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Agastache rugosa Extract and Its Bioactive Compound Tilianin Suppress Adipogenesis and Lipogenesis on 3T3-L1 Cells

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Abstract: *Agastache rugosa*, or Korean mint, is an herb used as a spice, food additive and traditional medicinal ingredient. It has desirable effects, such as its antibacterial, antifungal and antioxidant properties. *A. rugosa* contains many phenolic compounds studied for their various health benefits, with the primary components being tilianin. *A. rugosa* extract (ARE), which was extracted with ethanol and freeze-dried, contained 21.14 ± 0.15 mg/g of tilianin with a total polyphenol content of 38.11 ± 0.88 mg/g. Next, the antiadipogenic effect of *A. rugosa* and tilianin was clarified using 3T3-L1 cells, which differentiate into adipocytes and develop lipid droplets. 3T3-L1 cells were treated with ARE or tilianin and lipid accumulation (%) was calculated through oil red O staining. Tilianin elicited dose-dependent decrease in lipid accumulation (% of positive control) (30 μM 92.10 ± 1.19%; 50 μM 69.25 ± 1.78%; 70 μM 54.86 ± 1.76%; non-differentiation 18.10 ± 0.32%), assessed by oil-red-O staining, whereas ARE treatments caused consistent diminution in lipid accumulation regardless of dose (100 μM 86.90 ± 4.97%; 200 μM 87.25 ± 4.34%; 400 μM 88.54 ± 2.27%; non-differentiation 17.96 ± 1.30%), indicating that both compounds have anti-obesity effects on adipocytes. Treatment with ARE lowered the mRNA (PPARγ; C/EBPα; FABP4; SREBP1; ACC; FAS) and protein (PPARγ; C/EBPα; SREBP1) levels of adipogenesis and lipogenesis-related factors. Tilianin showed a greater effect on the mRNA levels compared with ARE. Thus, tilianin and ARE may have anti-adipogenic and anti-lipogenic effects on 3T3-L1 cells and be possible candidates of obesity-related supplements.

Keywords: tilianin; *Agastache rugosa*; Korean mint; anti-adipogenesis; anti-lipogenesis; 3T3-L1 cells

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1. Introduction

Obesity is typically induced by an imbalance in calorie uptake and usage, with surplus calories stored in the body as adipose tissue. Obesity can trigger serious medical issues, such as diabetes, cancer, hypertension or cardiovascular complications [1], and few studies also show that these symptoms can lead to cognitive disorders, including dementia [2].

Obesity can be treated with medication, and to date, several drugs were approved and prescribed to patients [3]. Alternatively, obesity and excess weight gain can be prevented by reducing dietary calorie intake, increasing the consumption of vegetables and whole grains, and engaging in regular exercise. Here *Agastache rugosa* extract (ARE) and its major bioactive components, tilianin, were studied and found to be possible candidates for nutraceuticals aimed at preventing obesity.

Peroxisome proliferator-activated receptors (PPARs) are a crucial class of ligand-dependent transcriptional regulators. PPAR-γ is a transcription factor expressed in mammalian adipocytes with major roles in differentiation and in controlling transcription factors related to lipid metabolism in adipose tissues [4].

Excess cholesterol can form sediments that destroy cells and can be fatal if developed in the bloodstream. Cholesterol almost solely exists in cell membranes, and its levels are monitored by sterol regulatory element-binding proteins (SREBPs), which transmit this

information to the nucleus to regulate the transcription of genes related to cholesterol uptake and synthesis [5]. SREBPs control transcription of enzymes involved in fatty acid uptake and biosynthesis, including acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), stearoyl CoA desaturase and lipoprotein lipase. SREBPs thus coordinate the synthesis of the two main components of membranes, i.e., fatty acids and cholesterol [6,7]. When SREBPs become overexpressed, the inhibition of adipocyte differentiation and production of a lipodystrophy occurs. In addition, the amount of white adipose tissue (WAT) is decreased and exhibits hyperglycemia and intensified fatty livers [8].

Polyphenols in plants are known for their various biochemical properties, including antioxidant effects. Tilianin (Figure 1 is a polyphenol antioxidant, and a naturally occurring phytochemical that modulates oxidative stress-related inflammation and apoptosis [9]. Tilianin also has health-promoting properties and exhibits cardioprotective and antihypertensive behavior. [10–13].

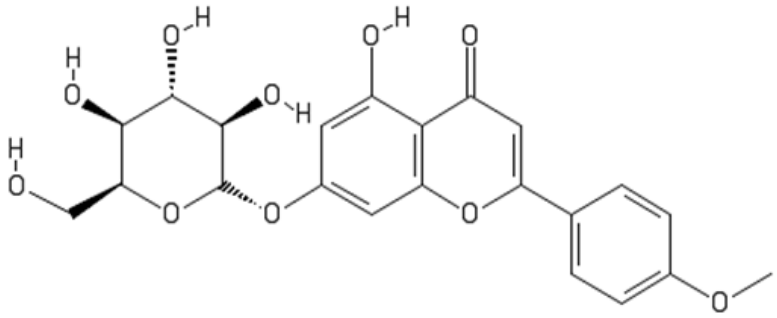

Figure 1. The chemical structure of tilianin. Tilianin is one of the major bioactive compounds in *Agastache rugosa* extract.

