.

3. Results

3.1. SGT decreases lipid accumulation in differentiated 3T3-L1 adipocytes

SGT was identified using HPLC analysis. Five components (hesperidin, naringin, 6-gingerol, honokiol, and magnolol) of SGT were identified based on the retention time of the standard sample (Fig. 1A–F). The chromatograms and chemical structures are presented in Fig. 1, and the identification results are listed in Table 3. To determine whether SGT has regulatory effects *in vitro*, an MTT assay was conducted to evaluate the cytotoxicity of SGT in 3T3-L1 preadipocytes. Since the results revealed that concentrations of 500 μg/mL and below had a cell viability higher than 80% (Fig. 2A), concentrations of 125, 250, and 500 μg/mL were used in further experiments. To assess the effect of SGT on lipid accumulation in 3T3-L1 adipocytes, the cells were fully differentiated for day 8. The amount of lipid droplets in 3T3-L1 adipocytes was notably increased in the differentiation group compared to the non-treated group, whereas SGT effectively decreased intracellular lipid accumulation in differentiated 3T3-L1 adipocytes (Fig. 2B and C).

3.2. SGT improves the increased body weight gain, serum TC and TG levels in HFD-induced obese mice model

To test whether SGT has anti-obesity effects *in vivo*, mice were fed HFD supplemented with 10% SGT. After 11 weeks of diet feeding, mice in the HFD group had significantly higher body weight and weight gain than those in the control group. However, SGT supplementation markedly ameliorated the increase in body weight and weight gain in the HFD-fed mice (Fig. 3A and B). There was no significant difference in food intake between the groups (Fig. 3C). Serum analysis demonstrated increased TC and TG levels in HFD-fed mice, whereas SGT supplementation significantly decreased the levels of TC and TG in HFD-fed mice (Fig. 3D and E).

3.3. SGT improves adipogenesis in eWAT of HFD-induced obese mice model

To check whether SGT reduces visceral adipose tissue in obsess mice model, eWAT weight and eWAT index (eWAT/body weight) were assessed. HFD feeding led to increased eWAT weight and eWAT index in mice, whereas significant decreases in eWAT weight and eWAT index were observed in SGT-supplemented HFD mice (Fig. 4A and B). Furthermore, histological examination of eWAT revealed a prominent reduction in adipocyte size after SGT supplementation (Fig. 4C and D). To further confirm whether SGT inhibits adipogenesis, we investigated the levels of adipogenic transcription factors in eWAT. As expected, SGT supplementation significantly suppressed the mRNA levels of *Pparγ*, *C/ebpα*, and *Srebp1* in the eWAT (Fig. 4E–G).

3.4. SGT improves adipogenesis in the liver tissue of HFD-induced obese mice model

As the liver is involved in the obesity-related adverse effects [30], the influence of SGT on the livers of HFD mice was examined. Histological examination by hematoxylin and eosin staining revealed greater sizes and elevated lipid droplets in the HFD-fed mice, but SGT supplementation remarkably reversed these changes in the livers of HFD-fed mice (Fig. 5A). Furthermore, the hepatic expression of adipogenic transcription factors was assessed. The augmented expression of the three adipogenic genes (PPARγ, C/EBPα, and SREBP1) in the livers of HFD mice was dramatically abolished by SGT supplementation (Fig. 5B). Moreover, the reduced protein expression of PPARγ, C/EBPα, and SREBP1 by SGT supplementation in the liver was consistent with the decreased hepatic mRNA levels of the three adipogenic genes in HFD-fed mice (Fig. 5C−E).

4. Discussion

SGT comprises 12 medicinal herbs that have many biological activities in obesity-related conditions, and several studies have demonstrated the inhibitory effects of *Cynanchum Wilfordii* Hemsley on hypertension, hypercholesterolemia, and gastric disorders in

Table 3
Identification of components in SGT by HPLC analysis.

Peak	t_R (min)	Content(mg)	Identification
1	18.223	8.93	Hesperidin
2	18.718	2.87	Naringin
3	32.729	0.30	Gingerol
4	42.595	0.09	Honokiol
5	44.822	0.02	Magnolol

SGT: Sipyimigwanjung-tang; HPLC: high-performance liquid chromatography; t_R: retention time.

