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목향 추출물의 항비만 활성 효과

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Anti-obesity Activity of Extract from *Saussurea lappa*

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ABSTRACT : Obesity has become one of the main public health problems. *Saussurea lappa* (Asteraceae), syn *Aucklandia lappa* and *Saussurea costus*, is a well-known herbal medicine that has been used for treating various ailments, such as inflammatory and gastrointestinal diseases. The present study examined the anti-obesity effect of *S. lappa* extract (SLE) in 3T3-L1 adipocytes and high fat diet (HFD)-induced obese mouse model. SLE significantly inhibited the differentiation from preadipocytes to adipocytes of cultured 3T3-L1 in dose-dependent manner. In addition, SLE significantly decreased the body weight gain and the food efficiency ratio of mice fed HFD during 9 weeks. Further study must be performed for the pharmacological mechanism and safety of SLE as well as the identification of active compound in SLE. Our results revealed that *S. lappa* suppresses the adipogenesis in cultured cells and the obesity in rodent models. Therefore, *S. lappa* may be useful toward the development of new potent anti-obesity drugs.

Key Words **:** *Saussurea lappa,* Anti-obesity, 3T3-L1, Adipocyte, Adipogenesis, High Fat Diet

INTRODUCTION

Obesity is a chronic metabolic disorder that results from the imbalance between energy intake and energy expenditure. It is mostly associated with a lot of pathological disorders, including diabetes (Sartipy and Loskutoff, 2003), hypertension (Pi-Sunyer, 2002), cancer (Lagra *et al*., 2004), and atherosclerosis (Wofford *et al*., 1999). It is characterized by enlarged fat mass and elevated lipid concentration in blood. The treatment of obesity targets the decrease of energy or food intake, the increase of energy expenditure, the decrease of preadipocyte differentiation and proliferation, the decrease of lipogenesis, and the increase of lipolysis and fat oxidation (Bray and Tartaglia, 2000). There is increasing interest in the use of natural resources as anti-obesity agents. The natural herb extracts that produce weight loss with minimal adverse effects have been used in the treatment of obesity. While several natural herb extracts, such as garcinia extract, hibiscus tea, marine algae, and green tea, have reported to be useful for the control of obesity with less significant side effects (Kim *et al*., 2007; Lee *et al*., 2008; Lee *et al*., 2003; Mochizuki and Hasegawa, 2004), the majority have yet to be scientifically or medically evaluated.

Saussurea lappa (Asteraceae), syn *Aucklandia lappa* and *Saussurea costus*, is a well-known traditional herbal medicine that is officially listed in the Korean Pharmacopoeia. The root of *S. lappa* has been used for the treatment of various diseases such as asthma, inflammation, dysentery, ulcer, and stomachache for thousands of years (Pandey *et al*., 2007). Previous studies reported that *S. lappa* has vasodilatory (Shoji *et al*., 1986), anti-inflammatory (Lee *et al*., 1995), anti-ulcer (Chen *et al*., 1994), anti-cancer (Ko *et al*., 2005), hepatoprotective (Chen *et al*., 1995), immunomodulatory (Taniguchi *et al*., 1995), and anti-microbial (Yu *et al*., 2007) activities. Sesquiterpenes lactones have been reported as the major phytoconstituents in the roots of *S. lappa* (Robinson *et al*., 2008). Especially, costunolide, dehydrocostus lactone, and cynaropicrin, which are isolated from this plant, show the pharmacological activity such as vasodilatory, anti-

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inflammatory, and anti-cancer activities (Cho *et al*., 2004; Cho *et al*., 1998; Jeong *et al*., 2002; Oh *et al*., 2004; Shoji *et al*., 1986). Besides saussureamines, costunolide, and dehydrocostus lactone showed anti-ulcer effect (Matsuda *et al*., 2000). Also costunolide and dehydrocostus lactone showed hepatoprotective and immunomodulatory effects (Chen *et al*., 1995; Taniguchi *et al*., 1995). Although a number of studies related to the physiological properties of *S. lappa* have been carried out, its anti-obesity effects have not yet been reported.