A. rugosa, or Korean mint, grows throughout East Asia, especially in Korea. *A. rugosa* is often used as a medication or gas-reducing agent in traditional medicine [14] and is known for its antifungal, anti-inflammatory, antioxidant and anti-atherogenic properties [15–19]. Therefore, based on the anti-atherosclerotic and anti-inflammatory activity of *A. rugosa*, we speculated the antiadipogenic predisposition of *Agastache rugosa* extract by considering the association with these previous studies [16–19].

In present study, to elucidate the anti-obesity activity as a new function of *A. rugosa*, we investigated the adipogenesis inhibitory activity of *A. rugosa* and its major active compound, tilianin, in murine adipocyte 3T3-L1.

2. Materials and Methods

2.1. Sample Preparation and Reagents

Agastache rugosa extract (ARE) was produced and provided by the Natural Medicine Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB) of the Korea in August 2019 Collected plants were identified and the voucher specimen (D 190724001) was deposited in KRIBB. ARE produced through the extraction process of *A. rugosa* leaves (air-dried in dark) at 50 °C–80 °C using 30–70% ethanol for an optimized amount of time. The ethanol was then collected and reused. ARE was then concentrated by evaporating the ethanol, drying the sample either by freeze-drying or spray drying, pulverizing the dried sample and storing the resulting extract at −4 °C for posterior use.

Tilianin was obtained from Ensol Biosciences (Daejeon, Korea). Oil-red-O, acacetin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dexamethasone (DEXA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Water and acetonitrile (HPLC grade) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Dulbecco's Modified Eagle's medium (DMEM), fetal bovine serum (FBS), bovine calf serum (BCS) and penicillin-streptomycin (P/S) were supplied by Hyclone (Logan, UT, USA). Trypsin-EDTA and insulin were purchased from Gibco. IBMX was obtained from Santa Cruz Biotechnology (Dallas, TX, USA).

2.2. HPLC Analysis

Measurements of tilianin and acacetin (well-known anti-adipogenesis component) levels were performed using HPLC-UV. The HPLC device (Nanospace Si-2; Shiseido, Tokyo, Japan) consisted of a pump, a UV spectrophotometric detector, a column oven and an autosampler. Separation was conducted using a Shiseido Capcell Pak column (UG 120 C18 250 × 4.6 mm, 5 µm; Shiseido) in a column oven at 35 °C. Injections were conducted using 10 µL of the sample and the flow rate was maintained at 1 mL/min. Detection was performed at 540 nm with a 55-min runtime. Mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) were degassed by sonication for 60 min and used in a gradient (% A–B: 80–20 for 5 min, 50–50 for 30 min, 2–98 for 10 min and 80–20 for 10 min).

2.3. Measurement of Total Polyphenol Levels

In a 10-mL volumetric flask, 0.005 g ARE was dissolved in distilled water. Next, 100 µL of ARE solution, 500 µL of 0.2 N folin–ciocalteu solution and 400 µL of 7.5% sodium carbonate was mixed and allowed to react for 30 min in the dark. OD_{765} value of samples was then measured using a multi-plate reader and the standard curve was calculated using gallic acid (1.2–250 ppm) dilutions. The total polyphenol content was defined in terms of gallic acid equivalents using a regression equation.

2.4. Cell Culture and Adipogenic Differentiation

The 3T3-L1 preadipocyte cell line was purchased from the American Type Culture Collection (Manassas, VA, USA) and grown in T75 cm^2 flasks (Sarstedt, Nümbrecht, Germany) containing high glucose DMEM supplemented with 10% (v/v) BCS and 1% (v/v) P/S at 37 °C in 5% CO_2. Adipocyte differentiation was induced when the cells had reached 100% confluence, and the initiation date of differentiation was designated day 0. Cells were fed for the first two days with DMEM (10% FBS, 1% P/S) containing 1 µM DEXA, 0.5 mM IBMX and 1 µg/mL insulin. After four days, the cells were treated with DMEM (10% FBS, 1% P/S) containing only 1 µg/mL insulin. Cell media were changed every other day. Treatment with tilianin and ARE was performed for six days once differentiation started.

2.5. Cell Viability Assay

MTT assays were used to determine the viability of 3T3-L1 preadipocyte cells treated with each compound. Cells were cultured in 96-well plates at a concentration of 1×10^5 cells/mL for 24 h and treated with various concentrations of tilianin and ARE for 48 and 72 h, respectively. The supernatant in each well was removed, and the cells were treated with 5 mg/mL MTT for 4 h. The MTT solution was discarded, and the resulting formazan was dissolved in dimethyl sulfoxide (DMSO). After 30 min, OD_{720} was measured using a microplate reader (fluostar omega, BMGlabtech, Offenburg, Germany).

