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Immature Persimmon (*Diospyros kaki* Thunb.) Ethanol Extract Ameliorates High-Fat Diet-Induced Obesity by Modulating Lipid Metabolism

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ABSTRACT: In this study, immature persimmon (*Diospyros kaki* Thunb.) ethanol extract was administered to an obese animal model fed a high-fat diet to measure weight change, adipose tissue weight, serum lipid level, and expression level of adipose-related genes to evaluate its efficacy. Administration of *D. kaki* ethanol extract (DKE) (100 and 500 mg/kg/d) decreased the body weight gain, adipose tissue weight, and serum triglyceride levels in mice fed a high-fat diet. Furthermore, it improved the leptin and adiponectin levels in the blood as well as gene expression in the liver. It also inhibited the expression of sterol regulatory element-binding protein-1c, inhibiting the production of triglyceride biosynthetic enzyme fatty acid synthesis and acetyl-CoA carboxylase, and decreased the expressions of peroxisome proliferator-activated receptor γ and CCAAT/enhancer-binding proteins that induce adipocyte differentiation. Therefore, these data suggest that DKE exerts beneficial effects on high-fat diet-induced obesity by modulating lipid metabolism in mice fed a high-fat diet.

Keywords: *Diospyros kaki* Thunb., high-fat diet, lipid metabolism, obesity

INTRODUCTION

Obesity is a chronic metabolic disease caused by an imbalance in the accumulation and consumption of excessive energy; it is characterized by an increase in fat and blood fat contents (Devlin et al., 2000; Haslam and James, 2005). Fat accumulation is defined as an increase in the number and size of adipocytes, and differentiated adipocytes store fatty acids in the cytoplasm in the form of triacylglycerol (Spiegelman and Flier, 1996). Obesity itself limits activities of daily living, but the greatest concern is the increased incidence of cardiovascular diseases, such as hyperlipidemia, hypertension, and arteriosclerosis (Kopelman and Grace, 2004; Gesta et al., 2007). Obesity increases the amount of triglycerides and cholesterol in the blood, leading to hyperlipidemia (Charlton, 2009), which accumulates triglycerides in the peripheral tissues and abdomen, thus increasing resistance to insulin, respiratory dysfunction, infertility, menstrual irregularities, osteoarthritis, and cancer (Doyle et al., 2012; Buras et al., 2019). Therefore, since obesity can cause various complications, continuous management, and treatment are necessary (Bray and Greenway, 1999). Consumption of antiobesity foods and food ingredients may help prevent obesity and other lifestyle-related diseases as they can effectively reduce visceral fat mass (Saito et al., 2005).

Immature persimmon (*Diospyros kaki* Thunb.) has a wide distribution in Korea, China, Japan, Brazil, Turkey, and Italy (Itamura et al., 2005). Its fruits and leaves have been widely used as herbal medicines (Matsuo and Ito, 1978). Consumption of ripe persimmon fruit has been practiced for a long time; contrarily, unripe, or immature, persimmon fruit is difficult to consume owing to its astringent texture; thus, it is not valuable as food. However, condensed tannins in unripe persimmon are known to be effective in improving lipid metabolism (Gorinstein et al., 1998), alcohol metabolism (Kim et al., 2001), anti-microbial properties (Harada et al., 2005), and antioxidant properties (Lee et al., 2001); they also have high potential to be used as functional food materials (Butt et al., 2015).

High-fat diet (HFD) has been commonly used to induce obesity in animal models because obesity exerts the same effect on both humans and animals (Hansen et al., 1997; Katagiri et al., 2007). Thus, this study investigated the

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antiobesity potential of immature persimmon extract in mice fed an HFD.

MATERIALS AND METHODS

Preparation of *D. kaki* ethanol extract (DKE)

Immature persimmon (*D. kaki* Thunb.) fruits used in this study were harvested in Jeju Special Self-Governing Province in August 2017. The whole fruit was washed, pulverized, and freeze-dried. The freeze-dried immature persimmon powder was extracted with 50% ethanol (Woori Ethanol Supplies Company) for 24 h. The resulting ethanol solution was filtered and then concentrated under reduced pressure to obtain the extract. The final yield of the *D. kaki* Thunb. ethanol extract was 30% (w/w) compared with that of the aforementioned immature persimmon powder.

Animals

This study was approved by the Institutional Animal Care and Use Committee of Jeju National University for the efficient management and review of the ethical and scientific validity of animal experiments (approval number: 2018-0043). Male 5-week-old C57BL/6 mice were supplied by a central animal laboratory (Central Lab. Animal Inc.), and the animals were sufficiently fed with solid food and water until the day of the experiment. In addition, the mice were acclimated for 1 week while maintaining the environment of the light-dark cycle for a temperature of 22±2°C, humidity of 55±15%. After adaptation, the experimental animals were divided into five groups [normal diet (ND) group, HFD (60% kcal, Research Diets, Inc.) group, positive control group (Orlistat, 30 mg/kg, Sigma-Aldrich), and DKE-administered groups (100 and 500 mg/kg)], and food and drinking water were given *ad libitum*. HFD was administered to the mice for 2 weeks, and when the average weight of over 26 g was reached, Orlistat and the DKE were forcibly administered orally into the stomach for 6 weeks. The dietary intake and body weight of the mice were measured and recorded at a fixed time each week. The food efficiency ratio (FER) was calculated as (total weight gain/total food intake) ×100.

