Effect of defatted pepper (*Capsicum annuum* L.) seed extracts on high-fat diet-induced obesity in C57BL/6J mice

Jeehye Sung, Jinwoo Yang, Younghwa Kim¹, Myunghee Kim², Heon Sang Jeong, and Junsoo Lee*

Division of Food and Animal Sciences, Chungbuk National University, Cheongju, Chungbuk 28644, Korea

¹School of Food Biotechnology and Nutrition, Kyungsoo University, Busan 48434, Korea

²Department Food Science and Technology, Yeungnam University, Gyeongsan, Gyeongbuk 38541, Korea

Received April 27, 2016
Revised July 8, 2016
Accepted July 21, 2016
Published online October 31, 2016

*Corresponding Author
Tel: +82-43-261-2566
Fax: +82-43-271-4412
E-mail: junsoo@chungbuk.ac.kr

pISSN 1226-7708
eISSN 2092-6456

© KoSfOST and Springer 2016

Abstract This study investigated the effect of defatted pepper seed ethanolic extract (DPSE) on high-fat diet-induced obesity in C57BL/6J mice. The mice were fed a normal diet (ND), high fat diet (HFD), or HFD in combination with DPSE (100 and 200 mg/kg body weight) for 7 weeks. Supplementing the HFD of mice with DPSE significantly decreased body weight gain and food efficiency ratio induced by HFD. The amount of epididymal fat and size of adipocytes were also dramatically reduced by DPSE supplementation. Although no statistical significance was found between the HFD and HFD plus DPSE groups in plasma fat deposition, liver weight, and hepatic cholesterol contents were significantly reduced in the DPSE-treated group. In conclusion, these results suggest that DPSE could prevent obesity and adiposity. These effects may be important in developing therapeutic agents against obesity in future.

Keywords: pepper seed, obesity, high-fat diet, epididymal fat, mice

Introduction

The prevalence of overweight and obesity has increased dramatically worldwide in the last decade (1). Obesity is simply defined as a condition of abnormal or excessive fat accumulation in the adipose tissues of the body due to an imbalance between energy intake and expenditure (2). Body fat accumulation leads to a risk of developing various obesity-associated chronic diseases, including glucose intolerance, cardiovascular diseases, non-alcoholic fatty liver, hypertension, and hyperlipidemia (3).

Recently, food or agricultural products have been attracting a lot of attention due to their non-toxicity and anti-obesity properties (4–6). Pepper (*Capsicum annuum* L.) seeds are by-products and wastes of pepper paste or powder processing, as well as underutilized agricultural products. These materials contain a wide variety of phytochemicals such as betulin, β -sitosterol, and myrtenal, which can be used for nutritional and biological potential (7,8). Previous studies have reported that pepper seeds exhibited several pharmacological actions, including antioxidant, anti-adipogenic, and antimicrobial activities (9,10). However, the anti-obesity effect of defatted pepper seeds in mice fed a high-fat diet has not been investigated.

Therefore, this study was aimed to examine the preventive effect of ethanolic extract from defatted pepper seed (DPSE) on high-fat diet (HFD)-induced obesity in C57BL/6J mice.

Materials and Methods

Preparation of DPSE Pepper seeds were collected from Yeoungdong area, Chungbuk, Korea, in 2014. To obtain the defatted pepper seeds, the oil in the seeds was extracted with *n*-hexane by shaking three times over 10 h at room temperature (Eyela Model MMS-300; Tokyo Rikakikai Co., Ltd., Tokyo, Japan). Defatted pepper seeds (200 g) were extracted with 2 L of 94% ethanol for 24 h at room temperature. After filtration with a Whatman No. 2 filter paper, the combined filtrate was dried under vacuum to remove ethanol. The residue was stored at -70°C until analysis.

