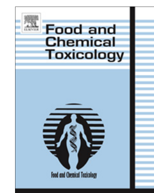
Food and Chemical Toxicology 58 (2013) 30–36

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox



Taraxacum official (dandelion) leaf extract alleviates high-fat diet-induced nonalcoholic fatty liver

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ARTICLE INFO

Article history:
Received 20 November 2012
Accepted 10 April 2013
Available online 18 April 2013

Keywords:
High-fat diet
Taraxacum official (dandelion)
Fatty liver
AMPK
Insulin resistance

ABSTRACT

The purpose of this study is to determine the protective effect of *Taraxacum official* (dandelion) leaf extract (DLE) on high-fat-diet (HFD)-induced hepatic steatosis, and elucidate the molecular mechanisms behind its effects. To determine the hepatoprotective effect of DLE, we fed C57BL/6 mice with normal chow diet (NCD), high-fat diet (HFD), HFD supplemented with 2 g/kg DLE DLE (DL), and HFD supplemented with 5 g/kg DLE (DH). We found that the HFD supplemented by DLE dramatically reduced hepatic lipid accumulation compared to HFD alone. Body and liver weights of the DL and DH groups were significantly lesser than those of the HFD group, and DLE supplementation dramatically suppressed triglyceride (TG), total cholesterol (TC), insulin, fasting glucose level in serum, and Homeostatic Model Assessment Insulin Resistance (HOMA-IR) induced by HFD. In addition, DLE treatment significantly increased activation of adenosine monophosphate (AMP)–activated protein kinase (AMPK) in liver and muscle protein. DLE significantly suppressed lipid accumulation in the liver, reduced insulin resistance, and lipid in HFD-fed C57BL/6 mice via the AMPK pathway. These results indicate that the DLE may represent a promising approach for the prevention and treatment of obesity-related nonalcoholic fatty liver disease.

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1. Introduction

Obesity is an alarming public health issue because it causes a broad range of chronic diseases and metabolic syndromes, such as type 2 diabetes, cardiovascular disease, hypertension, nonalcoholic fatty liver disease (NAFLD), and insulin resistance (Zimmet et al., 2001). NAFLD is a metabolic syndrome characterized by abnormal lipid formation in hepatocytes without excess alcohol intake. The primary danger of NAFLD is that it can lead to impaired glucose metabolism, inflammatory response, and insulin resistance, which produce elevated lipogenesis of free fatty acids (FFAs) and fat accumulation in the liver (Cornier et al., 2008; Hamaguchi et al., 2005; Sarafidis and Nilsson, 2006). Excessive dietary fat intake increases the concentration of FFAs in blood and leads to elevation of triglyceride (TG) stored in liver, which causes lipid accumulation and resulting hepatic steatosis (Jang et al., 2012).

Because of their safety and efficacy, and because they may possess potential bioactive components to treat or prevent the obesity and NAFLD (Jang et al., 2012; Melo et al., 2010), natural herbs and food factors have recently been the focus of many researchers. The common dandelion, *Taraxacum officinale* G.H. Weber ex Wiggers, a member of the Asteraceae/Composite family, is widely used as a traditional herb to treat various disorders such as liver disease, gallbladder disorders, digestive complaints, and arthritic and rheumatic diseases because of its anti-diabetic, anti-rheumatic, anti-inflammatory, anti-tumor, anti-cardiogenic and hypoglycemic properties (Bisset, 1994; Bradley, 1992; Koh et al., 2010; Racz-Kotilla et al., 1974; Schutz et al., 2005, 2006). Several reports have also verified that in rodent models, dandelion inhibits oxidative stress in CCl_4-induced acute liver injury, high cholesterol, streptozotocin-induced diabetes (Cho et al., 2002, 2003; Park et al., 2010), and fibrosis in CCl_4-induced acute liver injury (Domitrovića et al., 2010). Our own recent data showed that DLE had a protective effect against liver injury induced by methionine- and choline-deficient diet in mice (Davaatseren et al., 2013). However, the underlying mechanism of DLE in preventing obesity-induced hepatic steatosis and insulin resistance remains elusive.

In this study, we examined the protective effect and underlying mechanism of DLE on HFD-induced obesity. We demonstrated that DLE showed a remarkable effect on hepatic lipid accumulation and improved insulin resistance in HFD-fed mice by reducing lipid and

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http://dx.doi.org/10.1016/j.fct.2013.04.023

activating phosphorylation of adenosine monophosphate (AMP)–activated protein kinase (AMPK), suggesting that DLE has a potential protective effect against NAFLD.