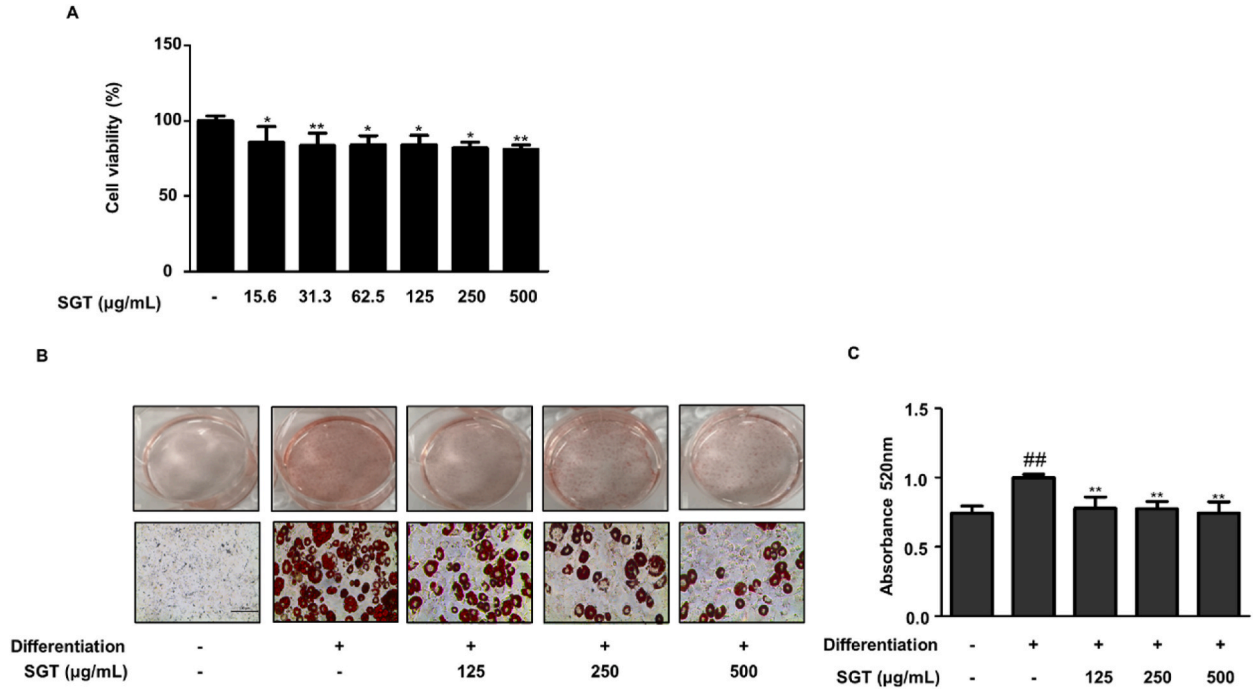

Fig. 2. Effect of SGT on lipid accumulation in 3T3-L1 adipocytes.
(A) 3T3-L1 preadipocytes were treated with various concentrations of SGT for 48 h and their viability was estimated using the MTT assay. The values are represented as mean ± S. D. *P < 0.05 and **P < 0.01 vs. untreated cells; significance was determined using one-way ANOVA followed by Dunnett's post hoc test. (B) 3T3-L1 preadipocytes were stimulated with a differentiation medium in the presence or absence of the indicated SGT concentrations for 8 days and subjected to Oil Red O staining. Stained cells were visualized under a microscope at 100× magnification. Non-adjusted images are provided as supplementary materials. (C) Oil Red O was dissolved in isopropanol and absorbance was measured at 510 nm. The values are represented as the mean ± S. D. ##P < 0.01 vs. non-treated cells; **P < 0.01, differentiated cells; significance was determined using one-way ANOVA followed by Dunnett's post hoc test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