2.6. Oil-Red-O Staining

After differentiation, cells were washed for five minutes and fixed for one hour with 10% formalin. The solution was then removed from each well, and cells washed with 60% isopropanol. Lipid droplets were stained using a working solution of oil-red-O for an hour. After coloring the lipid droplets, water was used to remove the remaining oil-red-O solution. Images were then taken through the water using a microscope, then the water was removed and the plates completely dried overnight. Isopropanol was then used to dissolve the oil-red-O dye in the cells, and absorbance was measured at 485 nm.

2.7. RNA Extraction and Qualitative Real-Time PCR (qRT-PCR) Analysis

For RNA isolation, total RNA was extracted using an AccuPrep Universal RNA Extraction Kit (k-3140; Bioneer, Daejeon, Korea). For the qRT-PCR, 1 µg of total RNA was reverse transcribed using a cDNA synthesis kit (K1641; Thermo Scientific, Waltham, MA, USA). qRT-PCR reactions were prepared using an SYBR green mix (A25741; Applied

Biosystems, Waltham, MA, USA) and consisted of pre-denaturation for 10 min at 95 °C followed by 40 cycles at 95 °C for 15 s, and 60 °C for 1 min. Analysis of qRT-PCR results was performed using Quantstudio 3 (Applied Biosystems, Waltham, MA, USA). Information about primers used in this experiment is provided in Table 1.

Table 1. Sequences of primers for mRNA used in RT-qPCR.

Target Gene	Forward	Reverse
C/EBPα	CGGGAACGCAACAACATCGC	TGTCCAGTTCACGGCTCAGC
CPT1	ACTCCTGGAAGAAGAAGTTCAT	AGTATCTTTGACAGCTGGGAC
FABP4	TGGGAACCTGGAAGCTTGTCTC	GAATTCCACGCCCAGTTTGA
FAS	TGGGCATAACGGTCTCTGGT	TCCATGTGCGGTGTGAAAAC
PPARγ	CGGAAGCCCTTTGGTGACTTTATG	GCAGCAGGTTGTCTTGGATGTC
SREBP1	CCCTGTGTGTACTGGCCTTT	ACGGTGTGTACCCGTAGCAT
β-actin	TGAGAGGGAAATCGTGCGTGAC	GCTCGTTGCCAATAGTGATGACC

2.8. Western Blotting

Cells were cultured in six-well plates, differentiation initiated using IBMX, DEXA and insulin (MDI), and cells treated with various concentrations of tilianin and ARE. At six days post-differentiation, the cells were washed with phosphate-buffered saline and proteins extracted using PRO-PREP for Cell/Tissue Protein Extraction Solution (iNtRON Biotech, Seognam-si, Korea) containing phosphatase inhibitor cocktails two and three. Supernatants were isolated, and protein concentrations were measured using bicinchoninic acid (BCA) assay. Cell lysates (20 µg of protein) were separated using SDS-PAGE, transferred onto a polyvinylidene fluoride membrane and then blocked for 20 min using EveryBlot Blocking Buffer (Bio-Rad, Hercules, CA, USA). The membrane was then incubated overnight with a primary antibody (in 1% bovine serum albumin) at 4 °C. After two hours of incubation with the secondary antibody (in 5% skim milk), EZ Western Lumi Femto (Dogenbio, Seoul, Korea) was used to create luminescence, which was detected using a chemiluminescence detector (iBright FL100; Invitrogen, Carlsbad, CA, USA).

2.9. Statistical Analysis

Results were expressed as mean ± standard deviation. Differences were analyzed with a one-way analysis of variance (ANOVA) followed by Duncan's test (n = 3, 4, 6). All analyses were conducted using SPSS v.26.0 (SPSS, Inc., Chicago, IL, USA). Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Tilianin, Acacetin and Total Polyphenol Content in ARE

As expected, ARE contains various plant-originated polyphenols, including flavonoids, which have numerous benefits for humans [15]. The total polyphenol content of ARE was determined to be 38.11 ± 0.88 mg/g. This figure means that it contains at least 2.05 times more polyphenols than green tea, a noted plant which contains abundant polyphenols from 1.17 to 18.59 mg/g [16].

The levels and identities of single compounds in ARE were determined using HPLC-UV. Tilianin and acacetin identification was verified by eluting the single standard and spiking methods. The mixture of the standards showed a clear chromatogram. The analysis method was modified from a previously published UPLC method [20]. Since the standard solution eluted clearly without any issues, this method was deemed verified.