Our study focuses on the anti-obesity activity of the root of *S. lappa* with respect to developing its medicinal applications and potential uses. In the present study, we investigated that the effect of *S. lappa* extract (SLE) on adipogenensis and obesity in 3T3-L1 adipocytes and high fat diet (HFD)-induced obese mouse model.

MATERIALS AND METHODS

1. Chemicals

Dulbecco's modified Eagle's medium (DMEM), calf serum (CS), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Invitrogen (Grand Island, NY, USA). Insulin, dexamethasone, isobutylmethylxanthine (IBMX), Oil red O, and other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2. Plant material

The roots of *S. lappa* (Asteraceae) were purchased from Omniherb (Oriental drug store, Yeongcheon, Korea) and were authenticated based on microscopic characteristics according to the 'Classification and Identification Committee of the Korea Institute of Oriental Medicine'. This committee was composed of nine experts in the fields of plant taxonomy, botany, pharmacognosy, and herbology. In addition, a voucher specimen (KIOM008174) was deposited at the herbarium of the Center of Herbal Resources Research at Korea Institute of Oriental Medicine (Daejeon, Korea).

3. Preparation of *Saussurea lappa* extract (SLE)

The dried root 100 g of *S. lappa* was refluxed three times in 1 L 70% ethanol for 2 h, and the extract was then concentrated under reduced pressure. The decoction was filtered, lyophilized, and stored at 4℃. The yield of dried extract from starting crude materials was 20.24% (W/W). The dried extract (SLE) was dissolved in phosphate-buffered saline (PBS) and filtered with 0.22-㎛ syringe filter to generate the stock solution.

4. Cell culture

3T3-L1 preadipocytes were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA) and maintained with DMEM containing 10% heat-inactivated CS, 100 units/㎖ penicillin, and 100 ㎍/㎖ streptomycin at 37℃ in a humidified atmosphere of 5% CO_2.

5. Induction of adipocyte differentiation and cytotoxicity assay

3T3-L1 preadipocytes were inoculated into twelve multidishes and incubated to confluent status. The differentiation to adipocyte was induced by the incubation in DMEM containing 10% FBS and hormone mixture MDI (0.5 mM isobutylmethylxanthine, 1 μM dexamethasone and 10 ㎍/㎖ insulin) for 2 days. Cells were then cultured in 10%FBS/DMEM supplemented with only 10 ㎍/㎖ insulin for 2 days. Thereafter, cells were re-fed with medium exclusive of insulin every other day. The various concentrations of SLE were added along with the differentiation medium. The same concentration of SLE was maintained when the culture medium was replaced. The differentiation, as evaluated by the expression of adipogenic markers and appearance of lipid droplets, was completed at day 8. The cytotoxicity test was performed to examine the effect of SLE on the viability of 3T3-L1 cells using MTT assay, which is based on the cleavage of tetrazolium salt by mitochondrial dehydrogenase in viable cells (Carmichael *et al*., 1987).

6. Oil red O staining

The differentiated cells were washed twice with PBS and fixed with 10% formalin for 30 min and then washed twice with PBS. The fixed cells were stained with 0.3% Oil red O-isopropanol for 1 hr and the excess of stain was removed by washing with distilled water three times. The cells were visualized by Olympus CKX41 microscope (Olympus, Tokyo, Japan), and photographed at 100× magnification by Motic image Plus 2.0 program (Motic, Causeway Bay, Hong Kong). To quantify the intracellular lipids, the stained lipid droplets were dissolved with isopropanol (500 ㎕) and calculated at 520 ㎚ using spectrophotometrical analysis

(spectaMAX 340 reader, Molecular Devices, Silicon Valley, CA, USA). The optical density of fully differentiated adipocyte was taken as 100% of relative lipid content. The results were represented as a percentage of relative lipid content found in each experimental group.