rats [18–20]. *Polygonum multiflorum* Thunberg showed therapeutic effects on hyperlipidemia and inflammation, and its compound stilbene also has hepatoprotective activity [21]. It was also reported that curcumin in *Alpinia officinarum* Hance. possesses anti-dyslipidemic bioactivity in high-fat diet (HFD)-fed hamsters [22]. Decades of evidence have identified the beneficial effects of *Zingiber officinale* Roscoe and its active compounds in the prevention of some metabolic syndromes, including diabetes, nonalcoholic fatty liver disease, and cardiovascular disease [23]. Herbal medication containing *Citrus unshiu* Markovich has anti-obesity effects in HFD-induced mice [24]. Researchers have reported that *Cyperus rotundus* possesses anti-inflammatory, antilipidemic, antidiabetic, and cardioprotective properties [25]. *Alpinia oxyphylla* Miquel has been reported to have pharmacological activities against inflammation, oxidative stress, and diabetes [26]. *Magnolia officinalis* Rehder et Wilson protected hepatocytes against oxidative stress [27]. Moreover, numerous investigations have demonstrated that *Areca catechu* L. has various properties such as digestion, cardiovascular systems, inflammation, and regulatory effects on blood glucose and lipids [28]. In the present study, HPLC analysis showed that SGT contains citrus flavonoids, hesperidin, and naringin, which are the main compounds in *Citrus unshiu* Markovich, but there were low levels of 6-gingerol (the main compound of *Zingiber officinale* Roscoe), magnolol, and honokiol (the main compounds of *Magnolia officinalis*) in SGT. Hesperidin [31] and naringin [32] have been shown to ameliorate weight gain and metabolic syndrome, including hepatic steatosis. Our study demonstrated that SGT effectively reduces weight gain and hepatic lipid accumulation in HFD-fed obese mice, and this strong effect seems to be correlated with the biological activities of herbal components and main compounds (hesperidin and naringin) in SGT on obesity-related conditions.

The main physiological feature of obesity is expanded adipose tissue (increase in the number and size of adipocytes), and adipocytes also undergo other functional changes of great importance [33,34]. Adipose tissue is a crucial component of metabolic control and an endocrine organ that secretes several adipokines known to mediate lipid metabolism, insulin resistance, and inflammation [35]. WAT is classified according to its location into subcutaneous and visceral WAT, and visceral WAT shows a higher correlation with obesity severity [36]. There is no argument that visceral adipose tissue represents a key organ for strategies designed to manage or prevent the health risks associated with abdominal obesity [37]. Because most visceral adipose tissue is drained by the portal vein, the hyperlipolytic state of hypertrophic adipocytes exposes the liver to any amount of free fatty acids and glycerol when consumption of high energy causes several impairments in liver metabolism, such as decreased hepatic extraction of insulin (exacerbating hyperinsulinemia), elevated production of triglyceride-rich lipoproteins, and augmented production of hepatic glucose [38]. In the present study, SGT supplementation effectively reduced HFD-induced white adipogenesis in eWAT and livers by controlling *Pparγ*, *C/ebpα*, and