2. Materials and methods

2.1. Materials

C2C12 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Dulbecco's modified Eagle's medium (DMEM), RMPI, and fetal bovine serum (FBS) were purchased from WelGene (Daegu, Republic of Korea). The compound, 2-[*N*-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino]-2-deoxy-d-glucose (2-NBDG), was purchased from Invitrogen (Carlsbad, CA, USA). Anti-phospho AMPK, anti-phospho acetyl-CoA carboxylase (ACC), anti-AMPK, anti-ACC, and anti-β-actin were purchased from Cell Signaling Technology (Beverly, MA, USA).

2.2. Cell culture, muscsle differentiation and glucose uptake asssay

For differentiation into myotubes, C2C12 cells were maintained in DMEM containing 10% FBS serum in a CO_2 incubator. Once the cells reached 100% confluence, the cell culture medium was changed to DMEM supplemented with 1% horse serum, and the cells were incubated for 4 days. Confluent cells were incubated overnight in low-glucose, serum-free medium followed by a 24 h treatment in the absence or presence of 50 μM 2-NBDG. Rosiglitazone (Rosi), a PPAR-γ agonist, was used as the positive control. The 2-NBDG uptake assay was performed with a fluorometer at excitation and emission wavelengths of 485 and 535 nm, respectively.

2.3. DLE preparation

Dandelion leaves were obtained from Yanggu Mindeulr (Yanggu, Korea) and dried and powdered using a stone mortar and pestle. A 10-fold volume of pure water was then added to the powdered leaves. The mixture was cooked at 100 °C for 4 h in a double boiler with a reflux condenser. Leaf extract was filtered through Whatman™ filter paper grade No. 4 after cooling at room temperature. The recovery rate of DLE was 29.2%.

2.4. High-performance liquid chromatography (HPLC) analysis for luteolin

HPLC was conducted using a JASCO liquid chromatography (JASCO, Tokyo, Japan) equipped with an autoinjector, a UV detector, and a YMC ODS-AM (250 × 4.6-nm I.D.; 5-μm particle size) reversed-phase column (YMC Co., Ltd., Kyoto, Japan). The mobile phase consisted of solvent A (0.1% acetic acid in water) and solvent B (0.1% acetic acid in acetonitrile). The following gradient conditions were used: initial (0 min) A:B (88:12), 18-min A:B (78:22), 28-min A:B (72:28), 35-min A:B (62:38), 48-min A:B (52:48), 54-min A:B (32:68), 58-min A:B (0:100), 60-min A:B (0:100), 62-min A:B (88:12). The column was equilibrated for 15 min prior to each analysis. The mobile-phase flow rate was 1.0 mL/min. Injection volume was 20 μl and temperature was 35 °C. UV wavelength was determined to be 285 nm.

2.5. Total phenol

The phenolic compounds were determined on basis of the Folin–Ciocalteu reagent reaction (Singleton et al., 1999; Ikawa et al., 2003; Everette et al., 2010). From each sample, 1-mL aliquots were mixed with 1 mL of 2% (w/v) Na_2CO_3 and incubated fo_r 3 min. Next, 0.1 mL of Folin–Ciocalteu reagent was added to each reaction mixture and incubated in the dark for 60 min. UV was recorded at 750 nm using UV spectrophotometry (JASCO, Easton, MD, USA) and then converted to total phenolic compound contents based on the standard calibration curve of chlorogenic acid.

2.6. Animal care and diet preparation

Forty 7-week-old male C57BL/6 mice were obtained from Charles River Korea (Seoul, Korea) and housed in the animal laboratory of Korea Food Research Institute (KFRI). Mice were given free access to water and kept at constant room temperature (22–26 °C) under 12/12-h light/dark cycles. Before starting the experiment, the mice adapted to their new environment and food for 1 week. They were randomly divided into 4 groups of 10 mice and supplied with 1 of 4 diets labeled according to their contents: normal chow diet (NCD; Dyets Inc., Bethlehem, PA, USA), high-fat-diet (HFD; Dyets Inc.), HFD supplemented with the diet was supplemented with 2 g/kg DLE (DL), and HFD was supplemented with 5 g/kg DLE (DH). Diets supplemented with DLE were prepared as follows: HFD was ground and mixed with a precise amount of dandelion leaf extract. Mixed diets were pressed using a pellet maker and kept frozen at −20 °C in a storage chamber until use. All 4 diet formulations were administered for 10 weeks. Food consumption and weight gain were measured daily and weekly, respectively. All mice were fasted for 18 h and underwent a glucose tolerance test (GTT) 2 days before sacrifice.

