Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.5327/fst.114222

Effect of *Centella asiatica* extract on anti-obesity suppression via inhibition of adipogenesis-related gene expression in preadipocyte

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Abstract

The present study was conducted to investigate the anti-oxidant and anti-obesity activities of *Centella asiatica* hot-water extract (CHE). Total polyphenol content and radical scavenging activity were evaluated for the anti-oxidant activity of CHE, and 14.4 mg GAE/g DM and 82.6% were measured, respectively. Lipase activity inhibition, an anti-obesity marker, was measured as 68.1%, confirming that triglyceride hydrolysis was significantly inhibited by CHE. To study the anti-obesity mechanism, mRNA expressions of peroxisome proliferator activated receptor-γ (*PPAR-γ*), CCAAT enhancer binding protein-α (*CEBP-α*), fatty acid synthase (*FAS*), and stearoyl-CoA desaturase1 (*SCD1*) in 3T3-L1 were evaluated. The level of mRNA expression was significantly suppressed by 1.52, 1.81, 1.13, and 1.18 times, respectively, compared to the control group, confirming that CHE had an anti-obesity effect by inhibiting adipocytes development and lipid accumulation. These results indicate that CHE can be used as a raw material for functional foods and pharmaceuticals with anti-oxidant and anti-obesity potential by reducing lipase activity and preadipocyte differentiation.

Keywords: *Centella asiatica*; 3T3-L1; anti-oxidant; anti-obesity; functional foods.

Practical Application: Overall, the results suggest that Centella asiatica hot-water extract (CHE) holds promise as a natural ingredient for the development of functional foods and pharmaceuticals targeting antioxidant and anti-obesity effects. Its ability to inhibit lipase activity and suppress adipocyte development and lipid accumulation makes it a valuable candidate for further research and product development in these areas.

1 Introduction

The World Health Organization defines obesity as a non-communicable disease that requires long-term and active treatment; moreover, it is a chronic condition that poses a serious threat to human health in the 21st century (King et al., 2021). There are different weight-management techniques to treat obesity, including exercise, dietary control, surgery, and medication (Salari et al., 2021). Among these, medication is widely utilized along with exercise, but anti-obesity medicines are usually accompanied by adverse effects and they vary in terms of effectiveness (Janakiraman et al., 2022). Xenical, a Food and Drug Administration-approved anti-obesity medicine, is a triglyceride analog that binds to the active site of triglyceride hydrolase to limit the formation of enzyme–substrate complexes (Zhang et al., 2020). However, frequent bowel movements, oily evacuation, oily rectal leakage, steatorrhea, and cardiovascular diseases have been reported as the common adverse effects of this medication. Overcoming these adverse effects is critical for the development of anti-obesity medicines, and various studies are being conducted to replace semtraditional drugs such as Xenical, Dexatrim, and Redux (Yildiz et al., 2021).

Obesity is induced by a combination of social, environmental, and hereditary features including cultural milieus, living environment, and eating habits (Santos et al., 2019). Additionally environmental factors, including ultraviolet (UV) radiation, air pollution, and chemical exposure, increase the risk of obesity by interfering with the obesity-related genes (Golden & Kessler, 2020). Numerous studies have found that obesity is induced by an imbalance in the bodily metabolic processes due to damaged proteins, lipids, and genes caused by reactive oxygen species (ROS). Remarkably, ROS production is triggered by stress, UV radiation, smoking, pollution, and an unhealthy diet. The ROS generated during biochemical reactions such as respiration and photosynthesis in mitochondria are eliminated by antioxidant enzymes, including glutathione peroxidase, catalase, and superoxide dismutase, which are present as intracellular defense systems or antioxidants in food, such as vitamins, tocopherol, catechin, retinol, resveratrol, and glutathione (Ba et al., 2022). Recent studies have reported a correlation between obesity and antioxidant levels. Specifically, the body mass index, which is a measure of obesity, reportedly decreases considerably as the levels of the main antioxidants increased in the blood of obese patients (Bonakdar et al., 2019).

In patients with obesity, the increase in adipocytes during lipid biosynthesis via preadipocyte differentiation causes hypertrophy and hyperplasia (White & Ravussin, 2019). When this differentiation is induced, cell proliferation halts due to the cell cycle arrest, and the proliferation of mature adipocytes, known as adipogenesis, occurs (Lee et al., 2018). It has been established that the suppression

Received 01 Nov., 2022
Accepted 26 Dec., 2022
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of preadipocyte development is a very successful strategy to prevent and treat obesity (Khandagale et al., 2022). The processes of preadipocyte initiation and differentiation involve numerous signaling pathways as well as multiple transcription factors and genes, such as isobutylmethylxanthine (IBMX), dexamethasone (DEX), and insulin (Choi et al., 2021). Various studies have demonstrated that cyclic adenosine monophosphate (cAMP) and the sterol regulatory element-binding protein-1c (*SREBP-1c*) are important transcription factors for lipid and glucose metabolism gene regulation. When the level of cAMP is increased by DEX during the early stage of differentiation, *SREBP-1c* expression is induced and the peroxisome proliferator activated receptor-γ (*PPAR-γ*) and CCAAT enhancer binding protein-α (*CEBP-α*) are mutually expressed. This promotes preadipocytes with fatty acid synthase (*FAS*) and stearoyl-CoA desaturase 1 (*SCD1*), enzymes involved in the synthesis of triglycerides from acetyl-CoA and malonyl-CoA (Sordi et al., 2021).

