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RESEARCH NOTE

Anti-obesity Activity of Methanol Extract from Hot Pepper (*Capsicum annuum* L.) Seeds in 3T3-L1 Adipocyte

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Abstract Anti-adipogenesis activity of methanol extract from hot pepper (*Capsicum annuum* L.) seeds (PSE) was evaluated. Exposure to PSE (25-200 μg/mL) for a 72 hr incubation period did not alter cell viability compared to the control. The adipocyte treated with PSE at the concentration of 200, 100, and 50 μg/mL showed decreased color intensity as compared to the control suggesting decreased lipid accumulation in the adipocyte. Moreover, the glycerol-3-phosphate dehydrogenase activity was significantly reduced. The expression of C/EBPβ, C/EBPα, and PPARγ, important adipogenic transcription factors, was significantly reduced when PSE was added to cells from day 0 to 6 as compared with the control group. The results suggest that PSE has significant health-promoting effects, having excellent anti-obesity activities.

Keywords: anti-obesity, hot pepper seed, adipocyte, lipid accumulation, 3T3-L1

Introduction

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. The fundamental cause of obesity and overweight is an energy imbalance between calories consumed on one hand, and calories expended on the other hand. Obesity is a serious health problem because it is implicated in various diseases including type 2 diabetes, hypertension, coronary heart disease, and cancer (1). Adipocyte is primary site for energy storage and accumulates triglycerides during nutritional excess. Adipose tissue growth involves formation of new adipocytes from precursor cells, further leading to an increase in adipocyte size. The transition from undifferentiated fibroblast-like preadipocytes into mature adipocytes constitutes the adipocyte life cycle and treatments that regulate both size and number of adipocytes may provide a better therapeutic approach for treating obesity (2). During adipocyte differentiation, members of CCAAT/enhancer-binding proteins (C/EBPs), and peroxisome proliferator-activated receptor (PPAR)γ are key regulators of the adipogenesis process (3). Exposure of preadipocytes to the adipogenic cocktail induces C/EBP, which in turn activate PPARγ (4). The amount of adipose tissue mass can be regulated by the inhibition of adipogenesis from fibroblastic preadipocytes to mature adipocytes and induction of apoptosis in adipose tissues (2). Products and programs that induce rapid weight loss and disturb metabolic homeostasis dominate the focus of marketers and consumers alike; however, rapid weight loss is potentially unhealthy and frequently induces undesirable rebound weight gain consequences. In addition, many anti-obesity pharmaceuticals are accompanied by adverse reactions, making the cure worse than the disorder itself; thus, it is very important to develop a strategic and therapeutic intervention using safe, novel, and natural supplements supported by credible research (5).

One of the agricultural by-products from pepper paste and powder factory is pepper seeds, which are an abundant, cheap, and readily available residue. The objective of this study was to evaluate pepper seeds from an agricultural waste as a new anti-obesity agent.

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Materials and Methods

Chemicals Dulbecco's modified Eagle's medium (DMEM)

and phosphate buffered saline (PBS) was purchased from HyClone (Logan, UT, USA). Bovine calf serum (BCS) and fetal bovine serum (FBS) were obtained from Gibco (BRL Life Technologies, Grand Island, NY, USA). Insulin, isobutyl methyl xanthine (IBMX), dexamethasone (DEX), trypsin, streptomycin, penicillin, dimethyl sulfoxide (DMSO), and thiazolyl blue terazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies specific for β-actin, C/EBPα, C/EBPβ, and PPARγ were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Preparation of hot pepper seed extract (PSE) Briefly, the pepper (*Capsicum annuum* L.) seed were extracted with methanol by shaking for 24 hr at room temperature and filtered through Whatman No. 2 filter paper. The extracts were evaporated under vacuum and then dissolved in DMSO.

Cell line and cell culture The mouse embryo 3T3-L1 cells (ATCC No. CL-173) were obtained from American Type Culture Collection (Manassas, VA, USA). Briefly, 3T3-L1 preadipocytes were incubated in DMEM containing 10% BCS, 1.5 g/L sodium bicarbonate, and 100 U/mL penicillin-streptomycin. Two days after postconfluence (day 0), the cells were treated with DMEM containing 10% FBS, 871 nM insulin, 0.5 mM IBMX, and 1 μM DEX for 48 hr (day 2). On day 2, differentiation medium was replaced with 10% FBS/DMEM medium containing 871 nM insulin and incubated for 48 hr (day 4), followed by culturing with 10% FBS/DMEM medium for an additional 4 day (day 8).