On the day of sacrifice, a laparotomy was performed under ketamine and xylazine anesthesia (intramuscular injection of 100 mg/kg body mass and 5 mg/kg body mass, respectively), and whole-blood samples were collected via cardiac puncture. To obtain serum for biochemical determination, blood samples were centrifuged at 1000*g* for 10 min. Livers were weighed and cut into several slices: 1 slice was fixed in 4% formalin for histological analysis; the other slices were snap-frozen in liquid nitrogen for other experiments. Serum and liver samples were then stored at −70 °C until required. All animal studies were performed in accordance with guidelines approved by the Animal Experimentation Ethics Committee of KFRI.

2.7. Histological examination

After livers were harvested and sliced, 1 slice from each liver was fixed in 4% formalin (paraformaldehyde [CAS Number 30525-89-4] in phosphate-buffered saline [PBS, pH 7.4]) overnight, after which the formalin was exchanged for fresh solution. Each formalin-fixed liver sample was embedded in paraffin and sliced into 4-μm-thick sections. Slides were hematoxylin and eosin (H&E) stained and evaluated by 3 investigators. H&E stained liver tissue sections were graded for hepatic steatosis and necroinflammation. Hepatic steatosis (percentage of liver cells containing fat) was graded as 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%), or 4 (>75%). Necroinflammation was graded as 0 (no inflammatory foci), 1 (mild), 2 (moderate), and 3 (severe). Images (5 different fields per sample) were obtained using a Nikon Eclipse 80i microscope (Nikon Imaging, Inc., Seoul, Korea) equipped with a digital charge-coupled device (CCD) camera.

2.8. Biochemical analysis

Serum TG and TC were estimated using the Asan Triglyceride and Total Cholesterol Kit (Asan, Seoul, Korea), and insulin level was determined using the Shibayagi Mouse Insulin ELISA Kit (Shibayagi, Shibukawa, Japan) according to the manufacturer's instructions.

Insulin resistance (IR) was evaluated by the homoeostasis model assessment (HOMA) method (Turner et al., 1979) and calculated as the product of fasting serum insulin in micro units per milliliter and fasting blood glucose in milligrams per deciliter divided by 22.5, or (HOMA-IR [mmol/L] = [fasting insulin × fasting glucose]/22.5). IR was defined as HOMA-IR $\geqslant$3.5.

2.9. Glucose tolerance test

All mice underwent a glucose tolerance test after an 18-h overnight fast. Basal fasting blood samples were obtained from the tip of the tail before administration of glucose injection. Glucose (1 g/kg body weight) was injected intraperitoneally. Blood glucose was determined 0, 30, 60, 90, 120, and 150 min after glucose injection. Blood glucose was determined by Accu-Chek Active (Roche, Mannheim, Germany) and the data plotted as blood glucose concentration over time.

2.10. Western blotting analysis

Cells were washed with ice-cold PBS and lysed with a radioimmunoprecipitation assay (RIPA) buffer containing 50 mM Tris-Cl, 1 mM EDTA, 1% Triton™ X-100, 0.5% sodium deoxycholate, 150 mM NaCl, 1 mM PMSF, 1 mM sodium orthovanadate, 1 mM NaF, and 0.2% protease inhibitor cocktail, pH 7.2. We used 1 mM AICAR as the positive control for AMPK activation. Each frozen liver and muscle tissue sample (10–20 mg) was homogenized in RIPA. The total protein concentration was quantified with Bradford reagent. The samples (50 μg of protein per lane) were mixed with sample buffer, boiled for 10 min, separated by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, and electroblotted onto nitrocellulose membranes. The membranes were incubated overnight at 4 °C with anti-pAMPK and p-ACC antibodies (dilution, 1:1000). The membranes were stripped and then-re-blotted with anti-AMPK, ACC, and actin antibodies (dilution, 1:2000).Bands were visualized using enhanced chemiluminescence (ECL) reagents (Amersham, Piscataway, NJ, USA).