Centella asiatica is an herbaceous perennial plant of the *Apiaceae* family distributed in wetlands in Brasil, Uruguay, Portugal, and Southeast Asia (Kant et al., 2019). It contains therapeutic compounds such as madecassoside, madecassic acid, and asiaticoside, which are reported to have wound-healing, anti-inflammatory, antioxidant, and anticancer effects (Tripathy et al., 2022). However, studies on the inhibition of preadipocyte differentiation and anti-obesity mechanisms of *C. asiatica* extract have not been widely conducted. Therefore, in this study, the antioxidant and lipase inhibiting activities of the *C. asiatica* hot-water extract (CHE) were investigated to evaluate the plant's anti-obesity effect. Furthermore, to elucidate the anti-obesity mechanism of the extract, the effects on the mRNA expressions of *PPAR-γ*, *CEBP-α*, *FAS*, and *SCD1*, which are major genes involved in preadipocyte differentiation were evaluated. The purpose of this study was to demonstrate the CHE treatment significantly decreased both *PPAR-γ* and *C/EBP-α* expressions, thereby affecting the expression of *FAS* and *SCD1*, sub-factor in the adipogenesis pathways.

2 Materials and methods

2.1 Preparation of CHE

C. asiatica was purchased from a local farm in Hongcheon (Gangwon-do, Korea) in 2021 and dried at 60 °C for 24 h in a forced convection oven (VS-1202D4N, Vision Bionex, Buchoen, Korea). Dried *C. asiatica* was pulverized using a food processor (HMF-3000S, Hanil, Seoul, Korea) and then passed through a 25 mesh sieve to collect particles less than 0.71 mm. The extraction was performed at 60 °C for 30 min using an ultrasound extractor (SD-250H, Mujigae Co., Seoul, Korea) after adding 10 mL of distilled water to 1.0 g of *C. asiatica*. Then, CHE was centrifuged at 2,800 g for 10 min (Labogene 1236R, Gyrozen Co., Daejeon, Korea) and stored at –21 °C.

2.2 Total Polyphenol Content (TPC) assay

TPC was determined by the modified Folin-Ciocalteu method (Semeniuc et al., 2018). 0.14 mL of CHE was mixed with 0.7 mL of 0.2 N Folin-Ciocalteu reagent (Sigma-Aldrich, St Louis, MO., USA). After 8 min, 0.56 mL of 7.5% Na2CO3 solution was added to the mixture. The absorbance was measured at 765 nm by a UV-vis spectrophotometer (Optizen 2120UV, Mecasys Co., Daejeon, Korea) after 60 min of reaction. The TPC was calculated on the basis of the calibration curve using gallic acid (Sigma-Aldrich, St Louis, MO., USA) and expressed as mg gallic acid equivalents (GAE)/g of dried matter (DM).

2.3 Radical Scavenging Activity (RSA) assay

RSA was assayed using the method by Yeom et al. (2022). 0.01 mM of DPPH (Sigma-Aldrich, St Louis, MO., USA) in methanol was prepared and 0.25 mL of CHE was added to 1.25 mL of DPPH solution. Then the reaction was carried out for 20 min in the dark condition at 25 °C. The absorbance of reactant was measured at 517 nm and RSA was calculated according to the below Equation 1.

$$\mathrm{RSA}(\%) = \left\{1 - \frac{\mathrm{Abs\ (sample)}}{\mathrm{Abs\ (control)}}\right\} \times 100 \quad (1)$$

2.4 Lipase Activity Inhibition (LAI) assay

Measurement of LAI was performed according to the modified method by Gam et al. (2021). Reaction mixture consisted of 0.1 mL of CHE, 0.2 mL of substrate solution (10 mM of *p*-nitrophenyl butyrate), and 0.2 mL of lipase. Enzymatic reactions were allowed to proceed at 37 °C for 30 min. LAI was determined by measuring the hydrolysis of *p*-nitrophenyl butyrate to *p*-nitrophenol at 405 nm. LAI was calculated according to the below Equation 2.

$$\mathrm{LAI}(\%) = \left\{1 - \frac{\mathrm{Abs\ (sample)}}{\mathrm{Abs\ (control)}}\right\} \times 100 \quad (2)$$

2.5 Measurement of preadipocyte differentiation

Media and reagents used for cell culture and differentiation include Dulbecco's modified eagle's medium (DMEM), new born calf serum (NBCS), fetal bovine serum (FBS), and 0.05% trypsin-EDTA were purchased from Thermo Fisher Sci. (Waltham, MA., USA). Preadipocyte with the inoculum density of 2.5×10^4 cells/mL was cultured in DMEM supplemented with 10% NBCS, 1% penicillin, and MDI (0.5 mM IBMX, 1.0 mM DEX, and 1.0 μg/mL insulin), and cultured in CO_2 incubator (MCO-18AIC, Sanyo, Osaka, Japan) set at 37 °C with 5.0% CO_2. After 48 h, cells were fed with DMEM containing 10% FBS and 10 μg/mL insulin and cultured for an additional 48 h. Subsequently, the media was replaced with DMEM containing 10% FBS every 48 h.