MTT assay Antiproliferative activity of hot pepper seed extracts on 3T3-L1 preadipocytes was measured by evaluating cell viability using the MTT assay (6). 3T3-L1 preadipocytes were plated into 96-well plates at a density of 2.5×10^3/well, and then samples (25, 50, 100, and 200 μg/mL) were added into the 96-well plate. After 48 hr of incubation, 20 μL of MTT reagent (5 mg/mL) was added and incubated for additional 4 hr, and the absorbance of formazan was determined at 550 nm. The cell viability (%) was obtained by comparing the absorbance between the samples and a control.

Oil red O staining Intracellular lipid accumulation was measured using Oil red O staining method. 3T3-L1 adipocytes were treated with control or sample (25, 50, 100, and 200 μg/mL) from days 0 to 6 of adipogenesis. In brief, cells were washed twice with cold PBS and fixed in 10% neutral formalin for at least 10 min at room temperature, and then washed with PBS twice. The lipid droplets in cells were stained with 0.3% Oil red O. The cells were washed

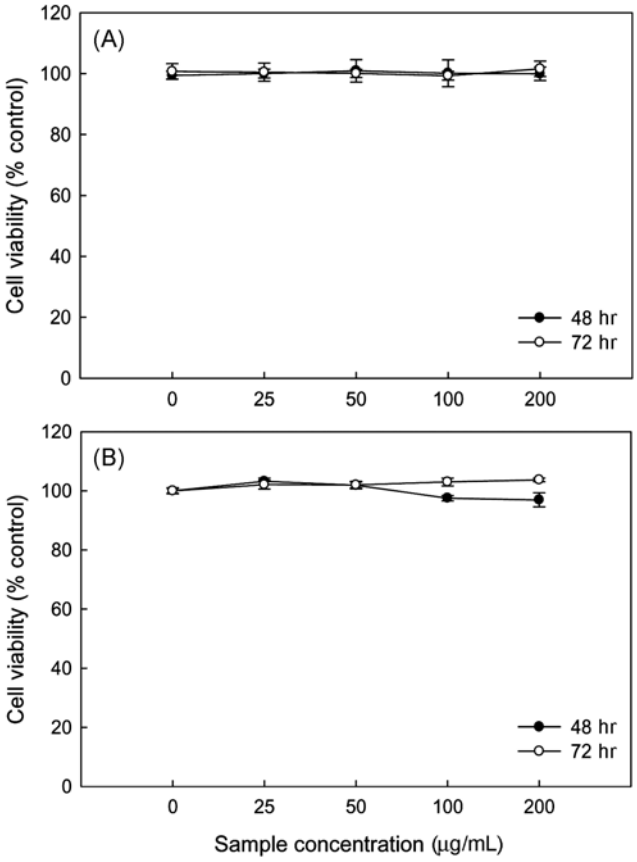

Fig. 1. Viability of preconfluent preadipocytes and maturing preadipocytes treated with different doses of hot pepper seed extract (PSE). (A) 3T3-L1 Preconfluent preadipocytes and (B) maturing preadipocytes were treated for 48 and 72 hr with the noted concentrations of PSE. Values are the mean of 3 measurements.

exhaustively with distilled water and the staining dye of cells was extracted with isopropyl alcohol and then the absorbance was measured at 490 nm using an enzyme-linked immunosorbent assay (ELISA) plate reader (Beckman Instruments Inc., Fullerton, CA, USA).

Glycerol-3-phosphate dehydrogenase (GPDH) assay 3T3-L1 Adipocytes were treated with control or sample (25, 50, 100, and 200 μg/mL) from day 0 to 6 of adipogenesis. Cells were washed twice carefully with cold PBS and then were collected in cold buffer containing 0.25 M sucrose, 1 mM ethylene diamine tetraacetic acid (EDTA), 5 mM Tris-base, and 1 mM dithiothreitol at pH 7.4. Harvested cells were lysed for 10 sec using a Vibra-Cell VCX 750 sonicator (Sonics & Materials, Inc., Newtown, CT, USA). Lysates were centrifuged at 10,000× g for 10 min at 4ºC, and the supernatants were immediately used for the protein and GPDH activity assay. GPDH activity was determined according to the procedure by Wise and Green (7). The protein concentration of each sample was determined using BCA protein assay reagent