2.11. Statistical analysis

All experiments were performed in triplicate and data expressed as mean ± standard deviation (SD). Differences between means were assessed by one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered statistically significant. Statistical analyses were conducted using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Luteolin and total phenol content

The luteolin concentration from DLE was 4.80 ± 0.01 μg/g, and total phenolic content was 8.017 ± 0.130 mg chlorogenic acid equivalents per gram of dried dandelion.

3.2. Glucose uptake by DLE in C2C12 myotubes

To examine the effect of DLE on glucose uptake, we determined the 2-NBDG uptake in C2C12 myotubes. Treatment of DLE increased 2-NBDG uptake in a dose-dependent manner. In particular, at doses of 400 and 800 mg/mL, DLE significantly increased glucose uptake (Fig. 1A).

3.3. Activation of AMPK/ACC by DLE in C2C12 myotubes

Next, we examined whether DLE-stimulated glucose uptake is accompanied by the activation of AMPK and its major substrate ACC in differentiated C2C12 myotubes. Activation of AMPKα was assessed by measuring phosphorylated AMPK (pAMPK) levels. DLE significantly increased the phosphorylation of AMPK in a dose-dependent manner; maximum increase was reached at a concentration of 800 μg/mL. Simultaneously, DLE also increased the phosphorylation of ACC in C2C12 myotubes. AICAR was used as a positive control of AMPK activation. This result demonstrates that AMPK/ACC phosphorylation is necessary for the DLE-stimulated glucose uptake in C2C12 myotubes (Fig. 1B).

3.4. Suppression of HFD-induced body and liver fat accumulation and reduction of HFD-induced serum TG and total cholesterol by DLE

The food intake of the HFD groups was significantly lower than that of the NCD group. However, there was no difference in HFD food intake between the DL and DH groups.

There were no significant differences in initial body mass between all groups. Final mean body mass of the HFD group was significantly increased compared to that of NCD group. The body mass increases resulting from the HFD were dramatically dose-dependently smaller in the DL and DH groups. The mean liver weight in the HFD group was significantly increased compared to that of the NCD group. The mean liver weight increases in the DL and DH groups were smaller than that in the HFD group (Table 1).

Next, the effect of DLE on HFD-induced lipid accumulation in liver tissue was analyzed with H&E staining. The lipid accumulation was highly amplified in the HFD group compared to that of the NCD group. The lipid accumulations induced by HFD were significantly reduced in the DL and DH groups (Fig. 2).

To examine the effect of DLE on biochemical changes, we determined the level of TG and TC in the serum. The TG and TC levels were significantly elevated in HFD group compared to those of the NCD group. The DL and DH groups showed significantly lower TG and TC levels in serum (Table 2).

3.5. Reduction of serum insulin, fasting glucose, and HOMA-IR with DLE

To examine the effect of DLE on HFD-induced insulin resistance, we determined the levels of insulin and fasting glucose from serum, then calculated HOMA-IR. Mean insulin and mean fasting glucose in serum were significantly increased in the HFD group compared to those of the NCD group. In the DL and DH groups, insulin and fasting glucose levels in serum were significantly and dose-dependently reduced compared to those in the HFD group. In addition, HOMA-IRs of the DL and DH groups were noticeably decreased (Table 3). Therefore, DLE decreases insulin and fasting glucose levels.

3.6. Improvement in glucose disposal and insulin sensitivity by DLE

To evaluate the effect of DLE on glucose homeostasis, we conducted a glucose tolerance test (GTT). The glucose level at 0 min was significantly higher in the HFD group than in the NCD groups. The highest peak was observed at 30 min and decreased in time-dependent fashion. Significant dose-dependent decreases were observed in the DL and DH groups' glucose levels compared to that of the HFD group (Fig. 3A). Furthermore, the area under the curve (AUC) was significantly decreased in the DL and DH groups even though it was elevated by HFD (Fig. 3B).

3.7. Reduced phosphorylation of AMPK by DLE

In order to examine whether DLE activates the AMPK pathway, we examined its effect on phosphorylation of AMPK in liver and muscle protein. Phosphorylation of AMPK was significantly decreased in the HFD group but significantly increased in the DL