2.6 Cell viability assay

The effect of CHE on the cell viability was determined using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (Hong et al., 2022). 3T3-L1 preadipocyte was cultured in 96-well plate and treated with concentration of 0.0 ~ 4.0 mg/mL after 24 h of inoculation. After 48 h of CHE treatment, 0.1 mL of MTT solution (0.25 mg/mL) was added and incubated at 37 °C for 4 h. Thereafter, the supernatant was

completely removed, and 0.1 mL of DMSO was added to dissolve the formazan crystal. Absorbance was read at 540 nm using a microplate reader (AMR-100, Allsheng, Seoul, Korea) and cell viability was calculated according to the following Equation 3.

$$\text{Cell viability}(\%) = \left\{1 - \frac{\text{Abs (sample)}}{\text{Abs (control)}}\right\} \times 100 \quad (3)$$

2.7 Lipid accumulation assay

Lipid accumulation of differentiated 3T3-L1 preadipocyte was measured by oil red O staining. The cells were washed with phosphate-buffered saline (PBS) and fixed with 10% formaldehyde solution for 30 min. Then, the cells were stained with oil red O solution for 60 min and washed with distilled water. The stained lipids were eluted with 99.5% isopropanol and measured using an UV-vis spectrophotometer at 497 nm by the following Equation 4.

$$\text{Lipid accumulation}(\%) = \left\{1 - \frac{\text{Abs (sample)}}{\text{Abs (control)}}\right\} \times 100 \quad (4)$$

2.8 Measurement of anti-obesity gene expression

RT-PCR was performed to determine the mRNA level of the *CEBP-α*, *PPAR-γ*, *FAS*, and *SCD1* anti-obesity genes in 3T3-L1 preadipocytes. Cells were cultured at an inoculum density of 3×10^5 cells/mL in a T-25 cell culture flask (SPL Life Sci., Seoul, Korea). After harvesting the cells, the total RNA was extracted using the AccuPrep® universal RNA extraction kit (Bioneer Co., Daejeon, Korea) and was reverse transcribed into cDNA using the amfiRivert cDNA synthesis platinum master mix (GenDEPOT Co., Greenville, SC., USA). Subsequently, cDNA was amplified using specific primers for the *CEBP-α*, *PPAR-γ*, *FAS*, and *SCD1* genes (Table 1). The PCR conditions were set as follows: initial denaturation at 94 °C for 5 min, followed by 30 cycles at 95 °C for 5 sec, 60 °C for 30 sec (*CEBP-α*), 59 °C for 30 sec (*PPAR-γ*), 54 °C for 60 sec (*FAS* and *SCD1*), and 72 °C for 60 sec. Each PCR product was subjected to electrophoresis on a 1.5% agarose gel and the intensity of bands was visualized using the Gel Doc™ XR + system (Bio-Rad Co., Richmond, CA., USA).

2.9 Statistical analysis

Data were expressed as the mean ± standard deviation (SD) for all experiments, and probabilities (p) of chance difference between groups were calculated according to Student's t-test. A statistically significant test means that the test hypothesis is false or should be rejected and the criteria were set at $p < 0.05$.

3 Results and discussion

3.1 Effect of the CHE on TPC and RSA

ROS are normal cellular metabolic byproduct of living cells and are readily eliminated by catalase and peroxidase to maintain homeostasis (He et al., 2021). However, under intense stress conditions, cells produce excessive ROS above the enzyme-induced homeostasis levels, resulting in lipid metabolic disorder, protein denaturation, and DNA damage, which may lead to obesity (Tam et al., 2020). Therefore, current research is the field is increasingly focusing on discovering natural antioxidants and developing an efficient extraction technology. In this study, the CHE was obtained using the ultrasound-assisted extraction (UAE) method to produce bioactive compounds with high antioxidant activity. The extraction was carried out at 60 °C and for 30 min based on the method reported by Ha et al. (2010). Under these conditions, the values of TPC and RSA, which are markers of antioxidant activity, were 14.4 ± 0.14 mg GAE/g DM and 82.6%, respectively, 1.3 times higher than the values reported by Shin et al., in a previous extraction of *C. asiatica* (Shin et al., 2020). These results indicated that polyphenol extraction via UAE produced strong antioxidant compounds and is a useful method to obtain extracts from natural sources.

Reactive oxygen species (ROS) could lead to the dysfunction of mitochondria by inhibiting the aerobic respiration process and, resulting in reduced energy expenditure in adipocytes (Wang et al., 2022). In addition, in hypoxic conditions, reactive nitrogen species (RNS) may also be produced during the respiratory chain reaction, and RNS may further lead to the production of reactive species such as reactive aldehydes, malondialdehyde, and 4-hydroxynonenal (Li et al., 2022). Excessive levels of ROS and RNS can cause damage to the adipocyte cellular structure and functions which may induce obesity and metabolic syndrome. Then, excessive production of ROS in adipocytes and adipose tissues may be deleterious if not removed quickly. Polyphenols can interact with ROS & RNS and thus terminate chain reaction before damage to adipocytes and adipose tissues (Colitti et al., 2019).

3.2 Effect of the CHE on LAI

Over 90% of dietary fat is composed of triglycerides and is micellized by pancreatic lipase, which hydrolyzes them into fatty

Table 1. Primer sequences used in reverse transcription-polymerase chain reaction (RT-PCR) of major genes related to anti-obesity.

Obesity-related genes	Forward primers (5'-3')	Reverse primers (5'-3')
[1)]***PPAR-γ***	ATT CTG GCC CAC CAA CTT CGG	TAA GAC CGG GTG GTT GAA GCC
[2)]***CEBP-α***	GGT TTA GGG ATG TTT GGG TTT T	CCA AAT CCC TAC AAA CCC AAA A
[3)]***FAS***	CCC TGA AAT CCC AGC ACT TC	GGG ACT CCA TTT TCG GCA AG
[4)]***SCD1***	CCG TGA AAT CCC AGC ACT TC	GGC ACT TTA GGG TCG GCA AG
β-actin	AAC GAC TAG GTG GAG ACG GT	TTG CTG ATC CAC ATC TGC TG

[1)]*PPAR-γ*: peroxisome proliferator activated receptor-γ. [2)]*CEBP-α*: CCAAT enhancer binding protein-α. [3)]*FAS*: fatty acid synthase. [4)]*SCD1*: stearoyl-CoA desaturase 1.

acids and monoglycerides, facilitating intestinal absorption and leading to obesity through the increased triglyceride metabolism (Zuin et al., 2022). Consequently, lowering lipase activity is expected to have an anti-obesity effect, resulting in a reduced generation of fatty acids through the inhibition of triglyceride hydrolysis. Therefore, for selection of effective anti-obesity medication candidates, evaluating the inhibition of lipase activity is an effective strategy to prevent lipid accumulation through the decrease in fatty acid absorption.

