Zanthoxylum piperitum DC Ethanol Extract Suppresses Fat Accumulation in Adipocytes and High Fat Diet-Induced Obese Mice by Regulating Adipogenesis

So Young GWON1, Ji Yun AHN1, Tae Wan KIM2 and Tae Youl HA1,*

1 Division of Metabolism and Functionality Research, Korea Food Research Institute, Seongnam 463–746, Republic of Korea
2 Department of Food Science and Biotechnology, Andong National University, Andong 760–749, Republic of Korea

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Summary This study was conducted to determine the anti-obesity effects of Zanthoxylum piperitum DC fruit ethanol extract (ZPE) in 3T3-L1 adipocytes and obese mice fed a high-fat diet. We evaluated the influence of the addition of ZPE to a high-fat diet on body weight, adipose tissue weight, serum and hepatic lipids in C57BL/6 mice. In addition, adipogenic gene expression was determined by Western blot and real-time reverse transcription-PCR analysis. We assessed the effect of ZPE on 3T3-L1 preadipocyte differentiation. ZPE reduced weight gain, white adipose tissue mass, and serum triglyceride and cholesterol levels (p<0.05) in high-fat diet-fed C57BL/6 mice. ZPE decreased lipid accumulation and PPARγ, C/EBPα, SREBP-1, and FAS protein and mRNA levels in the liver. ZPE inhibited in vitro adipocyte differentiation in a dose-dependent manner and significantly attenuated adipogenic transcription factors, such as PPARγ, C/EBPα, and SREBP-1 in 3T3L1 cells. These findings suggest that Z. piperitum DC exerts an anti-obesity effect by inhibiting adipogenesis through the downregulation of genes involved in the adipogenesis pathway.

Key Words 3T3-L1 adipocyte, high-fat diet, anti-obesity, Zanthoxylum piperitum DC fruit

Obesity is the most common metabolic disease in developed countries and has become a global epidemic in recent years (1). It is associated with numerous chronic diseases including type 2 diabetes, dyslipidemia, atherosclerosis, hypertension, cardiovascular diseases, stroke, and certain forms of cancer (2–5). Obesity can be defined as an increased fat mass due to an increase in the number and size of adipocytes. Adipogenesis, which is the process by which undifferentiated preadipocytes are converted to differentiated adipocytes, is closely related to the etiologies of obesity and obesity-related metabolic disorders (6). Controlling adipogenesis is a potential strategy for obesity prevention because adipocyte differentiation plays a key role in fat mass growth (7–9). The master adipogenic transcription regulators are CCAAT/enhancer binding protein α (C/EBPα) and peroxisome proliferator-activated receptor γ (PPARγ) (10). These factors modulate adipogenesis-related gene expression and lipid storage in adipocytes (11). Sterol regulatory element-binding protein (SREBP-1) also plays a role by upregulating many lipogenic genes, such as fatty acid synthase (FAS).

Zanthoxylum piperitum DC (ZPDC) has been used in Korea as a spice and as a traditional medicine for vomiting, diarrhea, and abdominal pain (12). The fruit and leaves of Z. piperitum contain aliphatic acid amides (13, 14), terpenoids (15, 16), flavonoids (17), an alkaloid (18), and other phenolics (19). Previous studies reported that a glycoprotein isolated from ZPDC possesses anti-inflammatory properties (20), and ZPDC fruits and leaves exhibit antioxidant and hepatoprotective effects (21). However, anti-obesity effects of Z. piperitum DC fruit have not yet been demonstrated. We hypothesized that ZPDC contains functional compounds that possess anti-obesity properties. Therefore, we evaluated the effects of an ethanol extract of Z. piperitum DC fruit in a 3T3-L1 cell culture system and investigated its effects on body weight gain, adiposity, liver steatosis, and gene expression in high-fat diet-induced obese mice.