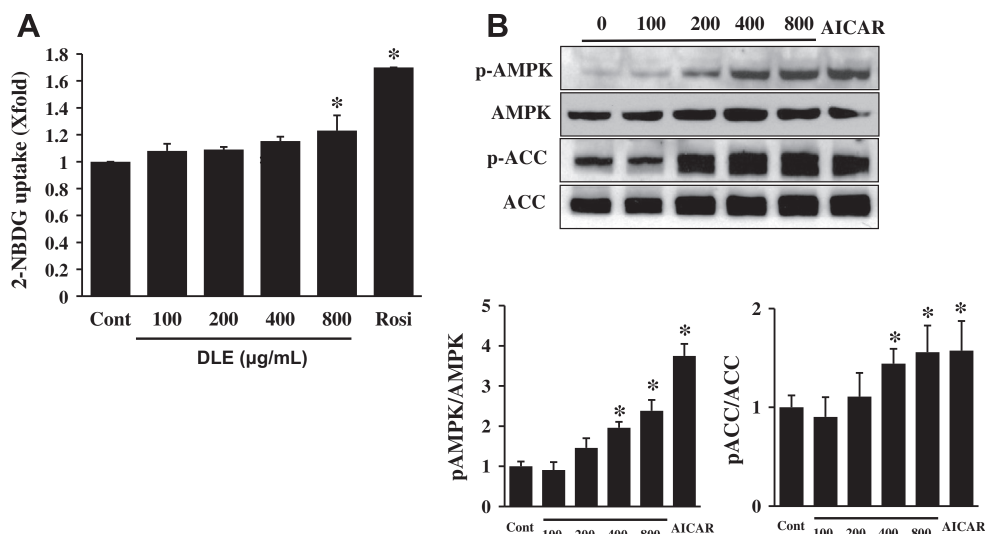


Fig. 1. Effect of DLE on glucose uptake, and AMPK/ACC phosphorylation in C2C12 myotubes. Cells were treated with the indicated DLE in a dose-dependent manner before the 2-NBDG uptake assay was performed. Rosiglitazone (Rosi), a PPAR-γ agonist, was used as the positive control. Data expressed as mean ± SD. $^{*}p < 0.05$ compared to Cont(A). Cells were treated with the DLE for 3 h in a dose-dependent manner. Western blot analysis was performed with phospho-AMPK, phospho-ACC, AMPK, and ACC antibodies in C2C12 myotubes (B). We used 1 mM AICAR as the positive control for AMPK activation. Western blotting bands were analyzed with ImageJ software, and relative ratios were calculated. Data expressed as mean ± SD. $^{*}p < 0.05$ compared to Cont.

Table 1
Effect of DLE on HFD-induced food intake, body mass, weight gain, and liver weight.

	NCD	HFD	DL	DH
Food intake (g/day)	3.19 ± 0.12	2.47 ± 0.21*	2.53 ± 0.21	2.57 ± 0.25
Body mass initial (g)	20.4 ± 1.1	19.9 ± 0.8	19.4 ± 0.6	19.4 ± 1.1
Body mass final (g)	27.8 ± 1.7	40.4 ± 2.1*	35.4 ± 2.3#	37.4 ± 2.4#
Weight gain (g)	7.6 ± 1.8	20.5 ± 1.6*	15.9 ± 2.5#	17.9 ± 1.7#
Liver (g)	0.96 ± 0.07	1.17 ± 0.13*	0.97 ± 0.09#	1.05 ± 0.10#

Notes: Diets were fed for 10 weeks. Food intake and body mass were determined daily and weekly respectively. Whole, wet livers were weighed just after sacrifice. Data are expressed as mean ± SD.
* $p < 0.05$ compared to NCD.
\# $p < 0.05$ compared to HFD.

Table 2
Effect of DLE on serum TG and TC induced by HFD.

	NCD	HFD	DL	DH
TG (mg/dl)	68.44 ± 9.84	78.49 ± 6.39*	61.15 ± 9.15#	72.77 ± 6.3#
Total cholesterol (mg/dl)	79.1 ± 2.98	94.8 ± 2.71*	93 ± 1.74	77.6 ± 2.12#

Notes: Serum TG and TC concentrations (estimated using the Asan Triglyceride and Total Cholesterol Kit according to manufacturer's instructions) in 4 groups; NCD; HFD; and DL and DH (HFD supplemented with low- and high-dose dandelion extract, respectively). Data expressed as mean ± SD.
* $p < 0.05$ compared to NCD.
\# $p < 0.05$ compared to HFD.