LAI is used as an important indicator to evaluate anti-obesity effects and it reached 68.1% in this experiment, which was 3.1 times higher than the value obtained for the hot-water extract of *Aceriphyllum rossii* leaves (21.9%), indicating that *C. asiatica* extract obtained via ultrasound can be used as an excellent anti-obesity agent to inhibit triglyceride hydrolysis (Lim et al., 2010). According to Gam et al., the mechanism underlying the anti-obesity effect of peanut shell extracts is due to the presence of polyphenol acting as an enzyme inhibitor for lipase, which in turn inhibits triglyceride hydrolysis and absorption (Gam et al., 2021). The lipase inhibitor, Orlistat is a commercially available medicine used to treat obesity that has a structure similar to that of triglycerides and acts as an inhibitor of triglyceride hydrolysis by competing with lipase (Shirai et al., 2019). Similar to Orlistat, the CHE's polyphenols are believed to exert anti-obesity effects by decreasing lipase activity through the competitive inhibition of triglycerides. Additional experiments need to be conducted to confirm the decrease in preadipocyte development following CHE treatment, as well as the effect of suppressing anti-obesity gene expressions to, validate the anti-obesity effect of the extract.

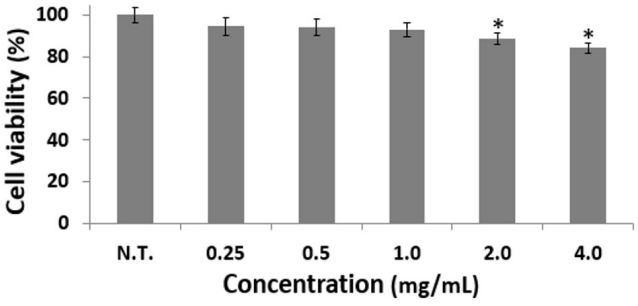

Figure 1. Effect of the CHE (0.0 ~ 4.0 mg/mL) and Non treated group (N.T.) on cell viability in 3T3-L1 preadipocytes. Bars represent the mean ± standard deviation of three independent experiments and asterisks indicate significant differences from the control (*$p < 0.05$).

3.3 Effect of the CHE on cell cytotoxicity

A cytotoxicity test was conducted to evaluate the effect of the CHE on adipocyte differentiation and triglyceride accumulation in 3T3-L1 cells (Figure 1). When preadipocytes were treated with the extract at concentrations from 0.0 to 4.0 mg/mL, cell viability significantly decreased as the concentration increased above 2.0 mg/mL ($p > 0.05$). Conversely, at a concentration of 1.0 mg/mL, cell viability was 92.8%, verifying that there was no inhibitory effect on the growth of preadipocytes in the treatments at or below 1.0 mg/mL. The CHE was shown to be less cytotoxic than conventional natural products, confirming that it is safer to use as a preadipocyte inhibitor. Therefore, in subsequent experiments, to evaluate the CHE's ability to inhibit preadipocyte differentiation and anti-obesity gene expression, the maximum concentration of the extract at which cell growth was not inhibited was set at 1.0 mg/mL. A previous study by Jeon *et al.*, where adipocytes were treated with 1.0 mg/mL of *Plantago asiatica* extract, reported a cell survival rate of 18.4%, which is lower than that obtained in the present study. Moreover, the extract was considered as a highly safe natural material due to its higher non-toxicity (Jeon et al., 2014).

3.4 Effect of the CHE on lipid accumulation

In this study, oil red O staining was performed to evaluate the effect of the CHE on preadipocyte differentiation. The cytoplasmic lipids accumulated in adipocytes are triglycerides surrounded by a monolayer of phospholipids. These lipid droplets are considered as dynamic and regulated intracellular organelles

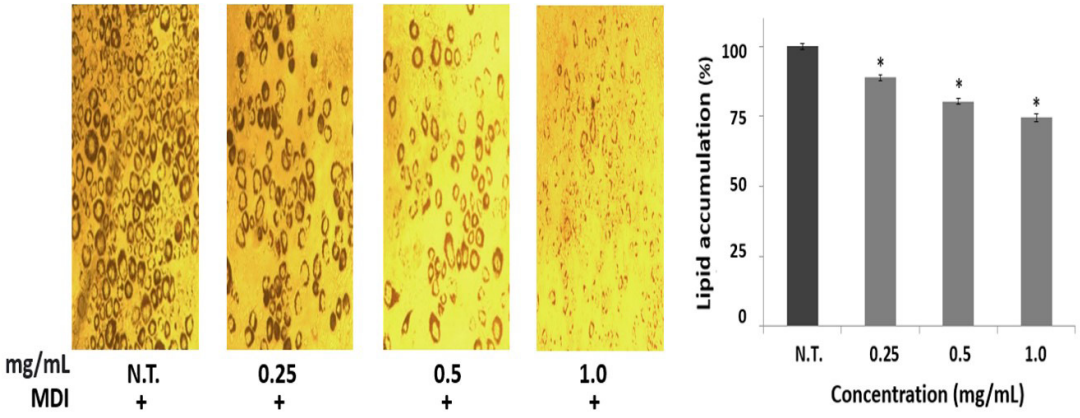

Figure 2. Lipid accumulation of CHE in 3T3-L1 preadipocytes exposed to methylisobutylxanthine, dexamethasone, insulin (MDI) medium Lipid droplets in adipocytes were stained with oil red O and were observed under a ×64 magnification microscope. Bars marked with asterisks indicate significant differences different from the control (*$p < 0.05$).