Lipid biochemical analysis in the serum

After the last experiment, blood was collected from the mice through cardiac puncture, and the serum was separated; subsequently, blood chemistry analysis was conducted. The contents of total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride in the isolated serum were measured using a quantitation kit (Sigma-Aldrich).

Analysis of leptin and adiponectin in the blood

Leptin and adiponectin in the isolated serum were analyzed. The diluted serum (100 μL) was dispensed into each well, reacted at 30°C for 1∼2 h, and washed twice with a washing buffer solution. Thereafter, the antibody avidin-horseradish peroxidase conjugate was treated and left to stand at room temperature for 1 h and then washed again. Next, 100 μL of the 3,3',5,5'-tetramethylbenzidine substrate was dispensed into the wells and then reacted in the dark for 30 min. Subsequently, 50 μL of the reaction stop solution was added, and absorbance was measured at a 450-nm wavelength using an enzyme-linked immunosorbent assay reader.

Obesity induction gene expression analysis

The gene expression patterns in liver tissues extracted from each experimental animal were analyzed through real-time polymerase chain reaction (PCR) amplification. After extracting RNA from each liver tissue using the RNAsol^{B} (Tel-Test Inc.) solution, cDNA and real-time PCR analyses were conducted using the KAPA SYBR® FAST qPCR Kit (Kapa Biosystems). RNAsol^{B} was added to the tissue, the tissue was crushed using a homogenizer, and chloroform was added to the mixture. After centrifugation (12,000 *g* at 15 min), the supernatant was recovered and mixed with 2-propanol; then, RNA was extracted, and cDNA was synthesized. Furthermore, real-time quantitative PCR was conducted using the iQ^{TM}5 Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories).

Statistical analysis

The results of each experimental group were statistically analyzed using unpaired Student's *t*-test, and significance tests were conducted at the level of $P<0.05$.

RESULTS AND DISCUSSION

Effect of DKE on body and fat weights in obese mice fed with HFD

Within the 6-week administration of DKE, a significant increase in mice body weight was observed after 2 weeks (Fig. 1). During the experiment, the body weight was 3.78±0.48 g in the ND group, 17.62±1.44 g in the HFD group, 6.88±0.64 g in the positive control group, as well as 6.91±1.97 g and 3.34±1.45 g in the DKE-administered groups (100 and 500 mg/kg, respectively). The DKE-administered groups had significantly decreased body weight than the HFD group, and the high concentration of 500 mg/kg was like the ND group. Furthermore, these groups had lower dietary efficiency than the HFD and positive control groups. In addition, the DKE-administered groups had similar or lower values in weight change, food intake,

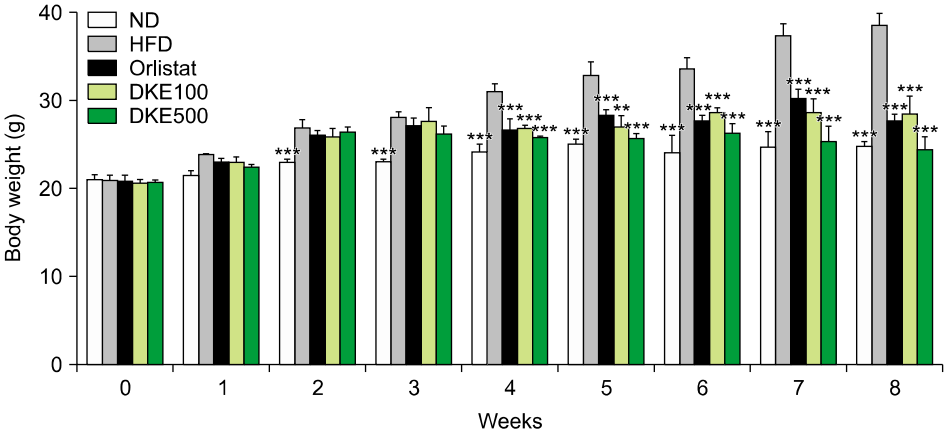

Fig. 1. Effect of *Diospyros kaki* extract (DKE) in high-fat diet (HFD)-fed C57BL/6 mice for the period of obesity induction. Changes in body weight were measured and recorded at a fixed time every week. Orlistat, a drug designed for the treatment of obesity, was a positive control. Values are presented as mean±SD. $^{**}P<0.01$ and $^{***}P<0.001$ compared with the HFD group. ND, normal diet; DKE100, DKE 100 mg/kg; DKE500, DKE 500 mg/kg (n=5 mice per group).

and FER than the positive control group (Table 1). These results indicate that DKE has similar efficacy to Orlistat, a drug that suppresses fat accumulation by inhibiting the absorption of nutrients that produce fat in the human body (Rössner et al., 2000).

When the intake of energy is greater than the consumption, excess energy accumulates as lipids, which are stored in the liver and abdomen (Fujioka, 2002). To confirm this, the antiobesity effect of DKE was explored by measuring the weight around the liver, abdomen, and kidneys of the mice in this animal experiment, which are presented in Table 1. The liver weight was decreased by both the 100- and 500-mg/kg concentrations of DKE, with the latter showing lower values than Orlistat, similar to the change in body weight ($P<0.001$). DKE also significantly inhibited the production of abdominal visceral and perirenal fats compared with HFD ($P<0.001$).