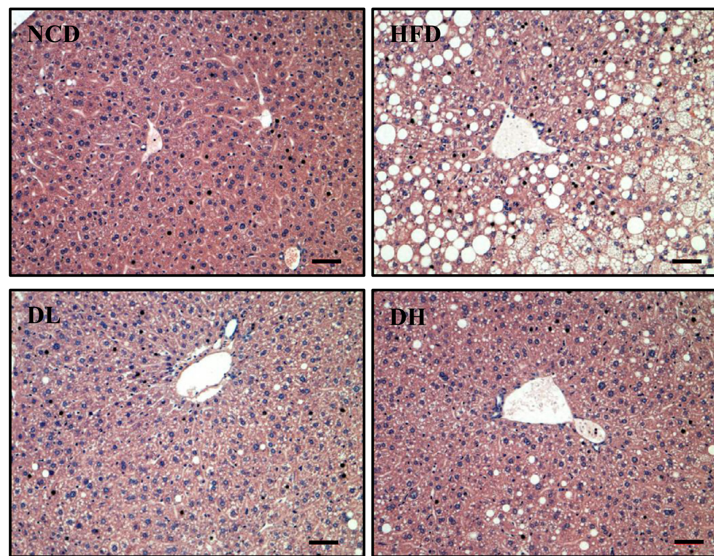
and DH groups compared to that of the HFD group (Fig. 4). Thus, DLE may have a beneficial effect on obesity induced by HFD. However, further experiments in this area should be performed in the future.

4. Discussion

NAFLD is a chronic liver disease characterized by abnormal lipid formation in hepatocytes without excess alcohol intake. NAFLD refers to wide spectrum of liver disease ranging from simple fatty liver to nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis, and is usually associated with obesity and type 2 diabetes. Fatty liver and insulin resistance are common clinicopathological conditions of both NAFLD and obesity because they involve lipid accumulation (steatosis), formation of lipid accumulation, and inflammation (hepatitis) in the liver due to dysfunction of adipose, liver, and muscle tissues and resultant imbalance of lipid metabolism (Polyzos et al., 2009; Després and Lemieux, 2006). Our data (Fig. 2) show that compared to the NCD group, the HFD group developed noticeably greater lipid accumulation in the liver tissue and that body and liver weights in the HFD group were also dramatically increased compared to those of the NCD group (Table 1). However, supplementation with DLE significantly lowered lipid accumulation in the liver and suppressed body and liver weight increases compared to those of the HFD group.

Obesity causes altered function of adipocytes, which leads to expanded adipocyte mass; increased insulin secretion; and

A



B

	NCD	HFD	DL	DH
Steatosis	0.3 ± 0.1	3.2 ± 0.5*	1.3 ± 0.4#	1.5 ± 0.3#
Necroinflammation	0.1 ± 0.1	2.1 ± 0.3*	1.5 ± 0.3#	1.6 ± 0.3#

Fig. 2. Effect of DLE on HFD-induced lipid accumulation. Liver tissues were fixed with 4% paraformaldehyde, embedded in paraffin, and H&E stained. Livers of the NCD group appeared normal, while livers of the HFD group showed accumulation of lipids. The accumulation of lipid induced by HFD was significantly decreased by supplementation of dandelion extract (DL and DH groups). This figure is representative of 5 different images taken of each sample under 100× magnification using a Nikon Eclipse 80i microscope. (B) Steatosis and inflammatory scores. Values of hepatic steatosis and necroinflammation are presented as mean ± SD ($n = 6$ per group). *$p < 0.05$ compared to NCD and #$p < 0.05$ compared to HFD.

Table 3
Effect of DLE on HFD-induced levels of serum insulin and fasting blood glucose.

	NCD	HFD	DL	DH
Insulin (ng/mL)	0.23 ± 0.06	2.27 ± 0.35*	1.29 ± 0.33#	1.27 ± 0.35#
Fasting blood glucose (mg/dl)	115 ± 3	201 ± 7.0*	168 ± 7.4#	170 ± 8.3#
HOMA-IR	1.95 ± 0.51	33.43 ± 5.45*	16.77 ± 5.3#	16.86 ± 5.48#

Notes: Fasting blood glucose was determined just before GTT. Insulin level was determined using the Shibayagi Mouse Insulin ELISA Kit according to the manufacturer's instructions. Data expressed as mean ± SD.
* $p < 0.05$ compared to NCD.
\# $p < 0.05$ compared to HFD.