Acacetin is another main compound in *A. rugosa*. It has numerous effects on human including anti-obesity [21]. Tilianin and acacetin from ARE eluted at average times of 19.42 and 32.82 min, respectively, during a total analysis time of 55 min. ARE contains 21.14 ± 0.15 mg/g of tilianin and 9.94 ± 0.08 mg/g of acacetin. In addition, a peak, eluted at approximately 23 min showed a similar area to that of tilianin and was identified as acacetin 7-O-(6″-O-malonyl)-β-D-glucopyranoside.

3.2. Effect of Tilianin and ARE on 3T3-L1 Preadipocyte Viability

MTT assays were performed after 24, 48 and 72 h of compound treatment. Data (Figure 2A–D) are shown just for 48 and 72 h of treatment because 24 h treatment did not cause any cytotoxicity.

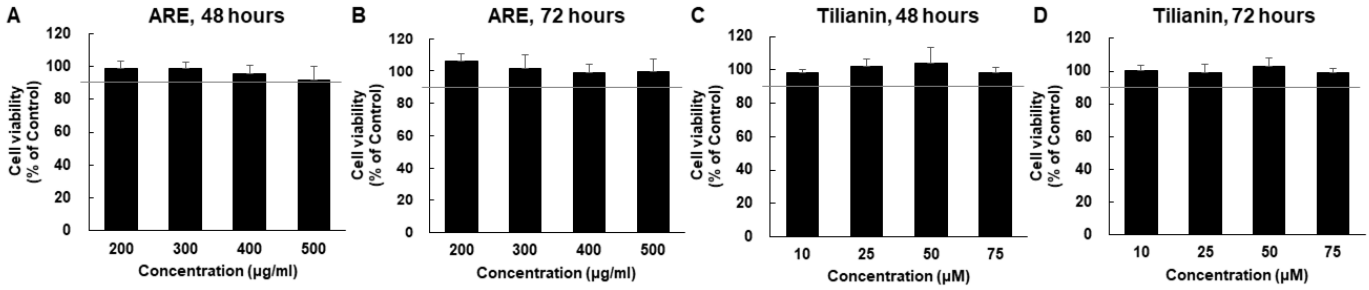

Figure 2. Cell viability (%) of 3T3-L1 cells in the presence of ARE and its bioactive compound, tilianin. 3T3-L1 cells were seeded on 96-well plate, and the cells were treated with different concentrations of ARE (200, 300, 400, 500 μg/mL) and tilianin (Tilianin, 10, 25, 50, 75 μM). The cytotoxicity was measured by MTT assay. ARE was treated for (**A**) 48 h and (**B**) 72 h with concentration of 200, 300, 400, 500 μg/mL. Treated tilianin on 3T3-L1 adipocytes for (**C**) 48 h and (**D**) 72 h. The results are expressed as the mean values ± SD (n = 6). Concentration which showed higher index than 90% was selected for further experiment.

ARE could not be solubilized in 30% ethanol at concentrations above 500 mg/mL; thus, the highest concentration used for the cell treatments was 500 μg/mL. Cytotoxicity was considered a loss in cell viability of more than 10%. In that the materials are treated for six days and the longest treatment for MTT assay is only 72 h, so the limit of cell viability set higher than regular research. The results indicate that ARE (at concentrations below 500 μg/mL) and tilianin were not cytotoxic against preadipocytes in all treatment times tested. ARE concentrations of 100, 200 and 400 μg/mL and tilianin concentrations of 30, 50 and 70 μM were, thus, used for subsequent experiment.

3.3. Inhibitory Effect of Tilianin and ARE on Intracellular Lipid Accumulation in Adipocytes

ARE-treated cells showed similar decreases in lipid droplet accumulation than the control groups at all concentrations tested (Figure 3A). These results confirm the study [22], which showed a 20.4% inhibition of intracellular lipid accumulation by ARE treatment. Lipid accumulation was markedly inhibited by tilianin in a dose-dependent manner.

As shown in Figure 3B, the number of stained lipid droplets decreased with increasing tilianin concentration. In contrast, treatments with 100, 200 and 400 μg/mL caused similar decreases in lipid accumulation compared with the controls. Two separate controls were used for this experiment: nondifferentiated cells, which were incubated in DMEM media with calf serum (BSM media) only, and a control group treated with MDI lacking ARE or tilianin. As there are very few eminent reports on the anti-obesity effect of tilianin, the inhibitory effect of tilianin on lipid accumulation is a notable discovery.