7. Animal study

Four-week-old male C57BL/6J mice of 19-20 g were obtained from Daehan Biolink Co. (Eumsung, Korea). The animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Korea Institute of Oriental Medicine. The mice were housed in a temperature- and humidity-controlled room under a constant 12-h light/12-h dark cycle. They were subjected to an adaptation period of one week. The mice were divided into three groups (n = 6) for the experiment. The commercial standard chow diet (Orient Bio Inc., Seongnam, Korea) was used for normal diet (ND) group. Rodent diet D12492 (Research diet, New Brunswick, NJ, USA), providing 60% of energy as fat, 20% as protein, and 20% as carbohydrate, was used to induce obesity for high fat diet (HFD) group. After 6 weeks of dietary manipulation with HFD, SLE was orally administered at doses of 200 ㎎/㎏/day for consecutive 9 weeks to the mice in HFD+SLE group. The concentration of SLE used in these experiments is a minimal dosage with effective as determined from the preliminary dose-response experiment (data not shown). In contrast, PBS was orally administered to the mice in control group. The body weight and food intake were measured two times weekly throughout the treatment period. The food efficiency ratio (FER) was calculated as follows: body weight gain/food intake.

8. Statistical analysis

All data were expressed as mean ± S.E. Statistical significance was analyzed by Student's *t*-test and ANOVA using SPSS ver.14 (SPSS Inc., Chicago, IL, USA). A *P*-value of less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Obesity is mostly associated with the development of a lot of pathological diseases, including diabetes, coronary heart disease, an increased incidence of certain forms of cancer, respiratory complication, and osteoarthritis of the large and small joints (Kopelman, 2000). The strategy for preventing and/or treating obesity includes the suppression of dietary intake, increased thermogenesis, and inhibition of adipocyte differentiation.

A variety of natural resources and these compounds have been found to have beneficial effects on health because of their relative safeness and accumulated evidence of physiological properties in animals and humans (Bhathena and Velasquez, 2002). In present study, the anti-obesity effect of *S. lappa* extract (SLE) was investigated *in vitro*/*vivo* studies.

As previously reported, confluent 3T3-L1 preadipocytes with MDI (0.5 mM isobutylmethylxanthine, 1 μM dexamethasone and 10 ㎍/㎖ insulin) initiated the adipocyte differentiation (Tontonoz *et al*., 1994). To investigate the effect of SLE on the differentiation to adipocyte, 3T3-L1 preadipocytes were treated with various concentrations of SLE during differentiation. SLE of testing concentrations did not affect the viability (data not shown). At day 8 of fully differentiated state, the differentiated adipocytes were stained with the fat-specific Oil Red O. The adipocyte differentiation was inhibited by the treatment of SLE as compared to the control treated with MDI alone (Fig. 1). When the inhibitory effect of SLE was calculated by spectrophotometrical quantification, SLE significantly decreased the lipid accumulation in adipocytes in a dose-dependent manner (Fig. 2). At 400 ㎍/㎖ concentration of SLE, lipid accumulation was inhibited to similar levels as compared to the vehicle. These results show that SLE effectively inhibits adipogenesis *in vitro*. The adipogenesis as a dynamic and plastic process, is now considered to lead the phenotype of mature adipocyte. Not only adipogenic transcription factors such as C/EBP α and PPARg, but also external hormones affect adipocyte differentiation at cellular and molecular levels (Rosen, 2005). The inhibition of differentiation to mature adipocyte is related to prevent and treat obesity (Feve, 2005).

Since the inhibitory activity of SLE on adipocyte differentiation was demonstrated *in vitro* study in a dose-dependent manner, we continued to evaluate the effect of SLE on obesity in mice induced by feeding a high fat diet (HFD). High energy diet has been widely accepted as a strategy to induce over-weight conditions and fat deposition in animal experimental model (Buettner *et al*., 2007).