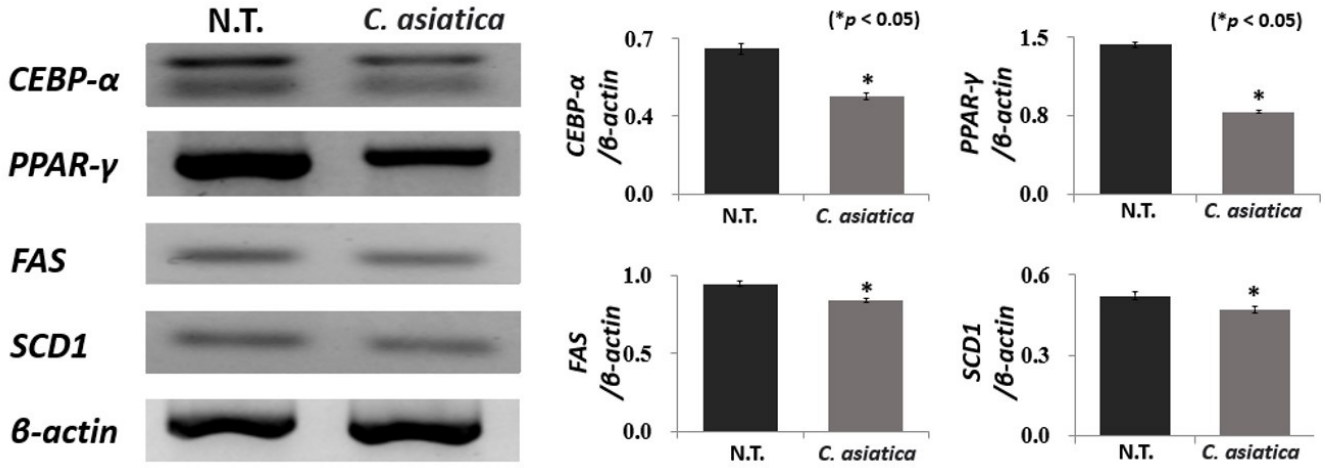

Figure 3. Effect of the CHE on *CEBP-α*, *PPAR-γ*, *FAS*, and *SCD1* mRNA expression levels in 3T3-L1 preadipocytes. Values are expressed as mean ± standard deviation of three experiments. Bars marked with asterisks are significantly different from the control (*$p < 0.05$).

that play an active role in fatty acid storage and mobilization (Pereira-Dutra et al., 2019). Triglycerides are selectively stained with oil red O, whereas phospholipids and free fatty acids remain unstained; therefore, oil red O staining allows to compare the degree of preadipocyte differentiation (Cheng et al., 2020).

As illustrated in Figure 2, the CHE treatment reduced lipid accumulation in a concentration-dependent manner specifically, by 1.26 times at 1.0 mg/mL, which is the concentration with no inhibitory effects on cell growth. This is consistent with the results reported in Kang et al. which indicated that adipocyte accumulation decreased when preadipocytes were treated with bellflower and turmeric extracts at a concentration ≤ 1.0 mg/mL (Kang et al., 2015).

Recent research showed that plant-derived polyphenols exert anti-obesity effects on preadipocyte differentiation and lipid accumulation by regulating the expression of *CEBP-α* and *PPAR-γ*, which are early transcription factors involved in preadipocyte differentiation. Considering this, it is hypothesized that polyphenols in the CHE contribute to inhibiting preadipocyte differentiation by regulating key adipogenesis genes (Stefania et al., 2021). Additionally, to confirm the mechanism underlying the extract's anti-obesity effect, it was necessary to evaluate the expression of major genes involved in preadipocyte differentiation.

3.5 Effect of CHE on anti-obesity gene expression

During preadipocyte differentiation, the transcription factors of the CCAAT enhancer binding protein (CEBP) promote adipocyte differentiation, while *CEBP-α* a key regulator of the glucose and lipid metabolisms in the liver is expressed by hormones such as IBMX, DEX, and insulin (Lin et al., 2021). Subsequently, *PPAR-γ*, a major gene regulating preadipocyte differentiation is expressed and the cell cycle is arrested again by the adipocyte induction complex (Kim et al., 2020). Furthermore, the interaction between *CEBP-α* and *PPAR-γ* induces the expression of *SCD1* and *FAS*, the enzymes involved in adipocyte differentiation and lipid synthesis, thus that intracellular lipids are accumulated and preadipocyte differentiation is completed (Gouthamchandra et al., 2019). It has been shown that reducing the excessive accumulation of triglycerides, which is the cause of obesity, is useful to treat this condition and can be achieved by modulating the expression of main factors involved in lipid precursor differentiation.