Animal studies Five-week-old C57BL/6J mice (Daehan Biolink Co., Eumsung, Korea) were used. All mice were housed in a controlled environment ($21\pm 2^{\circ}\text{C}$ at $50\pm 2\%$ relative humidity) with a 12-h light-dark cycle. The mice had free access to both food and water, and were handled according to the guidelines of the Ethics Committee of the Chungbuk National University (CBNUA-746-14-01). After 1 week of acclimation, the experimental mice ($n=32$) were randomly divided into four groups according to the daily food intake: normal diet (ND; 4.3 g% fat), high-fat diet (HFD; 24 g% fat), or HFD in combination with DPSE at 100 or 200 mg/kg doses for 7 weeks. DPSE was diluted with water and administered by oral gavage once a day while continuing the HFD. The ND and HFD groups were administered with vehicle

Table 1. Composition of experimental diets

	Normal diet	High fat diet
Formula (g%)		
Protein	19.2	24
Carbohydrate	67.3	41
Fat	4.3	24
Ingredient (g)		
Casein	200	200
L-Cystine	3	3
Corn starch	315	72.8
Maltodextrin 10	35	100
Sucrose	350	172.8
Cellulose, BW200	50	50
Soybean Oil	25	25
Lard	20	177.5
Mineral Mix S10026	10	10
DiCalcium phosphate	13	13
Calcium Carbonate	5.5	5.5
Potassium Citrate	16.5	16.5
Vitamin Mix V10001	10	10
Cholin Bitartrate	2	2
FD&C Red Dye #40	0.05	0.05
Total	1055.05	858.05
Kcal	4057	4057

alone. The experimental diet was obtained from Research Diets, Inc. (New Brunswick, NJ, USA) (Table 1). Body weight and food intake were measured twice and once per week, respectively, during the feeding period.

At the end of the 7-week treatment period, mice were fasted for 6 h and anesthetized by diethyl ether. Blood was collected in Vacutainer SST tubes (BD Bioscience, Franklin Lakes, NJ, USA) for plasma biochemical analysis. Plasma was obtained by centrifuging the blood at 900xg for 20 min at 4°C. The organs, including epididymal adipose tissues and liver, were immediately harvested, rinsed with physiological saline solution, weighed, and stored at -70°C until analysis.

Histological analyses Epididymal adipose tissue and liver were fixed with 10% neutral buffered formalin and embedded in paraffin and

Fig. 1. Effect of DPSE on body weight and food efficiency ratio (FER) in HFD-induced obese mice. (A) Body weight was measured twice per week throughout the study. (B) The FER was calculated based on the following equation: FER=body weight change (g/day) / total food consumption (g/day)×100. Each value is expressed as a mean value±standard error (n=8). Different letters above bars indicate significant differences by Duncan's test ($p<0.05$).

then stained with hematoxylin and eosin (H&E). The stained sections were examined under an optical microscope (Olympus, Tokyo, Japan) and photographed. The adipocyte size was measured using an image

Table 2. Effect of defatted pepper seed ethanolic extract (DPSE) on biochemical parameters in plasma of high-fat diet (HFD)-induced obese mice

	Normal diet	HFD	HFD+DPSE 100 mg/kg	HFD+DPSE 200 mg/kg
Total cholesterol (mg/dL)	100.25±2.94 ^{b1)}	137.63±4.97 ^a	143.00±4.48 ^a	151.13±8.91 ^a
Triglyceride (mg/dL)	78.38±1.66 ^{NS}	76.38±3.49	88.38±10.22	69.63±3.75
HDL-cholesterol (mg/dL)	97.38±2.65 ^b	130.13±4.31 ^a	132.13±3.50 ^a	138.50±7.18 ^a
LDL-cholesterol (mg/dL)	9.63±0.53 ^b	21.00±1.30 ^a	21.50±1.64 ^a	22.38±2.76 ^a
AST ²⁾ (IU/L)	46.13±2.34 ^{ab}	50.13±1.81 ^a	47.00±1.32 ^{ab}	42.00±1.03 ^b
ALT (IU/L)	19.38±1.24 ^{NS}	18.63±1.18	18.00±1.08	15.63±0.86
BUN (mg/dL)	25.06±0.62 ^a	20.94±1.30 ^b	20.13±0.61 ^b	20.91±0.77 ^b
Glucose (mg/dL)	148.38±2.20 ^b	208.50±8.15 ^a	177.38±13.28 ^{ab}	195.38±12.57 ^a
Creatine (mg/dL)	0.10±0.00 ^{NS}	0.10±0.00	0.10±0.00	0.10±0.00

¹⁾Each value is expressed as a mean value±standard error (n=8). NS; not significant. Different letters in a same row indicate significant differences by Duncan's test ($p<0.05$).