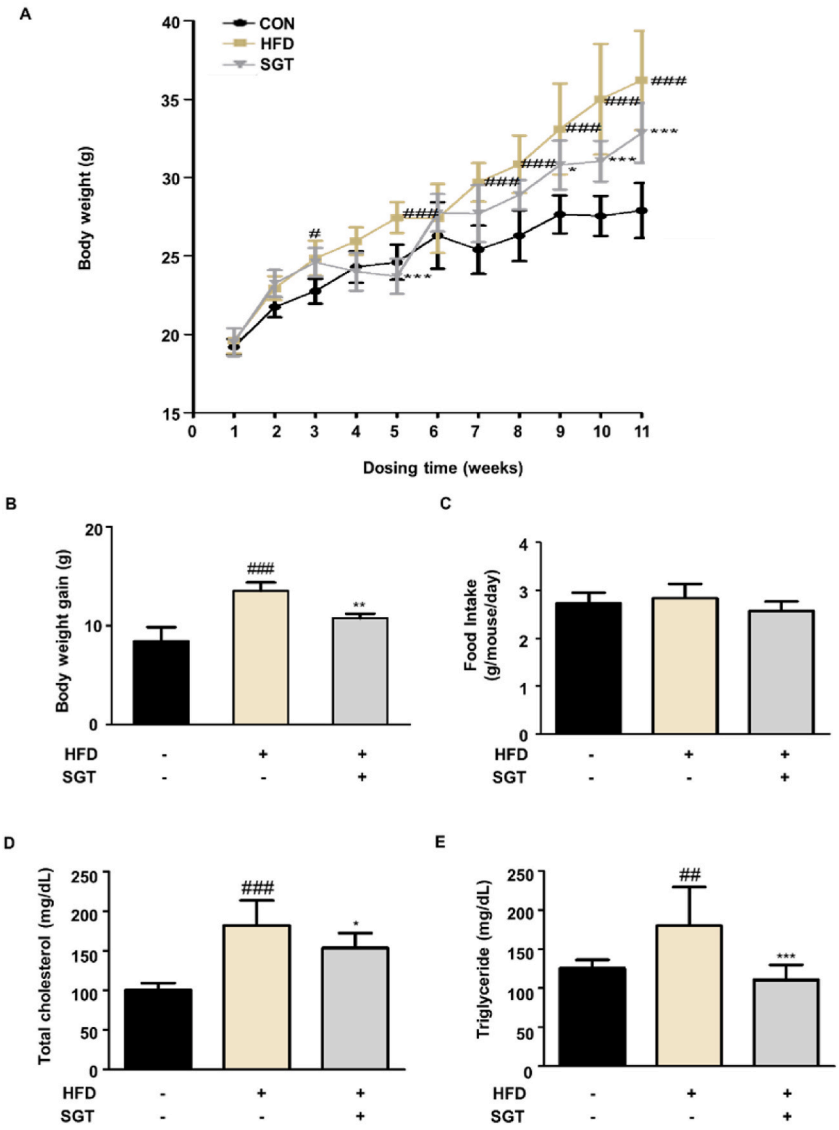

Fig. 3. Effect of SGT on body weight gain, serum TC and TG levels in HFD-induced obese mice.
(A) Mouse body weight was measured weekly for 11 weeks. (B) Body weight gain was calculated. (C) The food intake was measured weekly and calculated. (D) Serum total cholesterol and (E) triglyceride were detected. The values are represented as mean ± S. D. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ vs. CON; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs. HFD group; significances were determined using two-way ANOVA followed by a Bonferroni post hoc test, and one-way ANOVA followed by a Dunnett's post hoc test.

Srebp1 gene expression. These results indicate that SGT can prevent obesity by reducing adipogenesis in eWAT and thereby ameliorating hepatic steatosis. However, in order to conclusively affirm that the regulatory effect of SGT is on the inhibition of transcription of adipogenic genes, the assessment on their transcriptional activity (e.g. nuclear translocation) would be conducted in further study.

Many drugs have appetite suppressant effects via stimulation of the central nervous system (CNS); however, this action on the CNS may cause adverse effects, including restlessness, insomnia, reduced concentration capacity, and alteration of mood state [39]. In addition, a previous study suggested that the targets of anti-obesity drugs in the future are likely to change from CNS to peripheral mechanisms that are less likely to have side effects [40]. The present study revealed that SGT reduces weight gain by regulating adipogenesis through the inhibition of key adipogenic transcription factors. Although further investigations are needed to fully understand the mechanism of SGT, this study suggests the possibility of using SGT as a medication with fewer adverse reactions for the management of obesity.

5. Conclusions

Taken together, the present study provides considerable evidence that SGT prevents and manages obesity and thus could be a