Persimmon has various pharmacological activities based on its high antioxidant effect derived from different bioactive compounds, such as tannins (George and Redpath, 2008; Fukai et al., 2009). Immature persimmon fruit exhibits astringency due to the presence of tannins called kaki-tannin (Matsuo and Ito, 1978). Tannins have been reported to inhibit lipid droplet formation and stimulate lipolysis by increasing glycerol release in the 3T3-L1 adipocyte (Kim et al., 2008; Shin et al., 2014). Furthermore, a previous study demonstrated that supplementation with tannins tended to decrease body weight and liver lipids in an HFD-fed animal (Yugarani et al., 1992). Consistent with the results of this study, the tannin of DKE was found to reduce body weight in the experimental animals. Moreover, gallic acid produced by alkaline hydrolysis of tannins regulates body weight and glucose homeostasis through AMP-activated protein kinase activation and improves glucose tolerance and triglyceride concentration in mice with diet-induced obesity (Bak et al., 2013; Doan et al., 2015). Thus, it is suggested that the effects of DKE on body weight reduction may partly be due to polyphenols such as tannins contained in DKE. However, to elucidate the mechanism of the antiobesity action of DKE in animal experiments, further research on the active compounds contained in DKE is required.

Effect of DKE on blood biochemistry parameters in obese mice fed with HFD

These results suggest that DKE has the potential to improve obesity; therefore, we investigated the key downstream events related to lipid contents. When the amounts of total cholesterol and triglyceride in the blood increase, coronary artery disease occurs, which induces insulin resistance by accumulating triglycerides in the peripheral tissues (Bressler et al., 1996). LDL plays a role

Table 1. Changes in body weight gain, food intake, food efficiency ratio, liver weight, abdominal visceral fat weight, and perirenal fat weight in obese mice

Group	ND	HFD	Orlistat	DKE100	DKE500
Body weight gain (g/d)	3.78±0.48***	17.62±1.44	6.88±0.64***	6.91±1.97***	3.34±1.45***
Food intake (g/d)	3.11±0.29	2.69±0.54	2.36±0.39	2.57±0.46	2.24±0.57
Food efficiency ratio	0.78±0.43***	3.01±1.48	2.15±0.79**	2.05±0.82**	1.82±0.98***
Liver (g)	0.927±0.018	1.033±0.037	0.835±0.082**	0.786±0.067***	0.785±0.024***
Abdominal visceral fat (g)	0.422±0.073***	2.767±0.283	1.503±0.132***	1.276±0.316***	0.815±0.251***
Perirenal fat (g)	0.085±0.035***	1.055±0.060	0.552±0.018***	0.513±0.126***	0.289±0.102***

Values are presented as mean±SD. $^{**}P<0.01$ and $^{***}P<0.001$ compared with the HFD group.
Body weight gain and dietary intake were expressed as daily averages. Orlistat, a drug designed for the treatment of obesity, was a positive control.
ND, normal diet; HFD, high-fat diet; DKE100, *Diospyros kaki* extract 100 mg/kg; DKE500, *D. kaki* extract 500 mg/kg (n=5 mice per group).

in the transportation of cholesterol from the liver to the peripheral tissues, and high LDL-cholesterol (LDL-C) in the blood accumulates cholesterol in the coronary artery wall. In this study, the content of LDL-C in the blood was suppressed in the DKE-administered groups compared with the HFD group, but no significant results were observed (Fig. 2A). However, the triglyceride content was significantly lower in the DKE-administered groups than in the HFD group ($P<0.05$; Fig. 2B). In addition, the cholesterol ($P<0.05$) and HDL-cholesterol (HDL-C) ($P<0.01$) contents were significantly decreased in the DKE-administered group (500 mg/kg) than in the HFD group (Fig. 2C and 2D). In general, obese mice exhibit increased total cholesterol, LDL-C, and triglyceride concentrations and decreased HDL-C concentrations in the blood. However, recent studies have reported increased HDL-C concentration in the blood of obese mice (Williams et al., 2014; Ivanovic et al., 2015; Kim et al., 2016; Karandrea et al., 2017; Sun et al., 2017; Smoczek et al., 2020). Most of the extracts with antiobesity effects decrease body weight and total cholesterol, triglyceride, and LDL-C concentrations and increase HDL-C concentration. Nevertheless, consumption of methionine, conjugated linoleic acid, policosanol, or raw food, which exerts antiobesity effects like DKE, was found to reduce HDL-C concentration in the blood (Koebnick et al., 2005; Salas-Salvadó et al., 2006; Wendland et al., 2006; Velez-Carrasco et al., 2008; Yanai et al., 2015). Furthermore, changing food intake from a high-fat to a very-low-fat liquid formula diet, from a fixed 40% fat to polyunsaturated/saturated fat, or from a Western to a plant-based diet reduces HDL-C concentration in the blood (Shepherd et al., 1980; Nestel et al., 1981; Brinton et al., 1990; Mensink et al., 2003; Kent et al., 2013; Elliott et al., 2022). Previous studies have shown that the rate of mortality from heart disease and stroke increases when the HDL-C concentration in the blood of male and female is higher than 97 and 116 mg/dL, respectively, and HDL-C, which exerts an immunomodulatory effect, plays a role in redistributing a large amount of cholesterol, but when HDL is dysfunctional, results in loss of antiviral and antibacterial functions (Madsen et al., 2017). In addition, although the HDL-C concentration in the blood was high, the results indicated that if the LDL-C concentration was not reduced, heart disease could not be prevented (Madsen et al., 2018). That is, if the HDL-C concentration in the blood is excessively high, the mortality rate paradoxically increases; thus, it is more effective to reduce the cholesterol, triglyceride, and LDL-C concentrations while maintaining a certain level of HDL-C.