MATERIALS AND METHODS

Chemicals and reagents. Isobutyl-3-methylxanthine (IBMX), dexamethasone, insulin, and Oil Red O were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS), bovine calf serum, and antibi-
Preparation of ethanol extract from fruit of *Z. piperitum* DC (ZPE). The fruits of *Z. piperitum* DC originating from Korea were purchased from Khung-dong Oriental medicine market (Seoul, Korea) and identified by Professor Y. M. Park, Department of Life Science, Cheongju University. Voucher specimens (KFRI-ZPDC090705) were deposited in the Korea Food Research Institute. The dried fruits (1.0 kg) were soaked in 70% ethanol (10 L) at room temperature for 12 h. The ethanol extract was filtered through filter paper (Whatman Grade No. 2, New Jersey, USA), and concentrated under a vacuum at 40°C. The concentrated extracts were then freeze-dried. Finally, the dried extract (216.8 g) obtained from fruits of *Z. piperitum* DC (1,000 g) were stored at −20°C until use. The total polyphenol content in the extract was determined using the Folin-Ciocalteau method (22) and total flavonoid content was determined using the diethylene glycol method (23). Results were expressed in terms of gallic acid equivalents (GAE), and flavonoid content was expressed in terms of quercetin equivalents (QE). The main compounds in ZPE were analyzed using HPLC analysis. Samples were separated on a JASCO HPLC system (JASCO Corporation, Tokyo, Japan) and XTerra™ RP18 column (4.6 × 250 mm, Waters Corporation, Milford, MA, USA). The mobile phase consisted of solvents A (50 mM sodium phosphate, 10% methanol, pH 3.3) and B (70% methanol). The gradient elutes were filtered through a 0.45 μm Millipore filter and degassed prior to use. The linear gradient profile was from 70% A to 30% B in 7 min, 65% A to 35% B in 18 min, 60% A to 40% B in 20 min, 50% A to 50% B in 5 min, 100% B in 35 min and then 100% A in 25 min, followed by re-equilibration of the column to its initial conditions. The constituent was detected with UV wavelength at 280 nm.

Cell culture and differentiation. The 3T3-L1 mouse fibroblast cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium containing 10% calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM l-glutamine (Invitrogen, Carlsbad, CA, USA) at 37°C under 5% CO₂. On day 3 after confluence (day 0), the cells were exposed to differentiation medium (DMEM containing 0.25 mM 3-isobutyl-1-methylxanthine, 0.25 μM dexamethasone, and 1 μg/mL insulin [MDI] with 10% FBS) for 2 d. The cells were cultured for another 2 d in DMEM containing 1 μg/mL insulin and 10% FBS. Thereafter, the cells were maintained in postdifferentiation medium (DMEM containing 10% FBS) and the medium was replaced every 2 d. To evaluate the effects of ZPE on preadipocyte differentiation, the cells were cultured with differentiation medium in the presence of various concentrations of ZPE. The cells were harvested on day 8, when differentiation was complete.

**Oil Red O staining and cell quantification.** After differentiation was induced, the cells were stained with Oil Red O (0.2% Oil Red O in 60% isopropanol). The cells were washed twice with phosphate buffered saline (PBS), fixed with 10% formalin for 1 h, dried, and stained with Oil Red O for 10 min. The cells were washed with 70% ethanol and water, and then dried. The lipid content of stained cells was visualized by microscopy (Olympus IX71, Tokyo, Japan). The stained lipid droplets were dissolved in isopropanol and quantified by measuring absorbance at 500 nm.

**Animal experiments.** C57BL/6 male mice (4 wk old, n = 30) were purchased from Central Laboratory Animal (Seoul, Korea). After acclimation to the commercial chow for a week, they were divided into three groups according to diet: normal diet (ND), high-fat diet (HFD), or high-fat diet supplemented with 0.5% ZPE (ZPE). All mice were housed individually during the study. The experimental diets were based on the AIN-76 diet, and the high-fat diet contained 20% fat (lard 50 g/kg, coconut butter 70 g/kg, cocoa oil 30 g/kg, corn oil 50 g/kg) and 0.5% cholesterol (w/w). The energy content of the high-fat diet was 19 MJ/kg (4,625 kcal), whereas that of the normal diet was 16 MJ/kg (3,850 kcal). The animal room was maintained at 23 ± 1°C and 53 ± 2% humidity with a 12-h light/dark cycle. The mice had free access to water. The diet was given to the mice at 10:00 every day. The body weight was measured once a week, and food intake was measured every day by subtraction of pre- and post-weights of food jars for 6 wk. Energy efficiency was calculated as the energy intake divided by the body weight gain.

All animal experiments were approved by the Korea Food Research Institutional Animal Care and Use Committee.