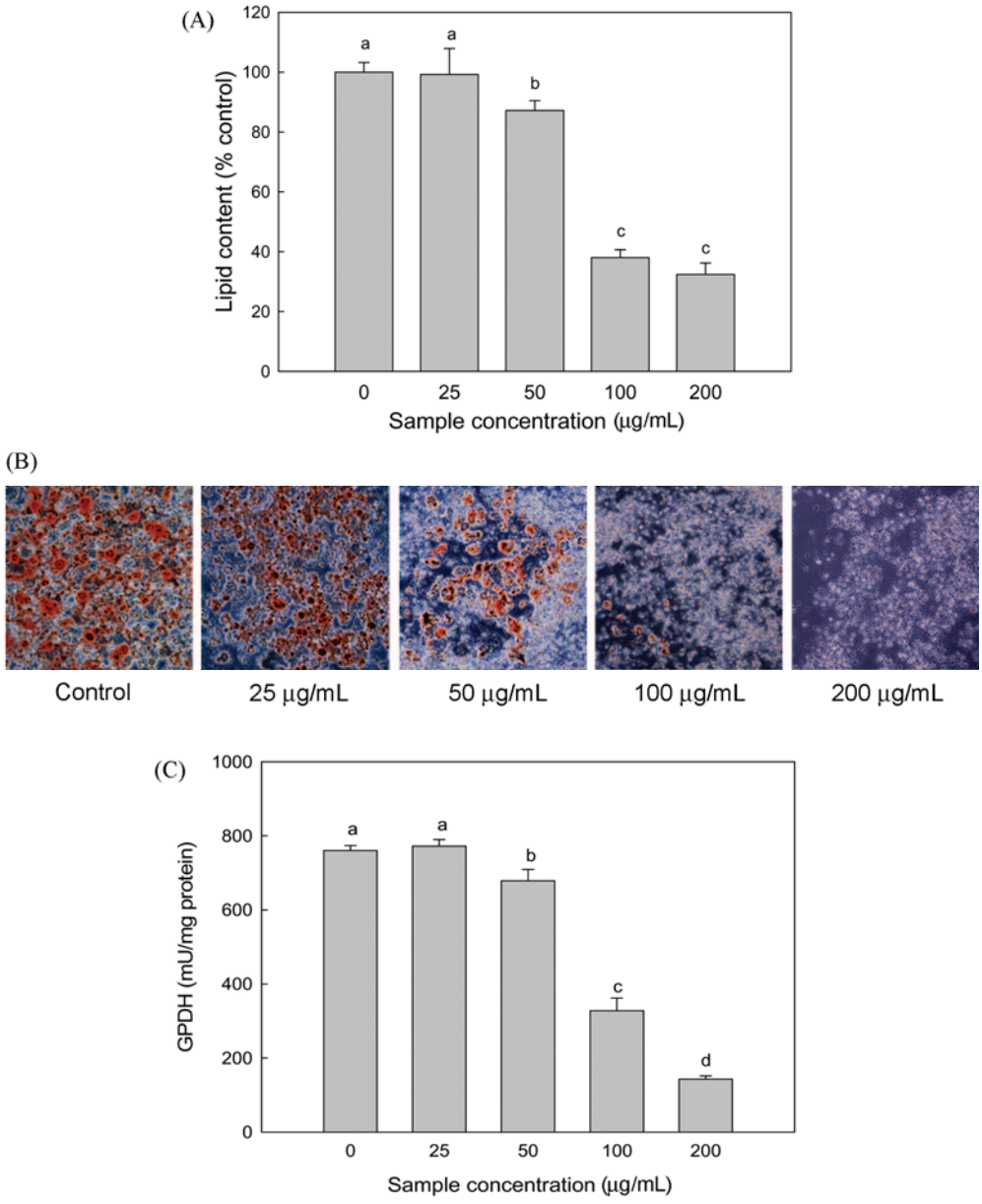

Fig. 2. Effect of PSE on lipid accumulation and GPDH activity during adipocyte differentiation. (A) Quantification of lipid in Oil red O stained adipocytes. (B) Representative images of Oil red O staining during differentiation. (C) GPDH activity in maturing preadipocytes expressed in mU/mg of protein. Values are the mean of 3 measurements. Results were analyzed by ANOVA and Duncan's test ($p<0.05$). Different letters above bars indicate significant difference.

(Pierce, Rockford, IL, USA). Enzyme activity was expressed as mU/mg protein.