The present study, evaluated of the effect of the CHE on the mRNA expressions of *CEBP-α*, *PPAR-γ*, *FAS*, and *SCD1*. As shown in Figure 3, these were inhibited during the 1.0 mg/mL treatment by 1.52, 1.81, 1.13, and 1.18 times, respectively, compared to the control group. The inhibition of *CEBP-α* expression in preadipocytes via CHE treatment was 2.2 times higher than that reported in Lee et al., where it was demonstrated that the inhibition of preadipocyte differentiation produced anti-obesity effects (Hwang et al., 2014). Additionally, Wang et al. (1995) confirmed that lipid accumulation did not occur when *CEBP-α* was eliminated from the in vivo model, further demonstrating that the inhibition of this gene is essential to obtain the anti-obesity effect as it upregulates preadipocyte differentiation (Wang et al., 1995). According to previous reports, *CEBP-α* is essential in the differentiation of preadipocyte through interaction with PPAR-γ. The present study demonstrates that the CHE treatment significantly inhibited both the *CEBP-α* and *PPAR-γ* expressions, thereby affecting the expression of *FAS*, a sub-factor of *CEBP-α* and *PRAR-γ*. Also, the extract inhibited the expressions of *CEBP-α*, *PPAR-γ*, *FAS*, and *SCD1* genes, which are involved in preadipocyte differentiation and it was confirmed that the accumulation of triglycerides in adipocytes could be inhibited by effectively suppressing *CEBP-α* and *PRAR-γ*.

4 Conclusion

This study measured the effect of the CHE on TPC, RSA, and LAI to confirm its antioxidant and anti-obesity effects. Additionally, the expressions of the *CEBP-α*, *PPAR-γ*, *FAS*, and *SCD1* genes, which are essential for preadipocyte differentiation, were assessed to further confirm the mechanism underlying the

anti-obesity effect. The CHE-treated TPC, RSA, and LAI showed values of 14.4 mg GAE/g DM, 82.6%, and 68.1%, respectively. Therefore, the extract could be used as an anti-obesity substance that competitively hinders the binding of triglycerides to lipase and removes ROS, which is one of the causes of obesity, via its antioxidant activity. During the treatment of preadipocytes at ≤ 1.0 mg/mL, the adipocyte formation was reduced in a concentration-dependent manner, and the anti-obesity effect was confirmed by the reduction of adipocyte accumulation. Additionally, in the treatment group, the expressions of the main anti-obesity genes *CEBP-α*, *PPAR-γ*, *FAS*, and *SCD1*, decreased significantly decreased compared to those in the control group. This research indicated that CHE with high TPC can be used as a natural antioxidant and anti-obesity agent, and it is expected to effectively inhibit the expression of *CEBP-α* and *PRAR-γ*, which is the major gene involved in the anti-obesity effect. Thus, CHE is predicted to have a high industrial application value as a functional food and pharmaceutical material.

References

Ba, L., Cao, S., Ji, N., Ma, C., Wang, R., & Luo, D. (2022). Exogenous melatonin treatment in the postharvest storage of pitaya fruits delays senescence and regulates reactive oxygen species metabolism. *Food Science and Technology*, 42, e15221. http://dx.doi.org/10.1590/fst.15221.

Bonakdar, S. A., Motlagh, A. R. D., Bagherniya, M., Ranjbar, G., Daryabeygi-Khotbehsara, R., Mohajeri, S. A. R., & Safarian, M. (2019). Pre-pregnancy body mass index and maternal nutrition in relation to infant birth size. *Clinical Nutrition Research*, 8(2), 129-137. http://dx.doi.org/10.7762/cnr.2019.8.2.129. PMid:31089466.

Cheng, Y., Pan, X., Wang, J., Li, X., Yang, S., Yin, R., Ma, A., & Zhu, X. (2020). Fucoidan inhibits NLRP3 inflammasome activation by enhancing p62/SQSTM1-dependent selective autophagy to alleviate atherosclerosis. *Oxidative Medicine and Cellular Longevity*, 2020, 3186306. http://dx.doi.org/10.1155/2020/3186306. PMid:33505579.

Choi, S.-I., Kwon, H.-Y., Han, X., Men, X., Choi, Y.-E., Jang, G.-W., Park, K.-T., Han, J., & Lee, O.-H. (2021). Environmental obesogens (bisphenols, phthalates and parabens) and their impacts on adipogenic transcription factors in the absence of dexamethasone in 3T3-L1 cells. *The Journal of Steroid Biochemistry and Molecular Biology*, 214, 105994. http://dx.doi.org/10.1016/j.jsbmb.2021.105994. PMid:34481040.

Colitti, M., Stefanon, B., Gabai, G., Gelain, M. E., & Bonsembiante, F. (2019). Oxidative stress and nutraceuticals in the modulation of the immune function: current knowledge in animals of veterinary interest. *Antioxidants*, 8(1), 28. http://dx.doi.org/10.3390/antiox8010028. PMid:30669304.

Gam, D. H., Hong, J. W., Yeom, S. H., & Kim, J. W. (2021). Polyphenols in peanut shells and their antioxidant activity: optimal extraction conditions and the evaluation of anti-obesity effects. *Journal of Nutrition and Health*, 54(1), 116-128. http://dx.doi.org/10.4163/jnh.2021.54.1.116.

Golden, A., & Kessler, C. (2020). Obesity and genetics. *Journal of the American Association of Nurse Practitioners*, 32(7), 493-496. http://dx.doi.org/10.1097/JXX.0000000000000447. PMid:32658169.

Gouthamchandra, K., Sudeep, H. V., Amritharaj, Lingaraju, H. B., & Prasad, K. S. (2019). SUNCA standardized extract from Helianthus annus L exerts enhanced digestive enzyme activity and subsides obesity through inhibition of C/EBP-α and PPAR-γ expression both in in vitro and in vivo model. *Pharmacognosy Magazine*, 15(66), 538. http://dx.doi.org/10.4103/pm.pm_204_19.

Ha, J.-H., Kwon, M.-C., Kim, S.-S., Jeong, M.-H., Hwang, B., & Lee, H.-Y. (2010). Enhancement of skin-whitening and UV-protective effects of Centella asiatica L. Urban by utrasonification process. *Korean Journal of Medicinal Crop Science*, 18(2), 79-85.