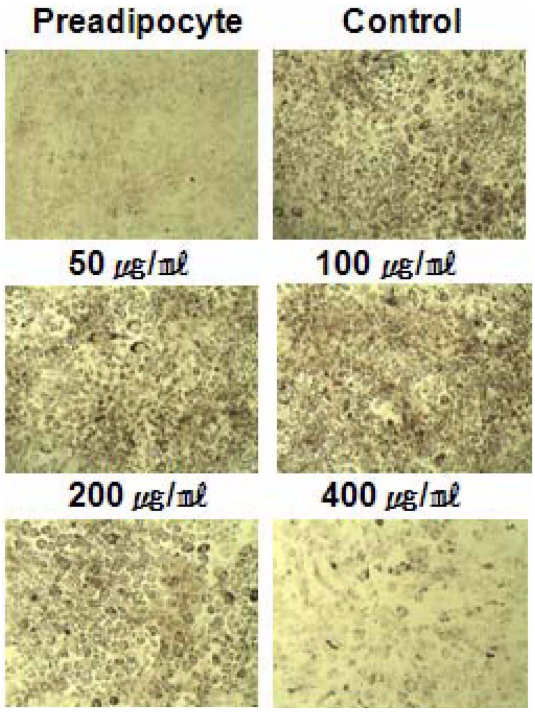

Fig. 1. Effect of SLE on the differentiation to adipocytes from 3T3-L1 preadipocytes. 3T3-L1 cells were treated with various concentrations (0, 50, 100, 200, or 400 ㎍/㎖) of SLE during the induction of differentiation, and then stained with Oil red O. Control is the fully differentiated adipocyte. The morphological changes were observed using microscope and photographed (100 × magnification).

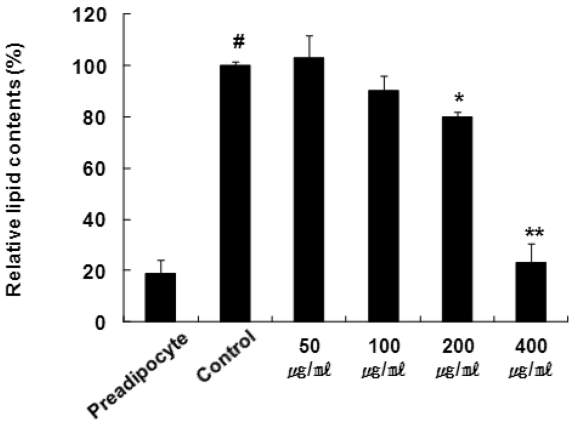

Fig. 2. Effect of SLE on lipid accumulation of the differentiated 3T3-L1 cells. 3T3-L1 cells were treated with various concentrations (0, 50, 100, 200, or 400 ㎍/㎖) of SLE during the induction of differentiation, and then stained with Oil red O. The stained oil droplets were dissolved with isopropanol and quantified by spectrophotometrical analysis at 520 ㎚. Control is the fully differentiated adipocyte. The results were represented as relative lipid contents. Each bar is the mean ± S.E. from three independent experiments. # $P < 0.001$ vs preadipocyte, *$P < 0.05$, **$P < 0.001$ vs control.

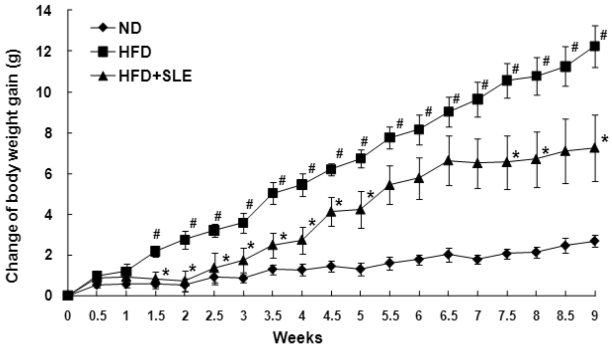

Fig. 3. Effect of SLE on body weight gain of mice fed with experimental diet. The mice were fed with normal diet (ND) or high fat diet (HFD) for consecutive 9 weeks. SLE was orally administered at the concentration of 200 ㎎/㎏/day to the mice in HFD+SLE group fed with high fat diet. The body weight gain was measured. Each point is mean ± S.E. (n = 6). # $P < 0.001$ vs ND, *$P < 0.05$ vs HFD.

During the experiment period, the amounts of body weight and food intake were monitored twice every week. The body weight was gradually increased with time, and the body weight of HFD-fed mice group became higher than that of normal diet (ND)-fed mice group for 6 weeks. Fig. 3 showed the changes in body weight gain of ND, HFD, and HFD+SLE group for consecutive 9 weeks. There were significant differences between HFD group and SLE-treated (HFD+SLE) group from week 1.5. The body weight gain of HFD group for 9 weeks was increased up to 5-fold compared to ND group. When SLE was administered orally for 9 weeks along with a high fat diet, the body weight gain induced by HFD was decreased to approximately 50% (Fig. 3). These results suggest that the treatment of SLE at 200 ㎎/㎏/day was effective in suppressing the body weight gain in obesity state.