Western blot analysis Expression levels of differentiation related proteins were examined by Western blot analysis. Preadipocytes or adipocytes were washed with cold PBS buffer and lysed with lysis buffer. The protein concentration of each sample was determined using BCA protein assay reagent. The cell lysates were diluted with sample buffer containing 1.28 M β-mercaptoethanol, and equal amounts of protein were separated by 12% acrylamide gels. The electrophoresed proteins were transferred to polyvinylidene difluoride (PVDF) membranes by semidry electrophoretic transfer. The PVDF membranes were blocked overnight with 5% skim milk in Tris-buffered saline, then incubated with primary antibodies diluted in Tris-buffered saline/Tween 20 (TBS-T) containing 5% skim milk for 2 hr and then incubated with the secondary antibody at room temperature for 1 hr. The specific protein bands were visualized on X-ray film activated by chemiluminescence using ECL reagent (Pierce, Rockford, IL, USA).

Statistical analysis The results were reported as mean± standard deviation (SD). The significance of differences

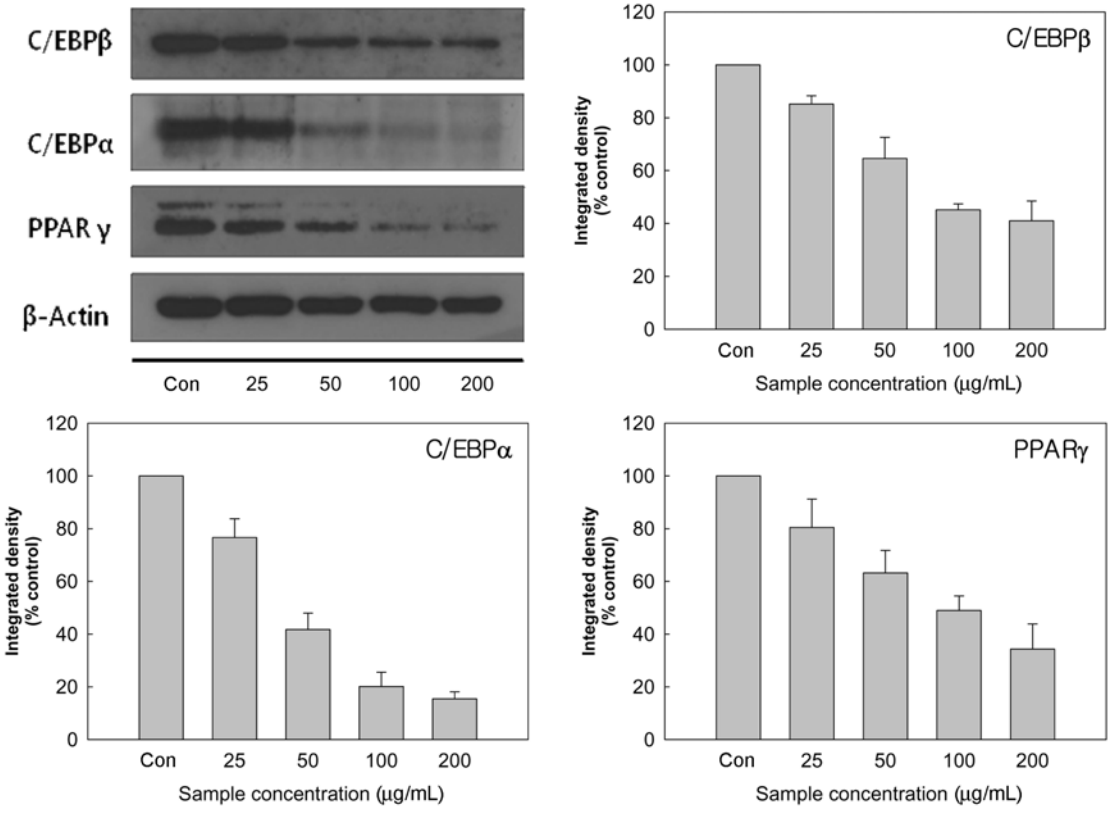

Fig. 3. Effect of PSE on expression of the adipocyte specific transcription factors, C/EBPβ, C/EBPα, and PPARγ. Values are the mean of 3 measurements.

among treatment means were determined by one-way analysis of variance (ANOVA) using SAS version 9.1 (SAS Institute, Cary, NC, USA) with a significance level of 0.05.