increased release of FFAs in the blood, which increases the amount of TG stored in the liver (Kim et al., 2012; Olefsky, 2008). The excess storage of TG in the liver develops into larger and more abundant lipid accumulations, resulting in fatty liver (Bradbury, 2006; Cho et al., 2010; Park et al., 2010). The liver metabolizes FFAs by 2 pathways, both of which are strongly related to hepatic steatosis (Browning and Horton, 2004). The first pathway is oxidation of FFAs to generate ATP. Mitochondrial β–oxidation of FFAs will produce ATP, some of which may be used for synthesis of phospholipids and mediators and some part of the carbon skeleton of an FFA used to synthesize glucose, cholesterol, and other compounds. The second pathway is esterification of FFAs to produce TG, which can be used for production of very-low-density lipoprotein (VLDL) particles (Bradbury, 2006). Since the obvious source of FFA increase is high dietary fat intake, obese subjects will have higher TG and TC levels compared to those of lean subjects. In this study, the levels of TG and TC in the HFD group were greatly increased compared to those in the NCD group. However, the levels of TG and TC were significantly decreased with DLE supplementation (Table 2). These results suggest that DLE supplementation has a preventive effect on HFD-induced lipid accumulation in the liver by controlling the metabolism of FFAs.

Insulin is a hormone that plays an important role in the control of blood glucose levels and regulation of lipid metabolism in liver, muscle, and fat tissue (Hegarty et al., 2002). Obesity reduced responsiveness to insulin in promoting glucose uptake and increased number of adipocytes, respectively, and are associated with inflammation and oxidative stress, resulting in type 2 diabetes. Our data show that mean serum insulin and glucose levels and HOMA-IR of the HFD group were significantly increased compared to those of the NCD group. However, increases in serum insulin and fasting glucose levels were significantly lesser with DLE supplementation. Calculated HOMA-IR was also significantly lower in the DL and DH groups than in the HFD group (Table 3). In addition, our GTT results demonstrate that the ability of insulin to regulate blood glucose was also improved by DLE supplementation, with blood glucose level in the DH group decreased to the level of the NCD group (Fig. 3). Therefore, these results suggest that DLE

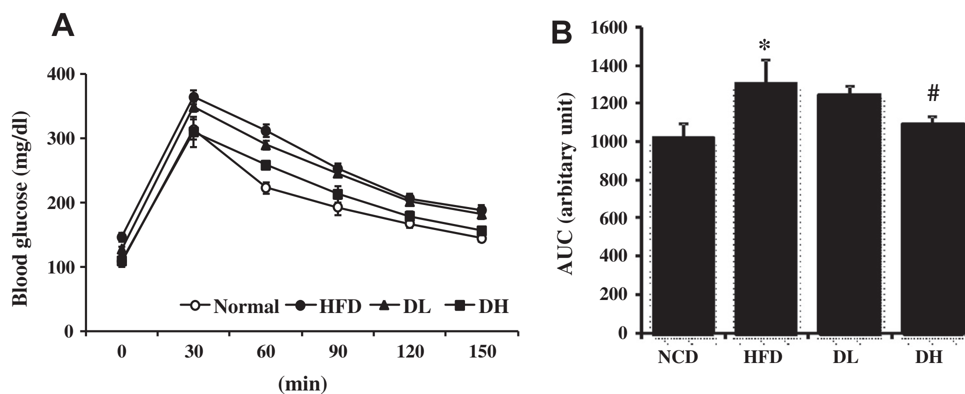


Fig. 3. Effect of DLE on glucose tolerance test. Blood glucose level (A) was determined using Accu-Check glucose determination kit, and AUC of glucose (B) was calculated. Data expressed as mean ± SD. $^{*}p < 0.05$ compared to NCD and $^{\#}p < 0.05$ compared to HFD.