**Biochemical analysis.** All mice were sacrificed after a 12-h fast. Blood was collected by orbital venipuncture and centrifuged at 3,000 rpm for 15 min to separate the serum, which was stored at −70°C until analysis. Triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were enzymatically analyzed with a commercial kit (Shinyang Chemical, Seoul, Korea). Low-density lipoprotein cholesterol (LDL-C) was calculated from TG, TC, and HDL-C concentrations using the following equation: LDL-C = TC − HDL-C − (TG/5) (24). The hepatic lipids were extracted using the Folch method (25). TG and TC concentrations were determined with an enzyme assay kit (Shinyang Chemical). Serum insulin levels and leptin levels were determined with an enzyme-linked immunosorbent assay kit (ELISA) according to the manufacturer’s protocol (Shinayagi Co, Tokyo, Japan). Serum glucose levels were enzymatically analyzed using a commercial kit (Shinyang Chemical).

**Histology analysis.** For hematoxylin and eosin (H&E) staining, liver and epididymal adipose tissues were fixed in 10% formalin for 1 d and processed in a routine manner for paraffin sections. Five-micrometer-thick sections were cut and stained with H&E for microscopic examination (Leica RM2235, Wetzlar, Germany). Images
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Protein extraction and Western blot analysis. For Western blot analysis, cells were washed with ice-cold PBS, collected, and centrifuged. The harvested cells were sonicated for 5 s at 40 W. Liver tissue (20 mg) was homogenized in 600 μL lysis buffer (PRO-PREP™, InTRON Biotechnology, Sungnam, Korea). Cell and liver tissue lysates were incubated for 20 to 30 min on ice, and then centrifuged at 13,000 rpm at 4˚C for 10 min. The protein concentration of the supernatant was determined with Bio-Rad Protein Assay Reagent (Bio-Rad Laboratories, Hercules, CA, USA) using bovine serum albumin as the standard. Total proteins (30 μg per lane) were separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membrane was blocked for 2 h at room temperature with 0.1% Tween 20 (Amresco Inc., Solon, OH, USA) using bovine serum albumin as the standard. Total proteins (30 μg per lane) were separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membrane was blocked for 2 h at room temperature with 0.1% Tween 20 (Amresco Inc., Solon, OH, USA) in Tris-buffered saline containing 5% skim milk. After overnight incubation at 4˚C with primary antibodies, membranes were incubated with a horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Immunodetection was carried out with ECL detection reagent (Amersham Biosciences, Uppsala, Sweden). All figures showing results of quantitative analysis include data from at least three independent experiments.

Quantitative RT-PCR analysis for expression of genes related to adipogenesis pathway. Total RNA was extracted from frozen liver tissue (30 mg) using the RNase kit (Nucleospin, InTRON Biotechnology), and used to synthesize cDNA for analysis by real-time reverse transcription-polymerase chain reaction (RT-PCR) (LightCycler® 480 System, Roche, Basel, Swiss). Equal amounts of total RNA were used for each analysis. Primer sequences were as follows: SREBP-1c forward 5’-CGG CGC GGA AGC TGT-3’; SREBP-1c reverse 3’-AGT CAC TGT CTT GGT TGT TGA TGA G-5’; FAS forward 5’-ATC CTG GAA CGA GAA CAC GAT CT-3’; FAS reverse 3’-AGA GAC GTG TCA CTC CTG GAC TT-5’; PPARγ forward 3’-CTG ATG ATG TGG AGA AGT ATT-5’; PPARγ reverse 3’-GAG AGG TCC ACA GAG GTG ATT-3’; C/EBPα forward 3’-GAC TTC AGC CCC TAC CTG GA-5’; C/EBPα reverse 3’-GTA GTC GTC GGC GAA GAG GT-5’.

Statistical analysis. Statistically significant differences were determined by analysis of variance (ANOVA) with the Tukey-Kramer test for post-hoc analysis using SAS 10.0 software. Data are expressed as mean±standard error of the mean (SE). p<0.05 was considered significant.

RESULTS

Components of ZPE and total polyphenol, flavonoids contents

The total polyphenol and flavonoid contents of ZPE were 742.8±3.97 mg GAE/g ZPE and 486.8±7.08 mg QE/g ZPE, respectively. As shown in Fig. 1, flavone was identified by its retention time of 64.9 min. ZPE contained 110 mg flavone. Therefore, flavone seemed to be the major component of ZPE.

Effects of ZPE on differentiation of 3T3-L1 preadipocytes

Differentiation of the 3T3-L1 preadipocytes was induced by MDI, which promoted accumulation of intracellular fat droplets (Fig. 2A and B). Oil Red O staining of the lipid droplets revealed that ZPE (0.25, 0.5, and
0.75 µg/mL) inhibited 3T3-L1 adipocyte differentiation in a dose-dependent manner.