²⁾AST, alanine aminotransferase; ALT, aspartate aminotransferase; BUN, blood urea nitrogen

analysis system (IPKR-1003; Saramsoft Co., Ltd., Anyang, Korea).

Measurement of plasma biochemical parameters Plasma triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, alanine transaminase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), creatinine, and glucose were performed using a Hitachi 7080 analyzer (Hitachi Medical Co., Tokyo, Japan).

Hepatic lipid extraction and measurement Lipids were extracted from livers as described by Semple *et al.* (11). Liver (50 mg) was homogenized in 1 mL of saline solution (0.1% NaCl). The homogenates were extracted with 3 mL chloroform/methanol (2:1). The chloroform layer was collected, dried, and resuspended in 1 mL of phosphate-buffered saline containing 1% Triton X-100. Triglycerides and total cholesterol levels in liver were analyzed using a Hitachi 7080 analyzer.

Statistical analysis Data were presented as the mean $\pm$ standard error (SE) ($n=8$). Statistical significance was analyzed by one-way analysis of variance using SAS version 8.1 (SAS Institute, Cary, NC, USA), followed by Duncan's multiple comparison test. A p -value <0.05 was considered statistically significant for all analyses.

Results and Discussion

Effects of DPSE on body weights and food efficiency ratio The HFD-induced obesity in mice has been widely used as an animal model because it mimics most of the phenotypes seen in human obesity (12). In this study, the effects of defatted pepper seeds on the development of obesity were investigated using HFD-induced C57BL/6J mice model. The initial body weights were not statistically different among the groups. After 7 weeks, the HFD-fed mice exhibited significantly higher body weights compared with the ND-fed mice, whereas the HFD+DPSE groups displayed remarkably lower body weights than the HFD group (Fig. 1A). During the feeding period, the food intake was higher in the ND group compared with that in the HFD group, but it did not significantly differ between the HFD and HFD+DPSE groups (data not shown). However, the food efficiency ratio (FER) calculating the body weight gain relative to food intake was significantly lower in mice fed HFD+DPSE groups than HFD fed mice group (Fig. 1B). These results show that DPSE effectively reduced the body weight on HFD-induced obesity in mice.

Effects of DPSE on the plasma biochemical parameters Plasma biochemical parameters are shown in Table 2. The contents of total cholesterol and LDL-cholesterol were higher in HFD group compared to those in ND group, but they were not significantly different between HFD and HFD+DPSE groups. Similarly, plasma glucose levels were not significantly different between HFD and HFD+DPSE groups. In terms of plasma parameters of liver injury (AST and ALT), DPSE intake

did not alter ALT and AST levels. Plasma parameters of kidney injury (BUN and creatinine) were not significantly different between HFD and HFD+DPSE groups. These data indicate that DPSE induced no detectable adverse toxic effects in the mice.

Fig. 2. Effect of DPSE on liver weight and hepatic lipid parameters in HFD-induced obesity in mice. (A) Liver weight was measured after 7 weeks. (B) Hepatic triglyceride and (C) total cholesterol were analyzed after 7 weeks. Each value is expressed as a mean value $\pm$ standard error ($n=8$). NS; not significant. Different letters above bars indicate significant differences by Duncan's test ($p<0.05$).

Effects of DPSE on liver weight and hepatic lipids The effects of DPSE on liver weight and hepatic lipids were examined. Liver weight in the HFD+DPSE group led to a significant decrease in its weight when compared to that in HFD group (Fig. 2A). Hepatic triglyceride contents were not significantly different among the experimental groups (Fig. 2B). Hepatic cholesterol levels in the HFD+DPSE (200 mg/kg) group were significantly decreased compared with those in the HFD group (Fig. 2C). H&E staining of the liver section indicated that DPSE successively ameliorate the development of white lipid droplets induced by the HFD fed mice (Fig. 3A).