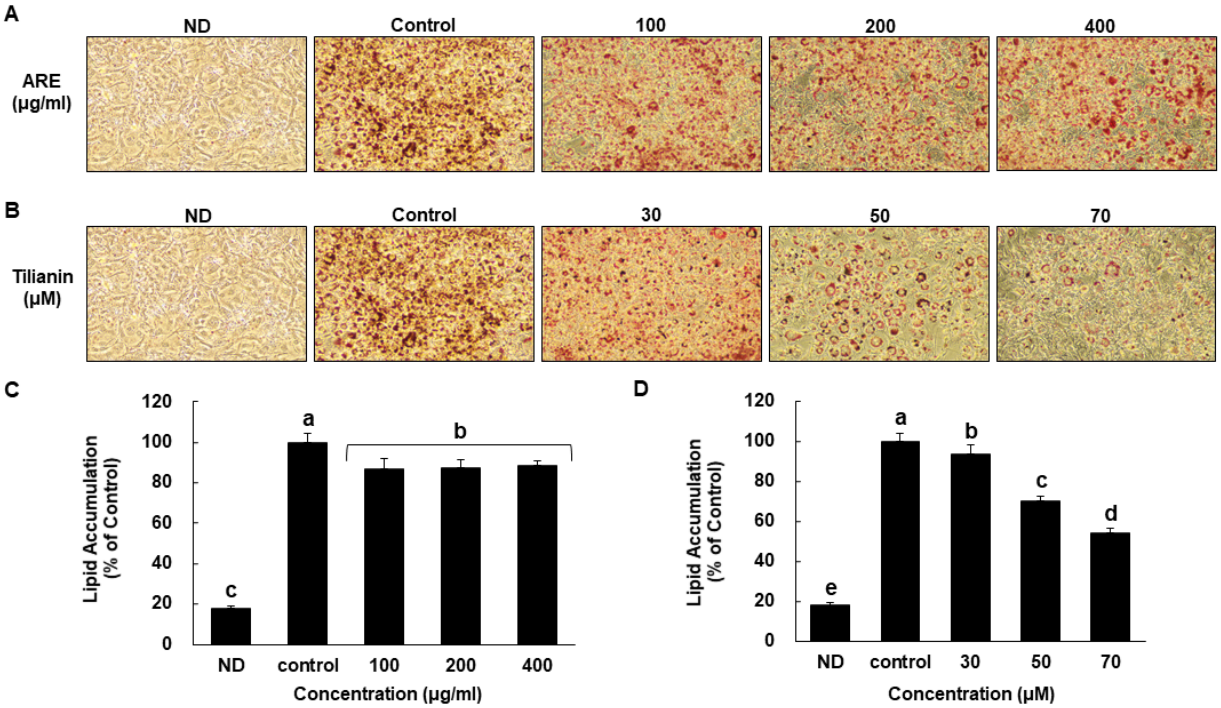

Figure 3. Antiadipogenic effect of ARE and tilianin. 3T3-L1 cells were seeded in 12 well and oil-red-O staining was assessed after treated with differentiation media. With DMSO, oil-red-o was dissolved and the absorption was estimated using spectrophotometer (OD_{675}). Effect of (**A**). ARE and (**B**). tilianin on adipogenic differentiation. Lipid accumulation (% control) quantification in the (**C**). ARE-, (**D**). tilianin-treated groups. ND, nondifferentiated; Control, untreated. Data are means of n = 6. Different letters (a–e) in each graph indicate significantly different values in samples ($p < 0.05$, ANOVA Duncan's test).

3.4. Anti-Adipogenic Effect of ARE and Tilianin on the mRNA and Protein Expression Level

PPAR-γ is a well-known master regulator of adipogenesis and is responsible for inducing the transcription of C/EBPα [23]. We can thus infer that diminution in PPAR-γ expression causes decreased levels of C/EBPα transcription, which was supported by the results of our experiments with ARE and tilianin treatments (Figure 4A,B). The mRNA levels of the three factors contributing to adipogenesis, PPAR-γ, C/EBPα and FABP4, were slightly lowered by treatment with ARE and significantly lowered by treatment with tilianin.

Expressed protein levels of PPAR-γ and C/EBPα were decreased with ARE concentration in dependent manner (Figure 4C). Our results also clearly confirmed the suppression of tilianin on PPAR-γ protein expression level (Figure 4D). As tilianin concentration increases, the levels of PPAR-γ decreased. Even though these levels were higher in cells treated with 30 μM tilianin compared with control cells. Since the Western blotting was performed with a few time points, it is possible that PPAR-γ levels varied with time.

3.5. Anti-Lipogenic Effect of ARE and Tilianin on mRNA and Protein Expression Level

Lipogenesis includes fatty acid biosynthesis involving the transcription factor SREBP1c and enzymes, such as ACC and FAS. Results in Figure 5A indicate that ARE treatment downregulated fatty acid synthesis and thus inhibited lipogenesis. As shown in Figure 5B, tilianin dose-dependently caused significant changes in the mRNA levels of the lipogenesis-related genes such as SREBP1, ACC and FAS in dose dependent manner. Figure 5D,E indicated the expression levels of SREBP1 protein regulated by ARE and tilianin, respectively. In addition, SREBP1 protein levels showed the same relationship with ARE and tilianin concentrations. Overall, ARE exhibits both anti-adipogenic and anti-lipogenic effects, which could be influenced by tilianin, a major component of ARE.