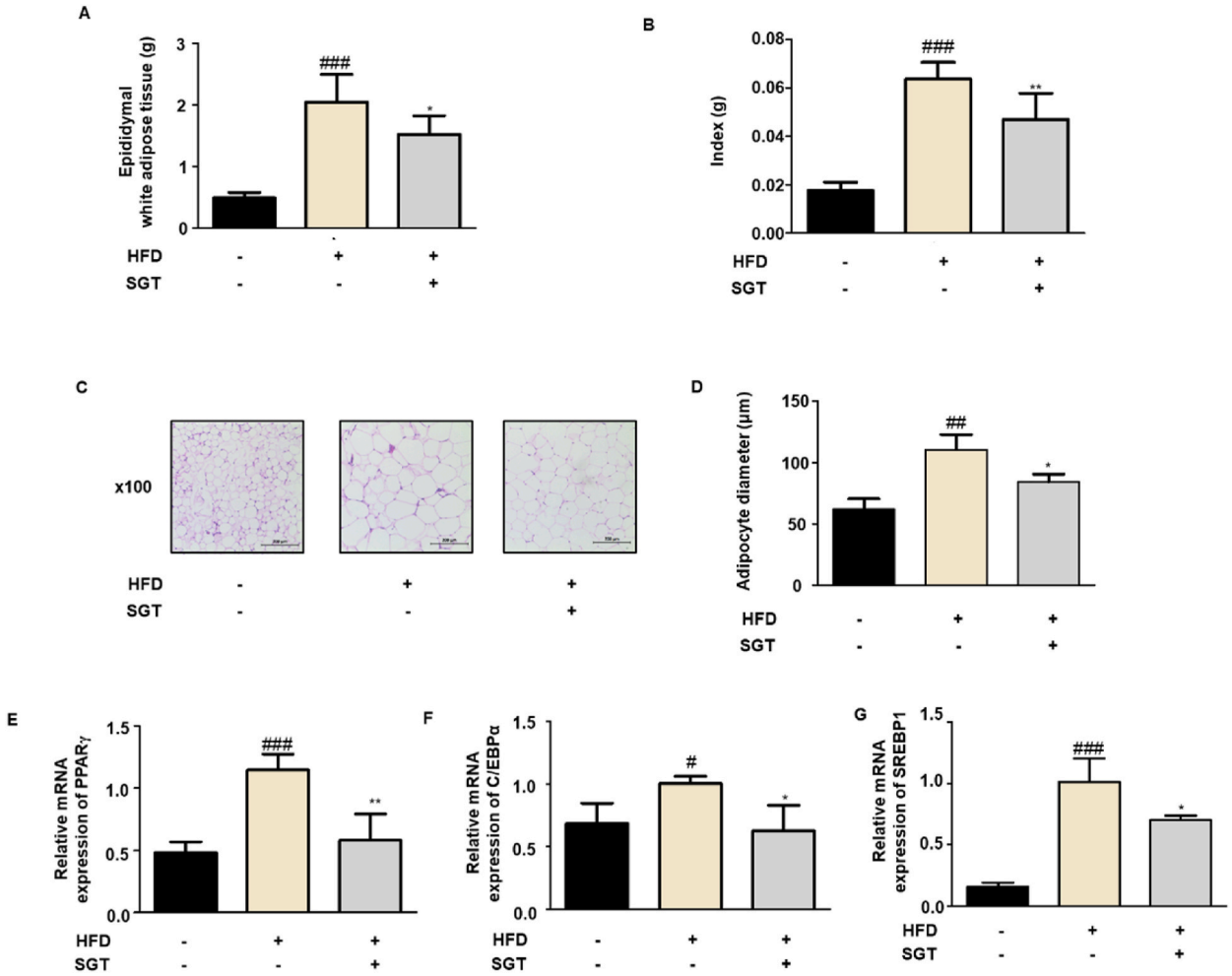

Fig. 4. Effect of SGT on adipogenesis in eWAT of HFD-induced obese mice.
At the end of the experimental period, the weights of (A) eWAT and (B) eWAT index were measured. The eWAT index is a measure of eWAT weight based on the body weight of the mice. (C) Hematoxylin/eosin staining images are shown at a magnification of 100. × (D) The average diameter of adipocytes in eWAT of each group. qRT-PCR was performed to determine mRNA levels of (E) *Pparγ*, (F) *C/ebpα*, and (G) *Srebp1*. The values are represented as mean ± S. D. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ vs. CON; $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. HFD group; significance was determined using one-way ANOVA followed by Dunnett's post hoc test.

promising treatment for obesity.

Ethics statement

All experiments were conducted with the approval of the Ethical Committee for Animal Care and the Use of Laboratory Animals of Sangji University (reg. no. 2018-21).

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Data availability statement

Data will be made available on request.