Hepatic steatosis is the major pathological stage of the nonalcoholic fatty liver disease (NAFLD), which is triggered by inhibition of fatty acid oxidation, enhancement of lipogenesis, and accumulation of cholesterol in liver (13). Therefore, hepatic triglyceride and cholesterol management may be a promising treatment target for NAFLD. In this study, DPSE exhibited the inhibitory effects of lipid accumulation in liver. Pepper seeds have been reported to be rich sources of phytochemicals such as phenolic compounds, flavonoids, phytosterols, and organic acids (7,8). Previous studies have shown that phenolic compounds suppress the increases in the levels of hepatic triglyceride and cholesterol induced by HFD in rodents (14,15). More recently, there has been an increasing evidence that dietary flavonoids exert multiple beneficial effects on the disorders associated with NAFLD (16,17). Therefore, DPSE can be used as a therapeutic agent to prevent the development of NAFLD and its associated diseases.

Effects of DPSE on adipose tissue weight and adipocyte size Histological analysis by H&E staining also revealed that the adipocyte size in the epididymal adipose tissue was larger in the HFD group than in the ND group (Fig. 3B). A significant decrease in adipocyte size was observed in the HFD+DPSE groups in contrast to that observed in the HFD group. The epididymal adipose tissue weight was significantly increased in the HFD group compared to that in the ND group (Fig. 4A). Administration of DPSE both at 100 and 200 mg/kg significantly reduced the epididymal adipose tissue weight. Consistent with the results in epididymal adipose tissue weight change, DPSE

Fig. 4. Effect of DPSE on adipose tissue weight and size in HFD-induced obese mice. (A) Epididymal adipose tissue weight was measured after 7 weeks (B) The adipocyte size was measured in the epididymal adipose tissue. Each value is expressed as a mean value±standard error ($n=8$). Different letters above bars indicate significant differences by Duncan's test ($p<0.05$)

administration obviously decreased the size of epididymal fat in HFD+DPSE groups (Fig. 4B).

Fig. 3. Effect of DPSE on histology changes in the adipose tissue and liver of HFD-induced obese mice. (A) Representative images of liver and (B) epididymal adipose tissue sections were stained with H&E and viewed under a microscope (200 ×).

Adipose tissue consisting of adipocytes has an important function for maintaining whole body energy homeostasis and stores dietary energy as triglyceride (18). Although adipose tissue is crucial for various normal processes, excess adipose tissue can have many negative implications for chronic disease states with obesity development (19). Obesity induced by HFD leads to store excessive lipids in adipocytes, resulting in the formation of hypertrophic and dysfunctional adipocytes (20). Adipocyte dysfunction is considered to be a major cause of many disorders, including insulin resistance, fatty liver, cardiovascular disease, and hypertension (21). In this study, the relatively lower amount of epididymal adipose mass was observed in the HFD+DPSE groups compared to that observed in the HFD groups. Therefore, it is plausible to suggest that DPSE can reduce body fat mass and the development of obesity-related diseases. In conclusion, this study demonstrated that administration of DPSE attenuated HFD-induced obesity through the decreases in body weight gain, hepatic fat deposition, adipose cell size, and body fat mass in C57BL/6J mice after 7 weeks. Although further studies are needed, these results indicate that DPSE might be beneficial for improving obesity and related metabolic disorders.

Acknowledgments This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (project number: 2012R1A1A4A0105273), Republic of Korea.

Disclosure The authors declare no conflict of interest.