Effect of DKE on leptin and adiponectin in obese mice fed with HFD

Leptin secreted from adipocytes is a hormone that regulates appetite suppression and energy metabolism (Friedman and Halaas, 1998). It is known to be an indicator of body fat mass, and it has increased secretion in individuals with obesity (Gil et al., 2008). Contrarily, adiponectin, a hormone that inhibits inflammation and promotes insulin sensitivity, has decreased concentration in the blood of individuals with obesity (Gil et al., 2008). Obesity is not simply a matter of increasing body weight and adipose tissue but is a chronic metabolic disease that affects systemic function. Among various symptoms caused by obesity, nonalcoholic fatty liver disease causes liver damage (Younossi et al., 2016). Obesity is often ac-

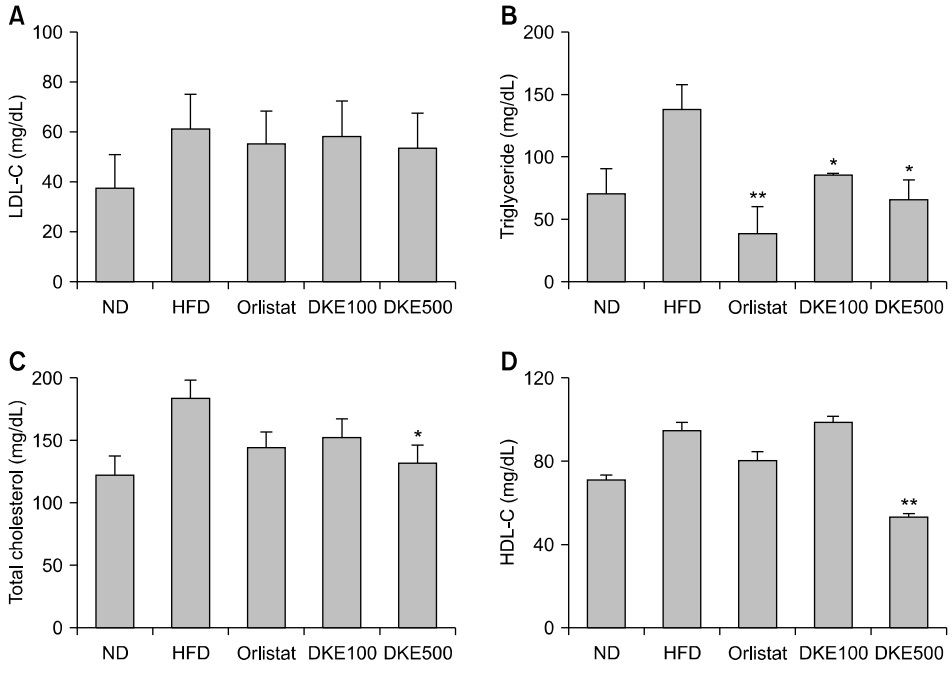

Fig. 2. Effect of *Diospyros kaki* extract (DKE) on blood biochemistry parameters in high-fat diet (HFD)-fed obese mice. Lipid biochemical analysis was conducted on the serum isolated from each experimental animal. The levels of (A) low-density lipoprotein-cholesterol (LDL-C), (B) triglyceride, (C) total cholesterol, and (D) high-density lipoprotein-cholesterol (HDL-C) were evaluated. Orlistat, a drug designed for the treatment of obesity, was a positive control. Values are presented as mean±SD. $^{*}P<0.05$ and $^{**}P<0.01$ compared with the HFD group. ND, normal diet; DKE100, DKE 100 mg/kg; DKE500, DKE 500 mg/kg (n=5 mice per group).

companied by a lipid metabolism disorder in the liver, which leads to liver damage due to lipid accumulation (Wu et al., 2020). Therefore, this study focused on obesity as a systemic disease and confirmed the expressions of genes related to adipocytes and lipid metabolism in the liver tissue. Compared with HFD, the leptin content in the blood of mice was significantly suppressed by the administration of DKE (100 and 500 mg/kg) ($P<0.01$ and $P<0.001$, respectively; Fig. 3A). The results of the gene expression analysis indicated that the leptin content in the liver of mice was increased in the HFD group but was significantly decreased in the DKE-administered groups (Fig. 3B). Furthermore, the adiponectin content in the blood of mice was significantly increased by the administration of DKE compared with HFD, but no significant differences were observed (Fig. 3C). However, the results of the gene expression analysis indicated increased adiponectin levels in the liver of mice in the DKE-administered groups (Fig. 3D).

Effect of DKE on gene mRNA expression in the liver of obese mice fed with HFD

Obesity is caused by the differentiation of fat cells and synthesis of fatty acids and cholesterol in the human body (Spiegelman and Flier, 1996; Jakab et al., 2021). The major lipid-producing transcriptional regulators are members of the CCAAT/enhancer-binding protein (C/EBP) family and peroxisome proliferator-activated receptor γ (PPARγ) (Rosen and Spiegelman, 2000; Payne et al., 2009; Oh et al., 2019). They act synergistically to induce obesity by expressing C/EBPα, PPARγ, and sterol regulatory element-binding protein-1c (SREBP-1c) (Kim and Spiegelman, 1996; Payne et al., 2009). Previous studies have demonstrated that PPARγ is necessary and sufficient to promote adipogenesis and that C/EBPα is influential in maintaining its expression (Barak et al., 1999; Wu et al., 1999; Wafer et al., 2017). Compared with HFD, the mRNA expressions of PPARγ and C/EBPs in the liver tissue of mice was suppressed by the administration of DKE, and significant differences were observed (Fig. 4A∼4C).