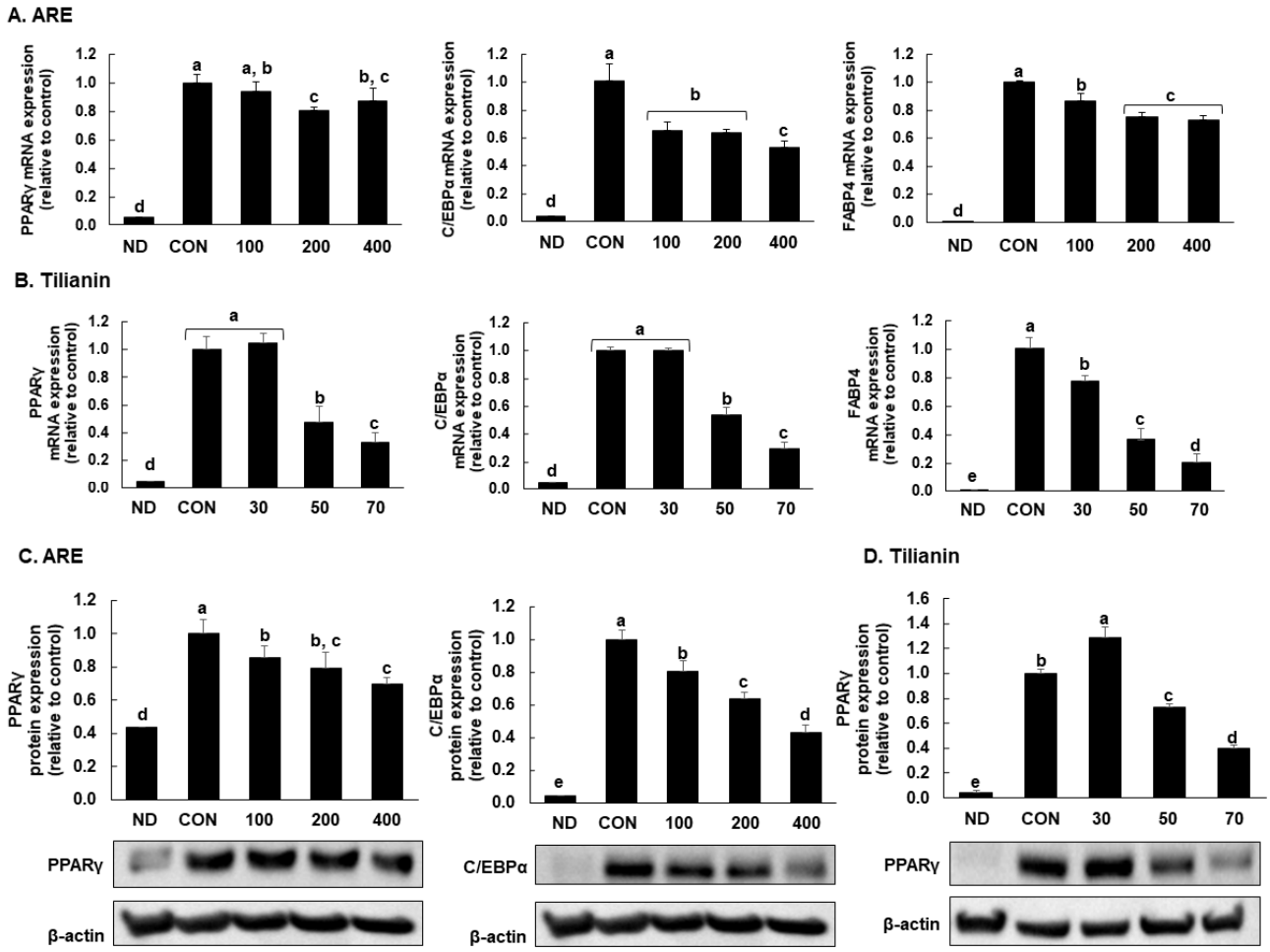

Figure 4. Anti-adipogenic effect of ARE and tilianin on mRNA and protein level of 3T3-L1 cells. (**A**). ARE-treated mRNA expression data (n = 3); (**B**). tilianin-treated mRNA expression data (n = 4). (**C**). ARE-treated protein expression data (n = 3). (**D**). Tilianin-treated protein expression data (n = 3). Different letters (a–e) in each graph indicate significantly different values in samples ($p < 0.05$, ANOVA Duncan's test).

3.6. Lipolytic Effect of Tilianin on mRNA Expression Level of 3T3-L1 Cells

Additionally, tilianin treatment dose-dependently increased the mRNA levels of carnitine palmitoyl transferase-1 (CPT1), a key oxidizer of fatty acids. Transportation of fatty acids into the mitochondria for oxidation is controlled by CPT1. There are three isoforms of CPT1—A, B and C—with CPT1A being the isoform expressed in white adipose tissue [24]. In the control group which is induced adipogenic differentiation, it was observed that the expression of CPT1 decreased. Meanwhile, the expression of CPT1 increased again by treatment with tilianin (Figure 5C).