To determine whether ZPE inhibits adipocyte differentiation by modulating the expression of the key transcriptional regulators, we determined protein levels of PPARγ and C/EBPα in the adipocytes. We found that levels of adipogenic transcription factors were dose-dependently reduced in ZPE-treated cells (Fig. 2C). To identify the upstream regulators involved in PPARγ downregulation, we then evaluated expression of SREBP-1 and its target FAS. Our results demonstrated that protein levels of SREBP-1 and FAS were attenuated

Table 1. Effect of *Zanthoxylum piperitum* DC fruit extract on body weight, food intake, and liver and adipose tissue weight in mice fed a high-fat diet for 6 wk.

<table>
<thead>
<tr>
<th></th>
<th>ND</th>
<th>HFD</th>
<th>HFD+ZPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight, g</td>
<td>16.07±0.90 NS</td>
<td>16.18±0.97</td>
<td>16.07±0.72</td>
</tr>
<tr>
<td>Body weight gain, g</td>
<td>8.48±1.88 c</td>
<td>14.31±1.57 a</td>
<td>11.81±2.19 b</td>
</tr>
<tr>
<td>Food intake, g/d</td>
<td>3.19±0.05 NS</td>
<td>3.06±0.06</td>
<td>3.07±0.05</td>
</tr>
<tr>
<td>Energy intake, MJ/42 d</td>
<td>2.13±0.04 b</td>
<td>2.44±0.05 a</td>
<td>2.45±0.04 a</td>
</tr>
<tr>
<td>Energy efficiency, g/MJ</td>
<td>3.94±0.07 c</td>
<td>5.94±0.11 a</td>
<td>4.83±0.08 b</td>
</tr>
<tr>
<td>Liver, g</td>
<td>0.80±0.05 c</td>
<td>1.23±0.10 a</td>
<td>1.06±0.11 b</td>
</tr>
<tr>
<td>Epididymal WAT, g</td>
<td>0.67±0.14 c</td>
<td>1.49±0.17 a</td>
<td>1.23±0.24 b</td>
</tr>
<tr>
<td>Perirenal WAT, g</td>
<td>0.24±0.09 b</td>
<td>0.63±0.08 a</td>
<td>0.51±0.10 a</td>
</tr>
<tr>
<td>Interscapular BAT, g</td>
<td>0.08±0.03 b</td>
<td>0.18±0.05 a</td>
<td>0.10±0.02 b</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SE (n=10).

a,b,c Values in a row with different superscripts are significantly different (Tukey-Kramer’s test: p<0.05).
a Body weight gain (g/42 d)/Energy intake (MJ/42 d).

ND, normal diet; HFD, high-fat diet; HFD+ZPE, high-fat diet plus 0.5% ZPE; WAT, white adipose tissue; BAT, brown adipose tissue.
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Reduction of body weight, adipose tissue, and adipocyte size by ZPE (Fig. 2C).

Table 1 shows the effect of ZPE on body weight, adipose tissue mass, and adipocyte size of C57BL/6J mice fed a high-fat diet for 6 wk. The mean body weight of the HFD group was significantly higher than that of the ND group. In contrast, the ZPE group's mean body weight was significantly lower than that of the HFD group (Fig. 3A). Food intake did not differ significantly among the groups. Energy efficiency was higher in the HFD group compared with the ND group. However, the HFD+ZPE group showed decreased energy efficiency compared with the HFD group (Table 1). Weights of white adipose tissue (epididymal) in the HFD group were significantly lower than those of the HFD group. In addition, the mean epididymal adipocyte size of the HFD+ZPE group (3,918±163 μm²) was lower than that of the HFD group (7,242±510 μm²) (Fig. 3B). These results showed that ZPE might have an anti-obesity effect.

Effects of ZPE on serum TG, TC, glucose, insulin, and leptin levels

Serum TG and TC levels were significantly increased by the high-fat diet (Table 2). However, ZPE supplementation significantly lowered serum TG levels in high-fat diet-fed mice. But the serum TC level did not differ significantly between the HFD group and the HFD+ZPE group. Likewise, serum insulin, glucose, and leptin levels were higher in the HFD group compared to the ND group, but ZPE supplementation decreased serum insulin levels by 59%, glucose levels by 11%, and leptin levels by 54% in mice fed the high-fat diet.