In the lipid synthesis process, the enzymes affecting the conversion to triglycerides are fatty acid synthesis (FAS) and acetyl-CoA carboxylase (ACC), and their expression is regulated by SREBP-1c acting as a transcription factor (Shimano, 2001). To confirm the correlation between these enzymes, the effect of DKE on the expression of obesity-related genes in the liver tissue was evaluated. The results indicated that the expression of SREBP-1c in the DKE-administered group was significantly decreased compared with the HFD group (Fig. 4D). The FAS and ACC gene expressions were also decreased in a concentration-dependent manner (Fig. 4E and 4F), suggesting that it is involved in the accumulation of lipids in the liver tissue of mice with HFD-induced obesity. Because overexpression of FAS contributes to the development of obesity and chronic fatty liver disease (Wueest et al., 2010), the reduction of FAS and ACC caused by the DKE administration is thought to inhibit fat accumulation in the liver. Thus, it was suggested that DKE decreased the expression of SREBP-1c, inhibiting the production of FAS and ACC and reducing the expressions of PPARγ and C/EBPs. These results indicate that immature persimmon exerts antiobesity effects by inhibiting the expressions of enzymes involved in lipid synthesis and adipocyte differentiation.

In conclusion, in HFD-fed mice, immature persimmon

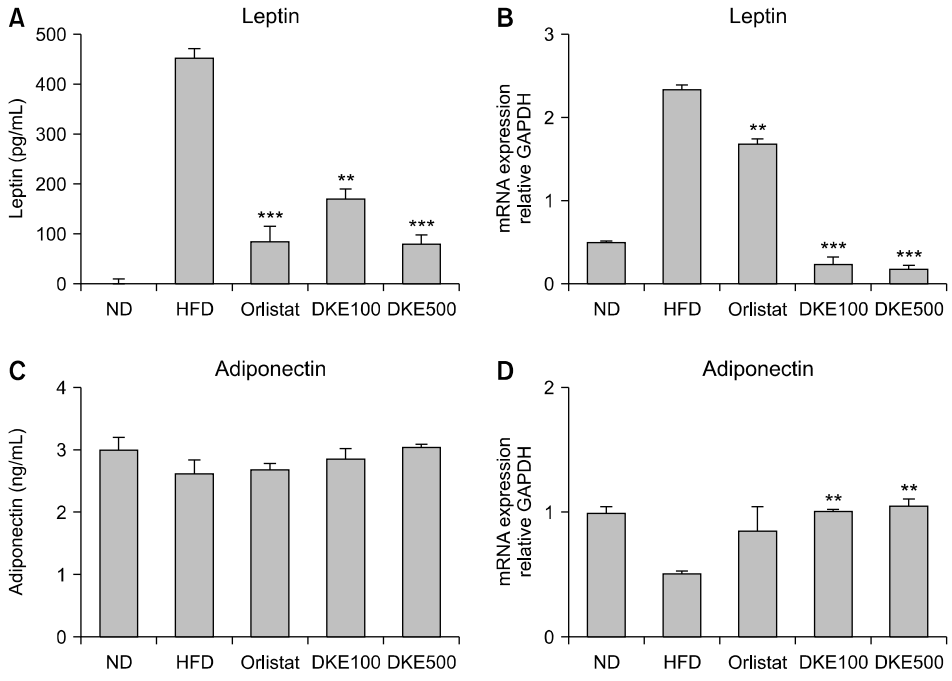

Fig. 3. Effect of *Diospyros kaki* extract (DKE) on leptin and adiponectin in high-fat diet (HFD)-fed obese mice. Leptin and adiponectin were measured in the serum isolated from each experimental animal. The mRNA expression in the liver tissues extracted from each experimental animal was analyzed through real-time polymerase chain reaction amplification. The levels of (A) leptin, (B) leptin mRNA, (C) adiponectin, and (D) adiponectin mRNA were confirmed. Orlistat, a drug designed for the treatment of obesity, was a positive control. Values are presented as mean±SD. $^{**}P<0.01$ and $^{***}P<0.001$ compared with the HFD group. ND, normal diet; DKE100, DKE 100 mg/kg; DKE500, DKE 500 mg/kg (n=5 mice per group); GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