Based on these results, ARE and tilianin both have anti-adipogenic and antilipogenic effects based on decreased PPAR-γ, C/EBPα, FABP4, SREBP1, ACC and FAS mRNA levels upon treatment, with tilianin also causing increases in CPT1A mRNA levels.

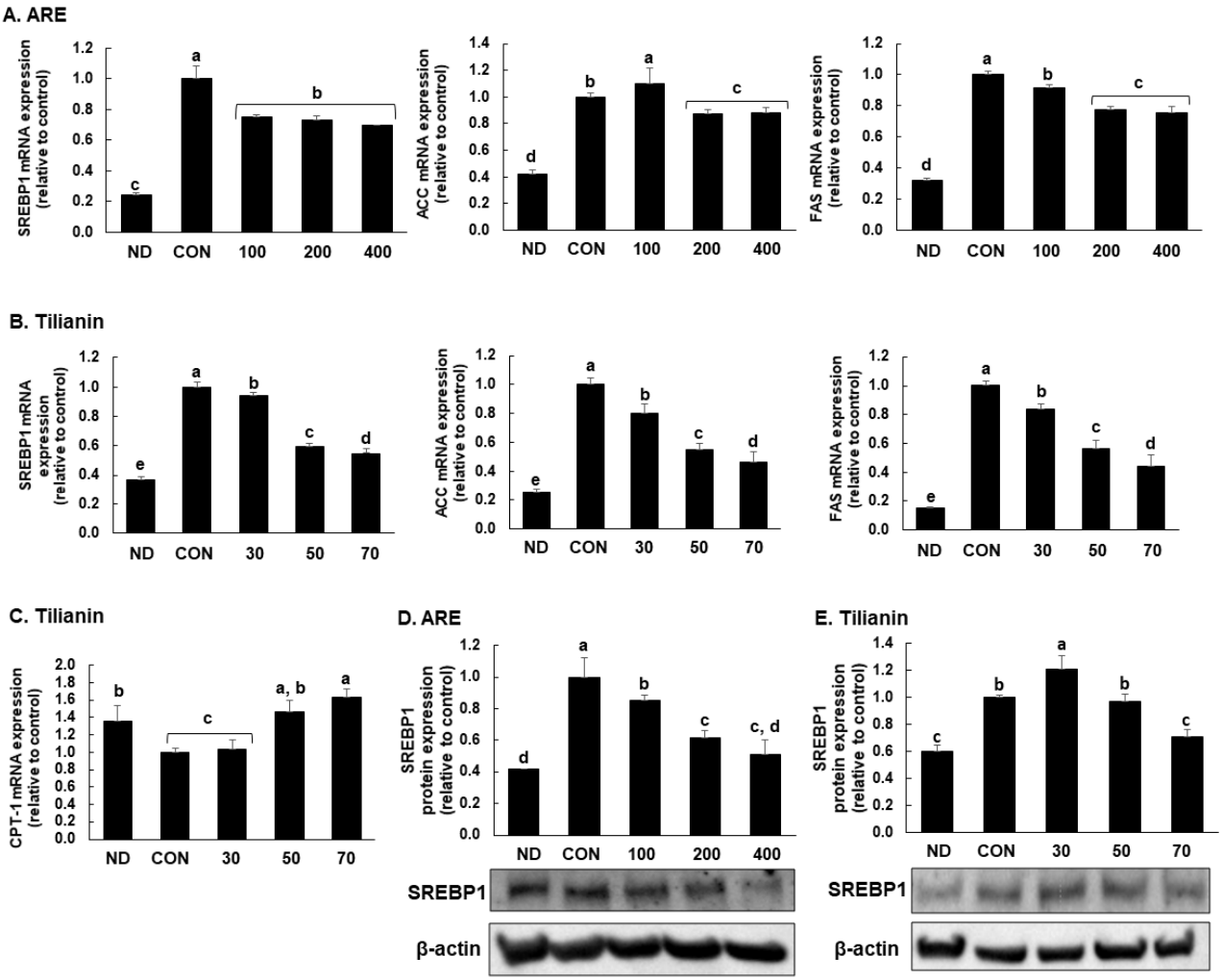

Figure 5. Anti-lipogenic effect of ARE and tilianin on mRNA and protein level and lypolytic effect of tilianin on mRNA level of 3T3-L1 cells. (**A**). mRNA expression of ARE-treated group (n = 3). (**B**,**C**). mRNA expression of tilianin-treated group (n = 4). (**D**). SREBP1 protein expression of ARE-treated group (n = 3). (**E**). SREBP1 protein expression of tilianin-treated group (n = 3). Different letters (a–e) indicate significantly different values in samples ($p < 0.05$, ANOVA Duncan's test).