He, Z., Xu, Q., Newland, B., Foley, R., Lara-Sáez, I., Curtin, J. F., & Wang, W. (2021). Reactive oxygen species (ROS): utilizing injectable antioxidative hydrogels and ROS-producing therapies to manage the double-edged sword. *Journal of Materials Chemistry. B, Materials for Biology and Medicine*, 9(32), 6326-6346. http://dx.doi.org/10.1039/D1TB00728A. PMid:34304256.

Hong, J. W., Park, H. Y., Park, J. H., Kim, S. H., Kim, H. A., & Kim, J.-W. (2022). Inhibition of lipase activity and preadipocyte differentiation in 3T3-L1 cells treated with Sargassum horneri extract. *Ocean and Polar Research*, 44(1), 61-67.

Hwang, J.-Y., Wu, Y.-X., Hwang, D.-I., Bae, S.-J., & Kim, T. (2014). Anti-obesity effect of Polygala tenuifolia. *Korean Journal of Food Preservation*, 21(1), 97-106. http://dx.doi.org/10.11002/kjfp.2014.21.1.97.

Janakiraman, S., Uma, K. S., Sasi, S. K., & Balagurusamy, K. (2022). Anti-obesity Siddha medicinal plants-a preclinical review. *International Journal of Pharmaceutical Sciences Review and Research*, 73(1), 53-63. http://dx.doi.org/10.47583/ijpsrr.2022.v73i01.011.

Jeon, S. Y., Park, J. Y., Shin, I., Kim, S. O., An, H. D., & Kim, M. R. (2014). Ethanol extract of Plantago asiatica L. controls intracellular fat accumulation and lipid metabolism in 3T3-L1 Adipocytes. *The Korea Journal of Herbology*, 29(4), 77-82. http://dx.doi.org/10.6116/kjh.2014.29.4.77.

Kang, Y. H., Kim, K. K., Kim, T. W., Yang, C. S., & Choe, M. (2015). Evaluation of the anti-obesity activity of Platycodon grandiflorum root and Curcuma longa root fermented with Aspergillus oryzae. *Korean Journal of Food Science Technology*, 47(1), 111-118. http://dx.doi.org/10.9721/KJFST.2015.47.1.111.

Kant, R., Srivastav, P. P., & Datta, A. K. (2019). The medicinal role of Centella asiatica and its applications in the Dahi: a research review. *Journal of Pharmaceutical Research International*, 28(6), 1-9. http://dx.doi.org/10.9734/jpri/2019/v28i630220.

Khandagale, T. T., Singh, K., Sinha, S., & Puri, A. (2022). In silico study of phytochemicals for anticholinesterase activity as a potential drug target against Alzheimer's disease. *Chemical Biology Letters*, 9(2), 310.

Kim, H.-Y., Kim, J. H., Zuo, G., & Lim, S. S. (2020). Anti-obesity effect of melandrium firmum rohrbach extract in 3T3-L1 cells and high-fat diet-induced obese C57BL/6N mice. *Food Science & Nutrition*, 8(5), 2251-2261. http://dx.doi.org/10.1002/fsn3.1466. PMid:32405382.

King, A. C., Perez-Escamilla, R., Vorkoper, S., Anand, N., & Rivera, J. (2021). Childhood obesity prevention across borders: the promise of US-Latin American research collaboration. *Obesity Reviews*, 22(Suppl. 3), e13238. http://dx.doi.org/10.1111/obr.13238. PMid:33949095.

Lee, J.-H., Cho, B.-Y., Choi, S.-H., Jung, T.-D., Choi, S.-I., Lim, J.-H., & Lee, O.-H. (2018). Sulforaphane attenuates bisphenol A-induced 3T3-L1 adipocyte differentiation through cell cycle arrest. *Journal of Functional Foods*, 44, 17-23. http://dx.doi.org/10.1016/j.jff.2018.02.021.

Li, B.-B., Pang, K., Hao, L., Zang, G.-H., Wang, J., Wang, X.-T., Zhang, J.-J., Cai, L.-J., Yang, C.-D., & Han, C.-H. (2022). Corosolic acid improves erectile function in metabolic syndrome rats by reducing reactive oxygen species generation and increasing nitric oxide bioavailability. *Food Science and Technology*, 42, e108821. http://dx.doi.org/10.1590/fst.108821.

Lim, S.-H., Kim, H.-Y., Park, M.-H., Park, Y.-H., Ham, H.-J., Lee, K.-Y., Kim, K.-H., Park, D.-S., & Kim, S.-M. (2010). Biological activities

of solvent extracts from leaves of Aceriphyllum rossii. *Journal of the Korean Society of Food Science and Nutrition*, 39(12), 1739-1744. http://dx.doi.org/10.3746/jkfn.2010.39.12.1739.

Lin, T., Zhang, Y., Zhang, T., Steckler, R. A., & Yang, X. (2021). Hop2 interacts with the transcription factor CEBPα and suppresses adipocyte differentiation. *The Journal of Biological Chemistry*, 297(5), 101264. http://dx.doi.org/10.1016/j.jbc.2021.101264. PMid:34600885.

Pereira-Dutra, F. S., Teixeira, L., Costa, M. F. S., & Bozza, P. T. (2019). Fat, fight, and beyond: the multiple roles of lipid droplets in infections and inflammation. *Journal of Leukocyte Biology*, 106(3), 563-580. http://dx.doi.org/10.1002/JLB.4MR0119-035R. PMid:31121077.

Salari, N., Jafari, S., Darvishi, N., Valipour, E., Mohammadi, M., Mansouri, K., & Shohaimi, S. (2021). The best drug supplement for obesity treatment: a systematic review and network meta-analysis. *Diabetology & Metabolic Syndrome*, 13(1), 110. http://dx.doi.org/10.1186/s13098-021-00733-5. PMid:34663429.