Results and Discussion

Effect of PSE on viability of adipocytes The amount of adipose tissue mass can be regulated by the inhibition of adipogenesis from fibroblastic preadipocytes to mature adipocytes (8) and induction of apoptosis (9). 3T3-L1 Preconfluent preadipocytes and maturing preadipocytes were treated with PSE at various doses for 48 and 72 hr. After treatment, the number of live cells was determined by MTT assay. Exposure to PSE (25-200 μg/mL) over a 72 hr incubation period did not alter cell viability compared to the control (Fig. 1).

Effect of PSE on intracellular lipid accumulation during adipocyte differentiation Pepper seed extract was added to the differentiation media on day 0 which is the day of the initiation of differentiation. Oil red O staining was used to assess the degree of differentiation and was performed on day 8 of differentiation. High color intensity suggests increased differentiation and triglyceride accumulation. PSE significantly inhibited differentiation at concentration of 200, 100, 50, and 25 μg/mL (Fig. 2A). The cells treated with PSE at 200, 100, and 50 μg/mL showed less color intensity as compared to the control suggesting decreased lipid accumulation and inhibition of differentiation.

Effect of PSE treatment on GPDH activity The cytosolic enzyme GPDH appears to have an important role in the conversion of glycerol into triglyceride. GPDH occupies a central position in the triglyceride synthesis pathway, at the point where it branches from the glycolytic pathway (7). Effect of PSE on GPDH activity is shown in Fig. 2C. The GPDH activity was significantly reduced when PSE was added to cells from day 0 (day of induction of differentiation) to 6 as compared with the group that was not treated with PSE. ($p<0.05$). Based on this result, we next studied whether treatment with PSE altered expression of adipocyte marker protein in 3T3-L1 cells.

Effect of PSE on the expression of adipogenic transcription factors C/EBPβ and C/EBPδ are the first transcription factors induced after exposure of the preadipocytes to the differentiation cocktail; they are therefore postulated to be involved in directing the differentiation process. In accord with this notion,

expression of C/EBPβ or C/EBPδ under the control of exogenous promoters of differentiation induces and accelerates adipogenesis in response to hormonal inducers. Then, it is believed that C/EBPβ induces PPARγ and C/EBPα followed by the expression of adipogenic genes such as GPDH and adipocyte selective fatty acid binding protein (aP2) to accumulate fat into cells (10). To determine whether PSE affects the expression of adipogenic transcription factors, Western blot analysis was performed. The expression of C/EBPβ, C/EBPα, and PPARγ, important adipogenic transcription factors, was significantly reduced when PSE was added to cells from day 0 to 6 as compared with the control group that was not treated with PSE (Fig. 3).

This study provides basic information of anti-obesity activity of PSE. Given that PSE suppresses adipocyte number and lipid accumulation in cell culture, it could be anticipated that PSE will influence these factors similarly *in vivo* and potentially help alleviate obese related diseases.

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References

1. Kopelman PG. Obesity as a medical problem. Nature 404: 635-643 (2000)
2. Rayalam S, Della-Fera MA, Baile CA. Phytochemicals and regulation of the adipocyte life cycle. J. Nutr. Biochem. 19: 717-726 (2008)
3. Gregoire FM, Smas CM, Sul HS. Understanding adipocyte differentiation. Physiol. Rev. 78: 783-809 (1998)
4. Gregoire FM. Adipocyte differentiation: From fibroblast to endocrine cell. Exp. Biol. Med. 226: 997-1002 (2001)
5. Bagchi D, Preuss HG. Obesity: Epidemiology, Pathophysiology, and Prevention. CRC Press. Boca Raton, FL, USA. pp. 233-238 (2007)
6. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods 65: 55-63 (1983)
7. Wise LS, Green H. Participation of one isozyme of cytosolic glycerophosphate dehydrogenase in the adipose conversion of 3T3 cells. J. Biol. Chem. 254: 273-275 (1979)
8. Roncari DA, Lau DC, Kindler S. Exaggerated replication in culture of adipocyte precursors from massively obese persons. Metabolism 30: 425-427 (1981)
9. Sorisky A, Magun R, Gagnon AM. Adipose cell apoptosis: Death in the energy depot. Int. J. Obes. Relat. Metab. Disord. 24: S3-S7 (2000)
10. Ntambi JM, Kim YC. Adipocyte differentiation and gene expression. J. Nutr. 130: 3122S-3126S (2000)