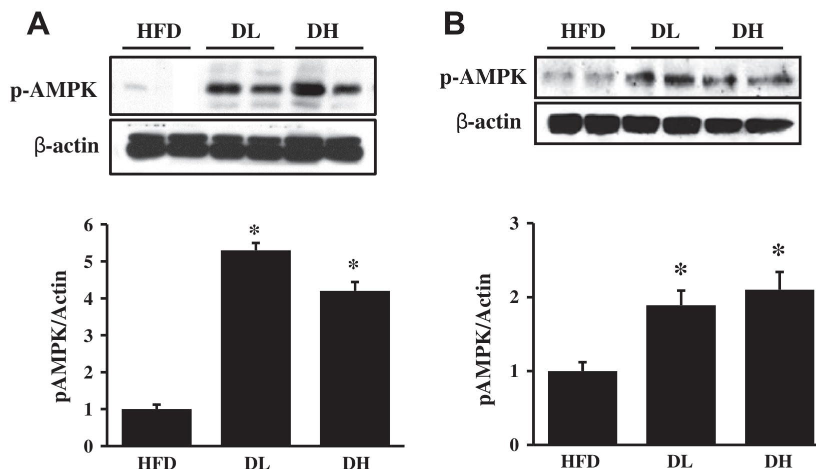


Fig. 4. Effect of DLE on AMPK signaling pathway. DLE activated AMPK signaling from liver (A), and muscle (B) tissue protein. Total protein was collected from liver and muscle tissue (10–20 mg). Western blotting bands were analyzed with ImageJ software, and relative ratios were calculated. Data expressed as mean ± SD. $^{*}p < 0.05$ compared to NCD and $^{\#}p < 0.05$ compared to HFD.

supplementation lowered serum insulin and fasting glucose levels, which indicates improved insulin sensitivity. It appears that DLE is not suitable for healthy or lean mice, whereas it is suitable for restoring insulin sensitivity in obese mice.

AMPK is one of the best-known target proteins regulating the metabolic pathway and functioning as an energy sensor (Hardie, 2008). Activation of AMPK occurs upon depletion of ATP and blocks energy-consuming processes such as glucose uptake and β-oxidation in fat, muscle, and liver. Hepatic AMPK is activated by adipocyte-derivative hormones, such as adiponectin, resistin, and leptin, under normal conditions (Mhairi et al., 2007).

In liver and muscle tissue, there is an inverse relationship between AMPK and elevation of sterol regulatory element binding protein-1c (SREBP-1c) activity (Kim et al., 2011). SREBP-1c leads to increases in fatty acid synthesis as a result of the induction of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC). AMPK decreases FAS and ACC by suppressing SREBP-1c (Goldstein et al., 2002; Ouadda et al., 2009). However, the mechanism underlying AMPK regulation of SREBP-1c activity in the control of lipid homeostasis, especially insulin resistance, is still unknown. Stimulation of lipoprotein secretion by these hormones may protect the liver from intracellular lipid accumulation. Muscle tissue is a primary site for insulin resistance and is the tissue responsible for glucose disposal stimulated by insulin. Therefore, activation of AMPK may contribute to the reduction of lipid accumulation in liver, glucose tolerance, and insulin resistance induced by HFD (Hiquchi et al., 2011). Our data show that phosphorylation of AMPK in the HFD group was significantly suppressed and supplementation with DLE greatly recovered the phosphorylation of AMPK in liver tissues (Fig. 4A). Moreover, our data show that DLE-stimulated glucose uptake is accompanied by the activation of AMPK and ACC in cultured muscle cells (Fig. 1). DLE treatment recovered significantly HFD-induced inhibition of AMPK phosphorylation in muscle tissues (Fig. 4B). These results suggest that DLE may have a suppressive effect on HFD-induced lipid accumulation in the liver and may ameliorate insulin-stimulated glucose and insulin resistance in the muscle via an AMPK/ACC-mediated pathway.

Numerous scientific reports cite the identification of several flavonoids and phytochemicals, including coumaric acid, chicoric acid, monocaffeoyltartaric acid, cinnamic acid, caffeic acid, chlorogenic acid, and luteolin, in dandelion leaf (Cho et al., 2010; Hu and Kitts, 2005; Huanga and Shenb, 2012; Schutz et al., 2006; Williams et al., 1996). Among these compounds, caffeic acid has been shown to improve glucose disposal in insulin-resistant mice (Huanga and Shenb, 2012), chlorogenic acid has been reported to have anti-obesity properties in HFD-induced obesity (Cho et al., 2010), and luteolin has been shown to have a therapeutic effect on CCl_4-induced liver fibrosis (Domitrovića et al., 2009). Our data show that DLE contains luteolin and chlorogenic acid; therefore, we hypothesize that the suppressive effect of DLE on HFD-induced hepatic lipid accumulation is due to these flavonoids.

In conclusion, DLE significantly suppressed lipid accumulation in the liver, reduced insulin resistance, and lipid in HFD-fed C57BL/6 mice via the AMPK pathway. These findings may provide molecular evidence for the use of DLE as a therapy in the management of fatty liver and obesity-relative disorders.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported by the Food Functionality Evaluation Program of the Ministry of Food, Agriculture, Forestry and Fisheries and by the Korea Food Research Institute.

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