However, the food intake of HFD+SLE group was not decreased as compared to HFD group (Fig. 4(A)). Additionally, the food efficiency ratio (FER) was higher in HFD group than in ND group, and was significantly decreased in HDF+SLE group (Fig. 4(B)). These results indicate that the inhibition of body weight gain by the treatment of SLE did not depend upon a decreased food or energy intake, because there was no significant difference in the diet intake between the control and experimental group.

It was reported that a continuous HFD may result in the fat accumulation, the increased serum leptin, and the decreased serum adiponectin (Matsubara, 2004; Uygun *et al.*,

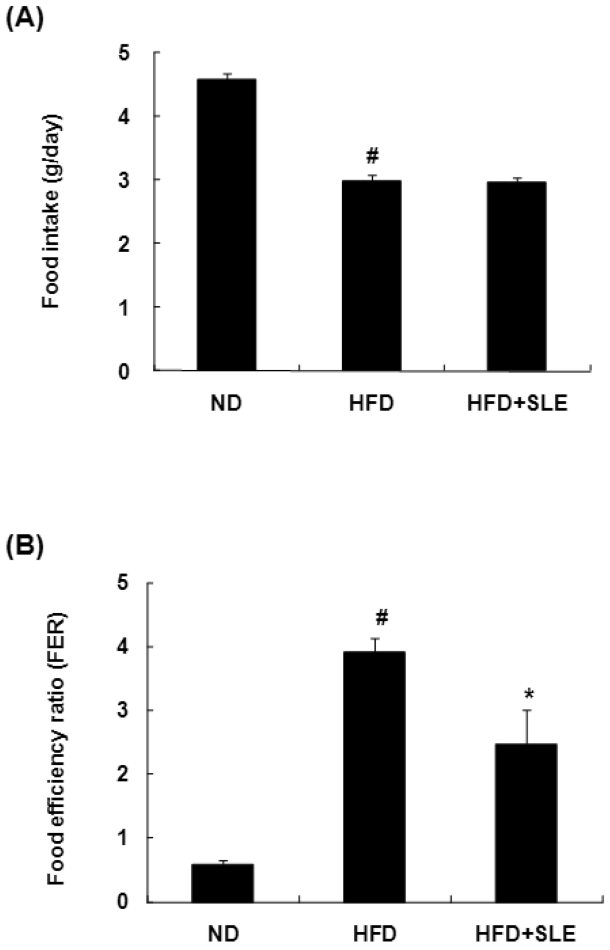

Fig. 4. Effect of SLE on food intake and food efficiency ratio of mice fed with experimental diet. The mice were fed with normal diet (ND) or high fat diet (HFD) for consecutive 9 weeks. SLE was orally administered at the concentration of 200 ㎎/㎏/day to the mice in HFD+SLE group fed with high fat diet. The food intake (A) was measured. The food efficiency ratio (FER) (B) was calculated as follows: body weight gain/food intake. Each bar is mean ± S.E. (n = 6). # $P < 0.001$ vs ND, *$P < 0.05$ vs HFD.

2000). Adipokines such as leptin and adiponectin, are secreted from adipose tissue, and their levels are closely correlated with the weight of adipose tissue (Friedman and Halaas, 1998). It has been reported that changes in the expression, secretion, and action of the adipokines involved in obesity may also be involved in the development of various disease (Mitchell *et al*., 2005) .

To clarify pharmacological mechanisms and safety of SLE, the further investigations must be performed. Also, the intensive researches need to be performed for the discovery of the new active components in SLE responsible for anti-obesity effect.

In conclusion, the present study reports for the first time that *S. lappa* has anti-obesity activity in 3T3-L1 adipocytes and high fat diet-induced obese mice. Thus, *S. lappa* may be a promising candidate for the treatment of obesity or may be useful towards the development of new anti-obesity therapy.

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