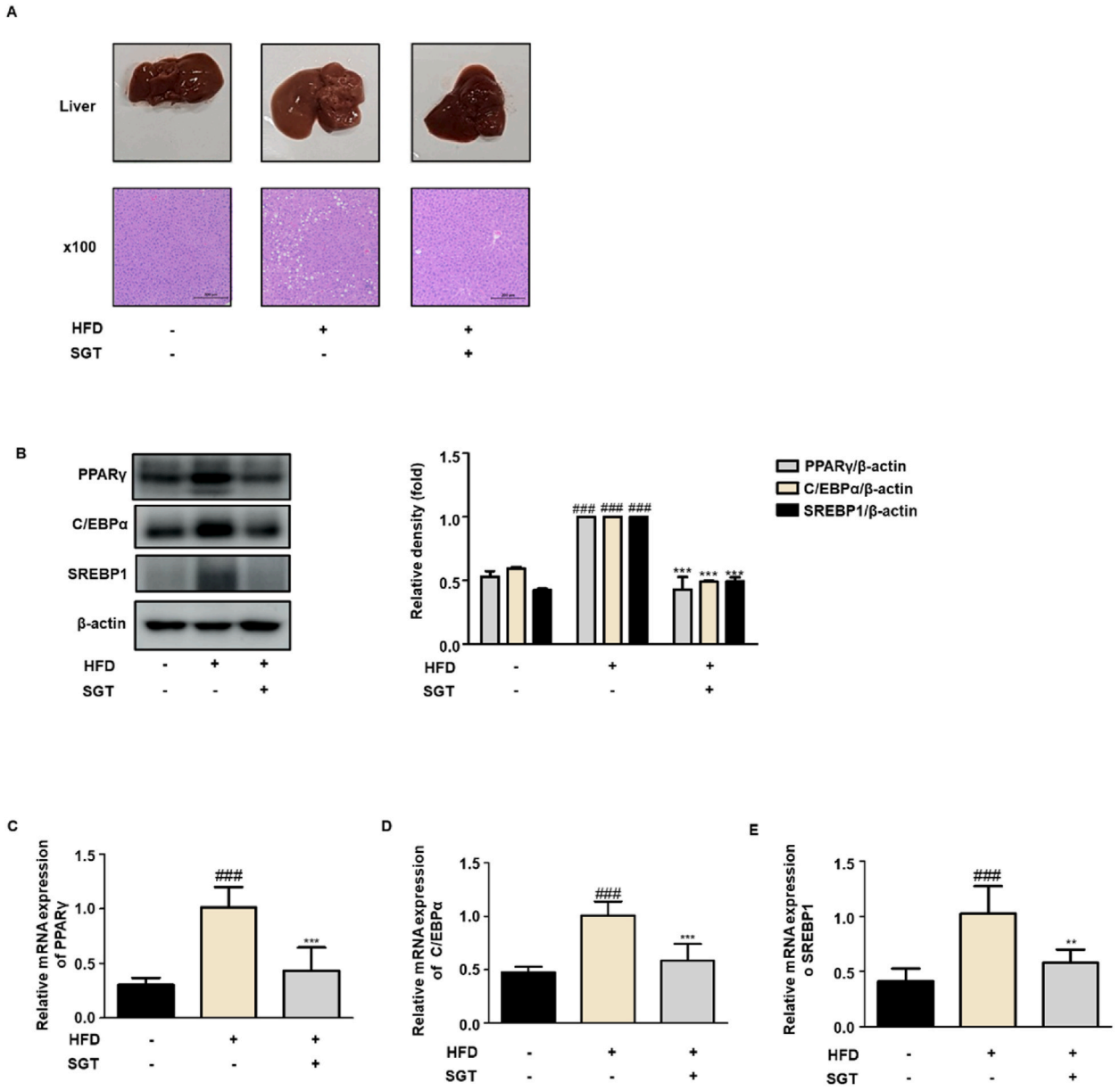

Fig. 5. Effect of SGT on adipogenesis in liver tissue of HFD-induced obese mice.
(A) At the end of the experimental period, the mouse liver was dissected for macroscopic analysis and subjected to hematoxylin/eosin staining. Images are shown at the magnification of 100×. (B) Western blot was performed to determine the protein level of PPARγ, C/EBPα, and SREBP1. The uncropped gel blots are provided as supplementary materials. qRT-PCR was performed to determine the mRNA level of (C) *Pparγ*, (D) *C/ebpα*, and (E) *Srebp1*. The values are represented as mean ± S. D. $^{###}$P < 0.001 vs. control; **P < 0.01 and ***P < 0.001 vs. HFD group; significances were determined using one-way ANOVA followed by a Dunnett's post hoc test.

CRediT authorship contribution statement

Yea-Jin Park: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Dong-Wook Seo:** Writing – original draft, Investigation, Conceptualization. **Tae-Young Gil:** Investigation. **Hyo-Jung Kim:** Investigation. **Jong-Sik Jin:** Resources, Methodology, Investigation. **Yun-Yeop Cha:**Investigation, Funding acquisition. **Hyo-Jin An:** Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27463.

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