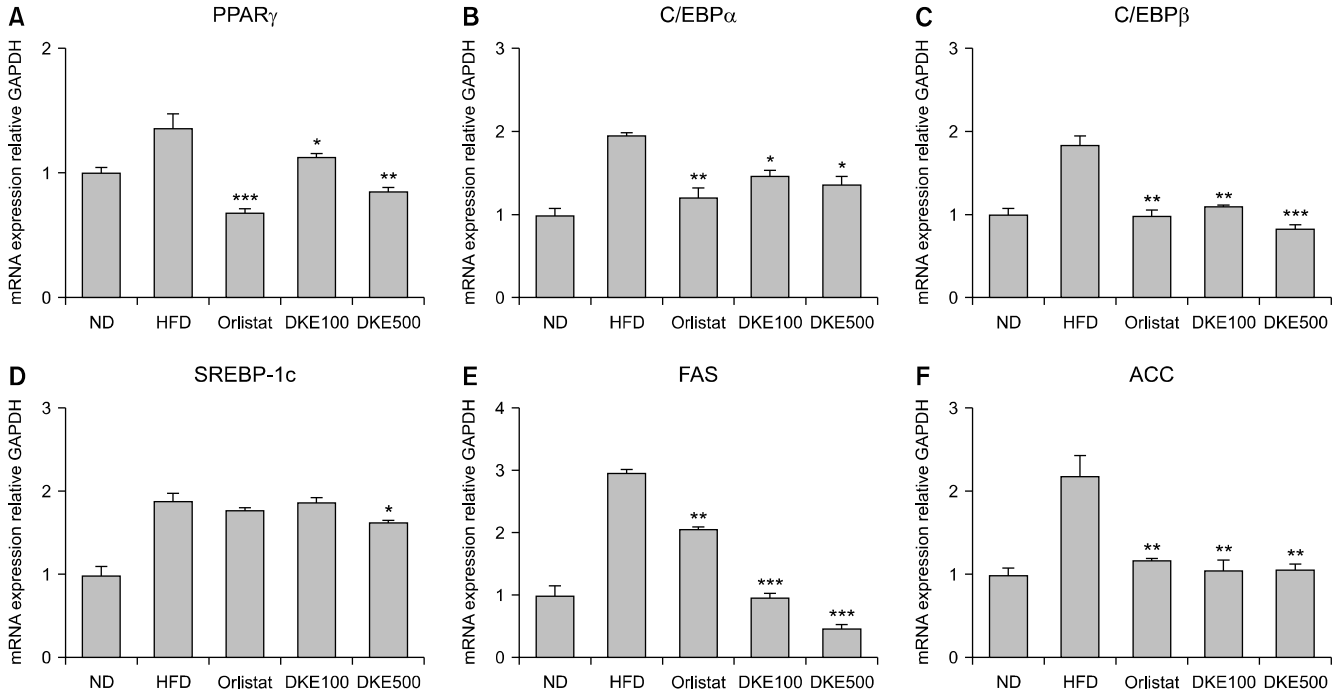

Fig. 4. Effect of *Diospyros kaki* extract (DKE) on the gene mRNA expression in the liver of high-fat diet (HFD)-fed obese mice. The gene expression patterns in the liver tissues extracted from each experimental animal were analyzed through real-time polymerase chain reaction amplification. The gene mRNA expressions in the liver of (A) PPARγ, (B) C/EBPα, (C) C/EBPβ, (D) SREBP-1c, (E) FAS, and (F) ACC were investigated. Orlistat, a drug designed for the treatment of obesity, was a positive control. Values are presented as mean±SD. *P<0.05, **P<0.01, and ***P<0.001 compared with the HFD group. ND, normal diet; DKE100, DKE 100 mg/kg; DKE500, DKE 500 mg/kg (n=5 mice per group); GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

reduced the body weight gain, adipose tissue weight, and serum triglyceride level. Furthermore, it decreased the expressions of genes involved in lipid biosynthesis and adipocyte differentiation by acting as a drug designed for the treatment of obesity. Therefore, extracts from natural resources like unripe persimmon are considered suitable for preventing or improving obesity through lipid metabolism modulation.

FUNDING

None.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: SAY, YMH, SCH. Analysis and interpretation: SAY, SCH, HBH, BG. Data collection: SCH. Writing the article: SAY. Critical revision of the article: YMH, YHJ, WJY. Final approval of the article: all authors. Statistical analysis: SCH, ESY. Overall responsibility: WJY.

REFERENCES

Bak EJ, Kim J, Jang S, Woo GH, Yoon HG, Yoo YJ, et al. Gallic acid improves glucose tolerance and triglyceride concentration in diet-induced obesity mice. Scand J Clin Lab Invest. 2013. 73: 607-614.

Barak Y, Nelson MC, Ong ES, Jones YZ, Ruiz-Lozano P, Chien KR, et al. PPARγ is required for placental, cardiac, and adipose tissue development. Mol Cell. 1999. 4:585-595.

Bray GA, Greenway FL. Current and potential drugs for treatment of obesity. Endocr Rev. 1999. 20:805-875.

Bressler P, Bailey SR, Matsuda M, DeFronzo RA. Insulin resistance and coronary artery disease. Diabetologia. 1996. 39:1345-1350.

Brinton EA, Eisenberg S, Breslow JL. A low-fat diet decreases high density lipoprotein (HDL) cholesterol levels by decreasing HDL apolipoprotein transport rates. J Clin Invest. 1990. 85:144-151.

Buras ED, Converso-Baran K, Davis CS, Akama T, Hikage F, Michele DE, et al. Fibro-adipogenic remodeling of the diaphragm in obesity-associated respiratory dysfunction. Diabetes. 2019. 68:45-56.

Butt MS, Sultan MT, Aziz M, Naz A, Ahmed W, Kumar N, et al. Persimmon (*Diospyros kaki*) fruit: hidden phytochemicals and health claims. EXCLI J. 2015. 14:542-561.

Charlton M. Obesity, hyperlipidemia, and metabolic syndrome. Liver Transpl. 2009. 15:S83-S89.

Devlin MJ, Yanovski SZ, Wilson GT. Obesity: what mental health professionals need to know. Am J Psychiatry. 2000. 157:854-866.

Doan KV, Ko CM, Kinyua AW, Yang DJ, Choi YH, Oh IY, et al. Gallic acid regulates body weight and glucose homeostasis through AMPK activation. Endocrinology. 2015. 156:157-168.