References

- Cornier MA, Marshall JA, Hill JO, Maahs DM, Eckel RH. Prevention of overweight/obesity as a strategy to optimize cardiovascular health. *Circulation* 124: 840-850 (2011)
- Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. *JAMA* 282: 1523-1529 (1999)
- Lee YJ, Lee JH, Kim YH, Kim JH, Yu SY, Kim DB, Lee JS, Cho ML, Cho JH, Kim BK, Lee BY, Lee OH. Assessment of the pectolinarin content and the radical scavenging-linked antiobesity activity of *Cirsium setidens* nakai extracts. *Food Sci. Biotechnol.* 24: 2235-2243 (2015)
- Jobu K, Yokota J, Yoshioka S, Moriyama H, Murata S, Ohishi M, Ukeda H, Miyamura M. Effects of Goishi tea on diet-induced obesity in mice. *Food Res. Int.* 54: 324-329 (2013)
- Misawa K, Hashizume K, Yamamoto M, Minegishi Y, Hase T, Shimotoyodome A. Ginger extract prevents high-fat diet-induced obesity in mice via activation of the peroxisome proliferator-activated receptor delta pathway. *J. Nutr. Biochem.* 26: 1058-1067 (2015)
- Pang J, Choi Y, Park T. *Ilex paraguariensis* extract ameliorates obesity induced by high-fat diet: Potential role of AMPK in the visceral adipose tissue. *Arch. Biochem. Biophys.* 476: 178-185 (2008)
- Silva LR, Azevedo J, Pereira MJ, Valentao P, Andrade PB. Chemical assessment and antioxidant capacity of pepper (*Capsicum annuum* L.) seeds. *Food Chem. Toxicol.* 53: 240-248 (2013)
- Sim KH, Sil HY. Antioxidant activities of red pepper (*Capsicum annuum*) pericarp and seed extracts. *Int. J. Food Sci. Tech.* 43: 1813-1823 (2008)
- Sung J, Lee J, Capsicoside G. A furostanol saponin from pepper (*Capsicum annuum* L.) seeds, suppresses adipogenesis through activation of AMP-activated protein kinase in 3T3-L1 cells. *J. Funct. Foods* 20: 148-158 (2016)
- Iorizzi M, Lanzotti V, Ranalli G, De Marino S, Zollo F. Antimicrobial furostanol saponins from the seeds of *Capsicum annuum* L. var. *acuminatum*. *J. Agr. Food Chem.* 50: 4310-4316 (2002)
- Semple RK, Meirhaeghe A, Vidal-Puig AJ, Schwabe JW, Wiggins D, Gibbons GF, Gurnell M, Chatterjee VK, O'Rahilly S. A dominant negative human peroxisome proliferator-activated receptor (PPAR) α is a constitutive transcriptional corepressor and inhibits signaling through all PPAR isoforms. *Endocrinology* 146: 1871-1882 (2005)
- Donado-Pestana CM, Belchior T, Genovese MI. Phenolic compounds from cagaíta (*Eugenia dysenterica* DC.) fruit prevent body weight and fat mass gain induced by a high-fat, high-sucrose die. *Food Res. Int.* 77: 177-185 (2015)
- Enjoji M, Yasutake K, Kohjima M, Nakamura M. Nutrition and nonalcoholic fatty liver disease: The significance of cholesterol. *Int. J. Hepatol.* 2012: 1-6 (2012)
- Yugarani T, Tan BK, Teh M, Das NP. Effects of polyphenolic natural products on the lipid profiles of rats fed high fat diets. *Lipids* 27: 181-186 (1992)
- Hsu CL, Wu CH, Huang SL, Yen GC. Phenolic compounds rutin and o-coumaric acid ameliorate obesity induced by high-fat diet in rats. *J. Agr. Food Chem.* 57: 425-431 (2009)
- Pisonero-Vaquero S, Gonzalez-Gallego J, Sanchez-Campos S, Garcia-Mediavilla MV. Flavonoids and related compounds in non-alcoholic fatty liver disease therapy. *Curr. Med. Chem.* 22: 2991-3012 (2015)
- Van De Wier B, Koek GH, Bast A, Haenen GR. The potential of flavonoids in the treatment of non-alcoholic fatty liver disease. *Crit. Rev. Food Sci. Nutr.* 21: in press (2015)
- Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat. Rev. Mol. Cell Bio.* 9: 367-377 (2008)
- Kadowaki T, Hara K, Kubota N, Tobe K, Terauchi Y, Yamauchi T, Eto K, Kadowaki H, Noda M, Hagura R, Akanuma Y. The role of PPARgamma in high-fat diet-induced obesity and insulin resistance. *J. Diabetes Complicat.* 16: 41-45 (2002)
- Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. *Nature* 444: 847-853 (2006)
- Pichiah PB, Moon HJ, Park JE, Moon YJ, Cha YS. Ethanolic extract of seabuckthorn (*Hippophae rhamnoides* L) prevents high-fat diet-induced obesity in mice through down-regulation of adipogenic and lipogenic gene expression. *Nutr. Res.* 32: 856-864 (2012)