Effects of ZPE on liver weight, hepatic lipids, and histology

The liver weight was significantly increased in the

Table 2. Effect of Zanthoxylum piperitum DC fruit extract on serum biochemical parameters in mice fed a high-fat diet for 6 wk.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ND</th>
<th>HFD</th>
<th>HFD+ZPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG (mg/dL)</td>
<td>73.92±13.2²</td>
<td>98.81±17.67¹</td>
<td>62.51±6.13³</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>91.06±10.31¹</td>
<td>166.71±15.61¹</td>
<td>155.78±12.66¹</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>46.27±5.56²</td>
<td>60.38±6.44¹</td>
<td>64.60±9.31¹</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>30.00±2.95²</td>
<td>95.19±4.17¹</td>
<td>75.57±4.94³</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>120.6±4.41¹</td>
<td>230.9±9.5²</td>
<td>204.24±4.63³</td>
</tr>
<tr>
<td>Insulin (pg/mL)</td>
<td>584.85±103.29²</td>
<td>2,141.4±232.37¹</td>
<td>858.75±113.86³</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>4.54±0.79¹</td>
<td>32.53±2.83¹</td>
<td>14.74±2.19³</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SE (n=10).

a,b,c Values in a row with different superscripts are significantly different (Tukey-Kramer’s test: p<0.05).

ND, normal diet; HFD, high-fat diet; HFD+ZPE, high-fat diet plus 0.5% ZPE supplemented diet; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
HFD group compared to the ND group, but liver weight in the HFD+ZPE group was significantly lower than in the HFD group (Table 1). Hepatic TG levels were also significantly higher in the HFD group than in the ND group (Fig. 4A). Compared to the HFD group, TG concentrations were significantly lower in the HFD+ZPE group. Hepatic TC levels appeared somewhat lower in the HFD+ZPE group compared to the HFD group, but this difference was not significant (Fig. 4B). Histologic assessment of H&E-stained liver sections is shown in Fig. 4C. The ND group showed normal histology, whereas the HFD group exhibited increased lipid accumulation. Lipid droplet accumulation was lower in the HFD+ZPE group compared with the HFD group.

Effects of ZPE on expression of adipogenic proteins and mRNA in liver

To determine whether the anti-obesity effects of ZPE are accompanied by changes in adipogenesis-related molecular factors, we determined the levels of protein and mRNA of adipogenic transcription factors and enzymes in liver tissues (Fig. 4D and E). Protein levels of the key transcription factors PPARγ and C/EBPα were elevated in the HFD group compared with the ND control. However, levels of these factors were lower in the HFD+ZPE group compared with the HFD group (Fig. 4D). In addition, expression of the upstream regulator SREBP-1c was decreased in the HFD+ZPE group compared with the HFD group. Further, ZPE significantly reduced the mRNA levels of these genes (Fig. 4E).

Effect of flavone on 3T3-L1 preadipocyte differentiation

Next, we investigated the effect of flavone, which is a main component of ZPE, on 3T3-L1 preadipocyte differentiation. After treatment with flavone at various concentrations, cell viability was determined by MTT assay to identify the optimal dose of flavone treatment to cells. Flavone did not affect the cell viability at concentrations of up to 0.5 μg (Fig. 5A). The effect of flavone on lipid accumulation was measured by Oil Red O staining. As shown in Fig. 5B, flavone treatment decreased lipid droplet accumulation in a dose-dependent manner.

**DISCUSSION**

In the present study, we evaluated the effect of ZPE on early stages of adipogenesis using the 3T3-L1 pre-
adipocyte an in vitro model of adipogenesis. We found that ZPE dose-dependently inhibited 3T3-L1 cell differentiation. Accordingly, protein levels of adipogenic transcription factors were markedly decreased by ZPE, demonstrating that ZPE inhibits differentiation of 3T3-L1 preadipocytes by suppressing PPARγ and C/EBPα. Further, downregulation of the upstream regulator SREBP-1 attenuated expression of its target gene FAS, which is involved lipid biosynthesis. These results demonstrate that ZPE inhibits differentiation of 3T3-L1 preadipocyte by regulating the expression of adipogenic and lipogenic transcription factors.

Because ZPE inhibited in vitro adipocyte differentiation, we evaluated the inhibitory effects of ZPE against high-fat diet-induced obesity. Therefore, experimental mice fed on a high-fat diet with or without ZPE supplementation for 6 wk and changes in body weight were examined. The mean body weight gain of the HFD+ZPE group was 17% lower than that of the HFD group, without a significant difference in food intake. In particular, perirenal and epididymal white adipose tissue mass was reduced by ZPE. ZPE supplementation lowered serum levels of TG, TC and leptin in high-fat diet-fed mice. Leptin appears to contribute to hepatic steatosis by promoting insulin resistance, and increased insulin levels result in free fatty acid accumulation in the liver (26). In the present study, glucose and insulin levels were significantly elevated in mice with diet-induced obesity. However, ZPE significantly lowered serum glucose and insulin levels. The adiponectin is secreted from adipocytes, and it was found to be decreased in obesity. Adiponectin has also been shown to improve insulin sensitivity, a key pathogenic factor of type 2 diabetes (27).