4. Discussion

Tilianin, a bioactive compound of *Agastache rugosa*, has been reported to have various functions as mentioned in introduction. However, the anti-obesity effect of tilianin remains unclear. *A. rugosa*, which has antifungal, antibacterial and antipyretic effects [25], also showed anti-adipogenic properties in this study. We evaluated the anti-adipogenic effects of ARE and tilianin using 3T3-L1 cells.

Tilianin is plant-derived flavonoids abundant in *A. rugosa*. Flavonoids exhibit antioxidant activities and play various biological and pharmaceutical roles as plant secondary metabolites. Moreover, some research suggest that plant-derived flavonoids are endowed with obesity prevention or treatment [26,27]. The effects of flavonoids on obesity that recently researched are: diminution of food intake and regulation of appetite; less intestinal fat absorption; control of adipocyte differentiation, adipogenesis and lipolysis [28].

Here, ARE and its flavonoid compound tilianin were clearly shown to have anti-obesity effects on 3T3-L1 adipocytes. More prominently, tilianin dose-dependently suppressed lipid accumulation in differentiated adipose cells. Lipid accumulation (%) is calculated by relative oil-red-o absorbance (485 nm) that eluted by treating 2-propanol on colored cells. Oil-red-O dyes lipid droplets which is known to be composed of neutral lipids, triglyceride and sterol esters. Most of the lipid droplets in white adipose tissues

consists of triglyceride [29]. Differentiated 3T3-L1 adipocytes which behaves similar to white adipose tissue form lipid droplets full of triglyceride. Therefore, we can conclude that lipid accumulation decreases, triglyceride content would decrease as well. Especially tilianin showed remarkable effects than ARE in lipid accumulation and mRNA expression levels. It can be assumed that single compounds may have more clear effects because it is a highly refined matter. The possibility cannot be ruled out that ARE is consisted of various compounds so other components may have offset the anti-obesity effects in ARE.

Inhibition of lipid droplet accumulation in adipocytes can have a variety of causes. Madsen et al. demonstrated that the key adipogenic transcription factors, PPARγ and C/EBP*a*, cooperate with the activation of adipocyte gene programs to result in adipogenic differentiation [30]. According to the related studies to date, it can be hypothesized that ARE and tilianin inhibit the differentiation of preadipocytes into adipocytes. In order to investigate the inhibitory activity of ARE and tilianin on the expression of PPAR-γ and C/EBPα, the expression of these factors was observed at the gene and protein level, and it was confirmed that the expression was reduced. Therefore, the hypothesis can be demonstrated that the inhibition of fat accumulation by ARE and tilianin is due to the inhibition of the adipogenic differentiation by these bioactive substances.

Our results showed that tilianin treatments clearly appeared significant diminution of the mRNA levels of the adipogenesis-related genes PPAR-γ, C/EBPα and FABP4 and the lipogenesis-related genes SREBP1, ACC and FAS. Additionally, the levels of CPT1, a lipolysis factor, increased following tilianin treatment. ARE exhibited anti-obesity effects, with ARE treatment causing decreases in lipid droplets by 20% compared with untreated cells and causing decreases in mRNA and protein levels of adipogenesis- and lipogenesis-related genes.

In particular, in the expression of PPAR-γ and SREBP1, tilianin showed an increased expression level compared to the control at a concentration of 30 μM (Figures 4D and 5E). In addition, as almost no effect was observed on the mRNA expression of CPT1 at a concentration of 30 μM (Figure 5C). Thus, it can be assumed that the threshold dose of tilianin in the 3T3 cell line is 30 μM or more.

Several studies have shown that other single compounds which can found in *Agastache rugosa* exert anti-obesity properties. For example, acacetin has anti-adipogenic effects on 3T3-L1 adipocytes and reduce lipid accumulation in high-fat-diet induced mice [21]. In addition, another component, rosmarinic acid, has been found to attenuate obesity in human adipocytes and HepG2 cells [31,32]. However, there are insufficient research on tilianin to date. Consequently, we have filed a patent regarding the results obtained in this study. Still, several experiments are remaining to explore. For example, to deeply understand our results at an in vitro level, other obesity factors should be investigated, such as the mitogen-activated protein kinase and extracellular-signal-regulated kinase pathway which are known to be closely related to triglyceride and hormones [33,34]. Moreover, research about mechanism of action should be investigated. Additionally, mitotic research could produce meaningful results, and lastly, in vivo experiments are required to clarify effects of tilianin and ARE on animals.

Natural products are more easily accessible than pharmaceutical products. In addition, there are many different dietary supplements; however, ARE does not occupy a large portion of the dietary supplement market. Through this study, the anti-obesity function of ARE and that of its major bioactive compound tilianin are shown, revealing their potential use as components for dietary products.

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Conflicts of Interest: The authors declare no conflict of interest.

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