Doyle SL, Donohoe CL, Lysaght J, Reynolds JV. Visceral obesity, metabolic syndrome, insulin resistance and cancer. Proc Nutr

Soc. 2012. 71:181-189.
Elliott PS, Kharaty SS, Phillips CM. Plant-based diets and lipid, lipoprotein, and inflammatory biomarkers of cardiovascular disease: a review of observational and interventional studies. Nutrients. 2022. 14:5371. https://doi.org/10.3390/nu14245371
Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature. 1998. 395:763-770.
Fujioka K. Management of obesity as a chronic disease: nonpharmacologic, pharmacologic, and surgical options. Obes Res. 2002. 10:116S-123S.
Fukai S, Tanimoto S, Maeda A, Fukuda H, Okada Y, Nomura M. Pharmacological activity of compounds extracted from persimmon peel (*Diospyros kaki* THUNB.). J Oleo Sci. 2009. 58:213-219.
George AP, Redpath S. Health and medicinal benefits of persimmon fruit: a review. Adv Hortic Sci. 2008. 22:244-249.
Gesta S, Tseng YH, Kahn CR. Developmental origin of fat: tracking obesity to its source. Cell. 2007. 131:242-256.
Gil JH, Lee JA, Kim JY, Hong YM. Leptin, adiponectin, interleukin-6 and tumor necrosis factor-α in obese adolescents. Korean J Pediatr. 2008. 51:597-603.
Gorinstein S, Bartnikowska E, Kulasek G, Zemser M, Trakhtenberg S. Dietary persimmon improves lipid metabolism in rats fed diets containing cholesterol. J Nutr. 1998. 128:2023-2027.
Hansen PA, Han DH, Nolte LA, Chen M, Holloszy JO. DHEA protects against visceral obesity and muscle insulin resistance in rats fed a high-fat diet. Am J Physiol. 1997. 273:R1704-R1708.
Harada M, Sakagami R, Watanabe T, Onizuka T, Kato H, Nagai A. Antibacterial and deodorizing effect of persimmon tannin (Pancil). Jpn J Conserv Dent. 2005. 48:314-319.
Haslam DW, James WP. Obesity. Lancet. 2005. 366:1197-1209.
Itamura H, Zheng Q, Akaura K. Industry and research on persimmon in Japan. Acta Hortic. 2005. 685:37-44.
Ivanovic N, Minic R, Dimitrijevic L, Radojevic Skodric S, Zivkovic I, Djordjevic B. *Lactobacillus rhamnosus* LA68 and *Lactobacillus plantarum* WCFS1 differently influence metabolic and immunological parameters in high fat diet-induced hypercholesterolemia and hepatic steatosis. Food Funct. 2015. 6:558-565.
Jakab J, Miškić B, Mikšić Š, Juranić B, Ćosić V, Schwarz D, et al. Adipogenesis as a potential anti-obesity target: a review of pharmacological treatment and natural products. Diabetes Metab Syndr Obes. 2021. 14:67-83.
Karandrea S, Yin H, Liang X, Slitt AL, Heart EA. Thymoquinone ameliorates diabetic phenotype in Diet-Induced Obesity mice via activation of SIRT-1-dependent pathways. PLoS One. 2017. 12:e0185374. https://doi.org/10.1371/journal.pone.0185374
Katagiri K, Arakawa S, Kurahashi R, Hatano Y. Impaired contact hypersensitivity in diet-induced obese mice. J Dermatol Sci. 2007. 46:117-126.
Kent L, Morton D, Rankin P, Ward E, Grant R, Gobble J, et al. The effect of a low-fat, plant-based lifestyle intervention (CHIP) on serum HDL levels and the implications for metabolic syndrome status—a cohort study. Nutr Metab. 2013. 10:58. https://doi.org/10.1186/1743-7075-10-58
Kim HJ, Yun YR, Song YB, Song YO. Anti-lipogenic effects of tannic acid in 3T3-L1 adipocytes and in high fat diet-fed rats. Food Sci Biotechnol. 2008. 17:362-366.
Kim JB, Spiegelman BM. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes Dev. 1996. 10:1096-1107.
Kim SG, Lee YC, Suh KG, Choi HS. Acetaldehyde dehydrogenase activator from persimmon and its processed foods. J Korean Soc Food Sci Nutr. 2001. 30:954-958.
Kim Y, Kwon MJ, Choi JW, Lee MK, Kim C, Jung J, et al. Anti-obesity effects of boiled tuna extract in mice with obesity induced by a high-fat diet. Int J Mol Med. 2016. 38:1281-1288.
Koebnick C, Garcia AL, Dagnelie PC, Strassner C, Lindemans J, Katz N, et al. Long-term consumption of a raw food diet is associated with favorable serum LDL cholesterol and triglycerides but also with elevated plasma homocysteine and low serum HDL cholesterol in humans. J Nutr. 2005. 135:2372-2378.
Kopelman PG, Grace C. New thoughts on managing obesity. Gut. 2004. 53:1044-1053.
Lee YC, Sa YS, Jeong CS, Suh KG, Choi HS. Anticoagulating activity of persimmon and it processed foods. J Korean Soc Food Sci Nutr. 2001. 30:949-953.
Madsen CM, Varbo A, Nordestgaard BG. Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies. Eur Heart J. 2017. 38:2478-2486.
Madsen CM, Varbo A, Tybjærg-Hansen A, Frikke-Schmidt R, Nordestgaard BG. U-shaped relationship of HDL and risk of infectious disease: two prospective population-based cohort studies. Eur Heart J 2018. 39:1181-1190.
Matsuo T, Ito S. The chemical structure of Kaki-tannin from immature fruit of the persimmon (*Diospyros kaki* L.). Agric Biol Chem. 1978. 42:1637-1643.
Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr. 2003. 77:1146-1155.
Nestel PJ, Billington T, Smith B. Low density and high density lipoprotein kinetics and sterol balance in vegetarians. Metabolism. 1981. 30:941-945.
Oh JH, Karadeniz F, Lee JI, Seo Y, Kong CS. *Artemisia princeps* inhibits adipogenic differentiation of 3T3-L1 pre-adipocytes via downregulation of PPARγ and MAPK pathways. Prev Nutr Food Sci. 2019. 24:299-307.
Payne VA, Au WS, Lowe CE, Rahman SM, Friedman JE, O'Rahilly S, et al. C/EBP transcription factors regulate SREBP1c gene expression during adipogenesis. Biochem J. 2009. 425:215-223.
Rosen ED, Spiegelman BM. Molecular regulation of adipogenesis. Annu Rev Cell Dev Biol. 2000. 16:145-171.
Rössner S, Sjöström L, Noack R, Meinders AE, Noseda G. Weight loss, weight maintenance, and improved cardiovascular risk factors after 2 years treatment with orlistat for obesity. European Orlistat Obesity Study Group. Obes Res. 2000. 8:49-61.
Saito M, Ueno M, Ogino S, Kubo K, Nagata J, Takeuchi M. High dose of *Garcinia cambogia* is effective in suppressing fat accumulation in developing male Zucker obese rats, but highly toxic to the testis. Food Chem Toxicol. 2005. 43:411-419.
Salas-Salvadó J, Márquez-Sandoval F, Bulló M. Conjugated linoleic acid intake in humans: a systematic review focusing on its effect on body composition, glucose, and lipid metabolism. Crit Rev Food Sci Nutr. 2006. 46:479-488.
Shepherd J, Packard CJ, Stewart JM, Vallance BD, Lawrie TD, Morgan HG. The relationship between the cholesterol content and subfraction distribution of plasma high-density lipoproteins. Clin Chim Acta. 1980. 101:57-62.
Shimano H. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. Prog Lipid Res. 2001. 40:439-452.
Shin YJ, Shon MS, Kim GN, Lee SC. Antioxidant and anti-adipogenic activities of persimmon tannins. Food Sci Biotechnol. 2014. 23:1689-1694.
Smoczek M, Vital M, Wedekind D, Basic M, Zschemisch NH, Pieper DH, et al. A combination of genetics and microbiota influences the severity of the obesity phenotype in diet-induced obesity. Sci Rep. 2020. 10:6118. https://doi.org/10.1038/s41598-020-63340-w
Spiegelman BM, Flier JS. Adipogenesis and obesity: rounding out the big picture. Cell. 1996. 87:377-389.