Santos, H., Reis, W. P., Ghamsary, M., Jackson, A., & Herring, P. (2019). Lifestyle and environmental factors associated with predictors of childhood obesity. *International Journal of Advanced Nutritional and Health Science*, 7(1), 313-324. http://dx.doi.org/10.23953/cloud.ijanhs.413.

Semeniuc, C. A., Socaciu, M.-I., Socaci, S. A., Mureşan, V., Fogarasi, M., & Rotar, A. M. (2018). Chemometric comparison and classification of some essential oils extracted from plants belonging to Apiaceae and Lamiaceae families based on their chemical composition and biological activities. *Molecules*, 23(9), 2261. http://dx.doi.org/10.3390/molecules23092261. PMid:30189606.

Shin, H.-Y., Kim, H., Jeong, E.-J., Kim, J.-E., Lee, K.-H., Bae, Y.-J., & Yu, K.-W. (2020). Bioactive compounds, anti-oxidant activities and anti-inflammatory activities of solvent extracts from Centella asiatica cultured in Chungju. *The Korean Journal of Food And Nutrition*, 33(6), 692-701.

Shirai, K., Fujita, T., Tanaka, M., Fujii, Y., Shimomasuda, M., Sakai, S., & Samukawa, Y. (2019). Efficacy and safety of lipase inhibitor orlistat in Japanese with excessive visceral fat accumulation: 24-week, double-blind, randomized, placebo-controlled study. *Advances in Therapy*, 36(1), 86-100. http://dx.doi.org/10.1007/s12325-018-0835-5. PMid:30535651.

Sordi, M. B., Curtarelli, R. B., Silva, I. T., Fongaro, G., Benfatti, C. A. M., Magini, R. S., & Cruz, A. C. C. (2021). Effect of dexamethasone as osteogenic supplementation in in vitro osteogenic differentiation of stem cells from human exfoliated deciduous teeth. *Journal of Materials Science. Materials in Medicine*, 32(1), 1. http://dx.doi.org/10.1007/s10856-020-06475-6. PMid:33469820.

Stefania, S., Clodoveo, M. L., Cariello, M., D'Amato, G., Franchini, C., Faienza, M. F., & Corbo, F. (2021). Polyphenols and obesity prevention: critical insights on molecular regulation, bioavailability and dose in preclinical and clinical settings. *Critical Reviews in Food Science and Nutrition*, 61(11), 1804-1826. http://dx.doi.org/10.1080/10408398.2020.1765736. PMid:32436425.

Tam, B. T., Morais, J. A., & Santosa, S. (2020). Obesity and ageing: two sides of the same coin. *Obesity Reviews*, 21(4), e12991. http://dx.doi.org/10.1111/obr.12991. PMid:32020741.

Tripathy, S., Verma, D. K., Thakur, M., Chakravorty, N., Singh, S., & Srivastav, P. P. (2022). Recent trends in extraction, identification and quantification methods of Centella asiatica phytochemicals with potential applications in food industry and therapeutic relevance: a review. *Food Bioscience*, 49, 101864. http://dx.doi.org/10.1016/j.fbio.2022.101864.

Wang, N.-D., Finegold, M. J., Bradley, A., Ou, C. N., Abdelsayed, S. V., Wilde, M. D., Taylor, L. R., Wilson, D. R., & Darlington, G. J. (1995). Impaired energy homeostasis in C/EBPα knockout mice. *Science*, 269(5227), 1108-1112. http://dx.doi.org/10.1126/science.7652557. PMid:7652557.

Wang, S., Zhou, D., & Lin, H. (2022). Ameliorative effects of phosphorylated peptide from Antarctic krill (*Euphausia superba*) against H_2O_2-induced damage in MC3T3-E1 cells. *Food Science and Technology*, 42, e64920. http://dx.doi.org/10.1590/fst.64920.

White, U., & Ravussin, E. (2019). Dynamics of adipose tissue turnover in human metabolic health and disease. *Diabetologia*, 62(1), 17-23. http://dx.doi.org/10.1007/s00125-018-4732-x. PMid:30267179.

Yeom, S.-H., Gam, D.-H., Kim, J.-H., & Kim, J.-W. (2022). Development of ultrasound-assisted extraction to produce skin-whitening and anti-wrinkle substances from safflower seed. *Molecules*, 27(4), 1296. http://dx.doi.org/10.3390/molecules27041296. PMid:35209083.

Yildiz, E., Guldas, M., Ellergezen, P., Acar, A. G., & Gurbuz, O. (2021). Obesity-associated pathways of anthocyanins. *Food Science and Technology*, 41(Suppl. 1), 1-13. http://dx.doi.org/10.1590/fst.39119.

Zhang, C., Sheng, L., Yuan, M., Hu, J., Meng, Y., Wu, Y., Chen, L., Yu, H., Li, S., Zheng, G., & Qiu, Z. (2020). Orlistat delays hepatocarcinogenesis in mice with hepatic co-activation of AKT and c-Met. *Toxicology and Applied Pharmacology*, 392, 114918. http://dx.doi.org/10.1016/j.taap.2020.114918. PMid:32045588.

Zuin, J. C., Gandra, R. L. P., Ribeiro, A. P. B., Ract, J. N. R., Macedo, J. A., & Macedo, G. A. (2022). Comparing chemical and enzymatic synthesis of rich behenic lipids products: technological and nutritional potential. *Food Science and Technology*, 42, e105821. http://dx.doi.org/10.1590/fst.105821.