We also evaluated the effects of ZPE on high-fat diet-induced fatty liver, which is strongly associated with obesity and insulin resistance. Fatty liver is the result of increased uptake of circulating free fatty acids and elevated endogenous hepatic fatty acid synthesis (28). Histologic examination of liver tissue revealed a marked accumulation of fat droplets, indicating fatty liver; however, hepatic steatosis was prevented by ZPE. In addition, ZPE markedly lowered hepatic TG levels in mice fed a high-fat diet, which limits the availability of long-chain fatty acids required for hepatic lipid synthesis.

To gain more mechanistic insights into the signaling pathway affected by ZPE, we determined the expression of PPARγ, C/EBPα, SREBP-1, and FAS consistent with our in vitro results, liver tissue protein levels of PPARγ, C/EBPα, and SREBP-1 were elevated in the HFD group, and ZPE significantly reduced both protein and mRNA levels of these genes. Generally, PPARγ is expressed at very low levels in the liver, whereas PPARα is predominantly expressed in the liver as a sensor for fatty acid (29). In the insulin resistance state and fatty liver, PPARγ expression markedly increased, consistent with our observations of the HFD group. However, ZPE supplementation has been shown to reduce insulin levels and PPARγ expression. These results suggest that ZPE
improved insulin sensitivity, and also attenuated fatty liver induced by HFD.

Adipogenesis is a complex cellular pathway requiring sequential regulation of adipogenic and lipogenic genes (11). PPARγ, a member of the nuclear-receptor superfamily, is a master regulator of adipogenesis and both necessary and sufficient for adipogenesis (10). PPARγ and C/EBPα mediate the transcription of terminal adipocyte differentiation marker genes (11). SREBP-1c activates PPARγ by inducing its expression as well as by promoting production of an endogenous PPARγ ligand (10). In the present study, ZPE downregulated SREBP-1c significantly, thereby attenuating PPARγ expression. PPARγ induces C/EBPα expression by binding to its promoter region. Thus the ZPE-induced downregulation of adipogenic transcription factors, suppressed FAS mRNA expression and resulted in inhibiting adipogenesis (Fig. 6).

In previous studies, various natural extracts containing polyphenols were shown to decrease lipid accumulation and stimulate lipolysis in preadipocytes and adipocytes (30, 31). ZPDC was shown to possess high polyphenol content and the highest radical scavenging activity of the 11 Korean salad plants evaluated (21). The ethanol extract of ZPDC used in the present study contained 742.85 mg/g of total phenolic compounds, 486.82 mg/g of flavonoid compounds and 110.11 mg/g of flavone. The result revealed that flavone accounts for approximately 14.82% of the phenolic compounds, suggesting that ZPE contains substantial amounts of flavone. Flavone is a class of flavonoids found in vegetables and fruits. It has been widely studied and has shown beneficial health effects including anticancer, antiallergic, and anti-inflammatory effects (32). Also, flavone has been reported to induce lipid lowering action in hyperlipidemia rats (33). Our results showed that flavone significantly reduced the lipid accumulation and induced inhibition of adipocyte differentiation (Fig. 5B). Our results are consistent with other previous reports on the antiobesity effects of flavone such as baicalin (34), naringenin (35). Flavone might be one of the candidates for an active component in ZPE. SREBP1 was regulated by flavone, which led to down regulation of transcription factors and FAS. Taken together, these results demonstrate that the inhibitory effect of ZPE on HFD-induced obesity may be partially attributed to flavone. Further study is needed to determine whether flavone can improve the serum lipid profile of diet-induced obese mice.

In conclusion, ZPE significantly inhibits 3T3-L1 preadipocyte differentiation and decreases weight gain, hepatic lipid accumulation, adipose tissue mass and adipocyte size induced by a high-fat diet in mice. These anti-obesity effects of ZPE may be mediated by downregulating adipogenic transcription factors. Further studies are needed to demonstrate the antiobesity effect of bioactive compounds in Z. piperitum DC.

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