Sun D, Zhang L, Chen H, Feng R, Cao P, Liu Y. Effects of Antarctic krill oil on lipid and glucose metabolism in C57BL/6J mice fed with high fat diet. Lipids Health Dis. 2017. 16:218. https://doi.org/10.1186/s12944-017-0601-8

Velez-Carrasco W, Merkel M, Twiss CO, Smith JD. Dietary methionine effects on plasma homocysteine and HDL metabolism in mice. J Nutr Biochem. 2008. 19:362-370.

Wafer R, Tandon P, Minchin JEN. The role of peroxisome proliferator-activated receptor gamma (PPARG) in adipogenesis: applying knowledge from the fish aquaculture industry to biomedical research. Front Endocrinol. 2017. 8:102. https://doi.org/10.3389/fendo.2017.00102

Wendland E, Farmer A, Glasziou P, Neil A. Effect of alpha linolenic acid on cardiovascular risk markers: a systematic review. Heart. 2006. 92:166-169.

Williams LM, Campbell FM, Drew JE, Koch C, Hoggard N, Rees WD, et al. The development of diet-induced obesity and glucose intolerance in C57BL/6 mice on a high-fat diet consists of distinct phases. PLoS One. 2014. 9:e106159.

Wu Y, Tan F, Zhang T, Xie B, Ran L, Zhao X. The anti-obesity effect of lotus leaves on high-fat-diet-induced obesity by modulating lipid metabolism in C57BL/6J mice. Appl Biol Chem. 2020. 63:61. https://doi.org/10.1186/s13765-020-00541-x

Wu Z, Rosen ED, Brun R, Hauser S, Adelmant G, Troy AE, et al. Cross-regulation of C/EBPα and PPARγ controls the transcriptional pathway of adipogenesis and insulin sensitivity. Mol Cell. 1999. 3:151-158.

Wueest S, Rapold RA, Schumann DM, Rytka JM, Schildknecht A, Nov O, et al. Deletion of Fas in adipocytes relieves adipose tissue inflammation and hepatic manifestations of obesity in mice. J Clin Invest. 2010. 120:191-202.

Yanai H, Katsuyama H, Hamasaki H, Abe S, Tada N, Sako A. Effects of dietary fat intake on HDL metabolism. J Clin Med Res. 2015. 7:145-149.

Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016. 64:73-84.

Yugarani T, Tan BK, Teh M, Das NP. Effects of polyphenolic natural products on the lipid profiles of rats fed high fat diets. Lipids. 1992. 27:181-186.