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제주 자생 식물들에 대한 항비만 효능에 대한 연구

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The Study on Anti-obesity Activity of the Wild Plants of Jeju Island

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요 약: 본 연구에서는 제주도에서 자생하는 30여 종의 식물추출물에 대한 항비만 효능을 조사하였다. 항비만 효능은 *in vitro* oil red-O staining 방법을 이용하여 지방전구세포 3T3-L1에서 분화억제력을 측정하였다. 그 결과, 30여 종의 식물 중에서 약도라지, 호장근, 유근피 등을 포함한 6종의 식물이 지방전구세포 3T3-L1의 분화를 억제하였다. 지방전구세포의 분화를 억제하는 물질 중 유근피, 약쑥, 호장근, 후박 4종은 우수한 항산화 효능도 동시에 가지고 있음을 확인하였다. 이러한 결과를 통해, 본 실험에서 확보된 추출물이 항비만 물질로 사용될 수 있는 가능성을 확인하였다.

Abstract: In this study, we investigated the anti-obesity activity of extracts collected from wild plants in Jeju island. The inhibitory effect of plant extracts on the differentiation of preadipocyte 3T3-L1 was examined by oil red-O staining. We found that extracts collected from 6 plants among 31 plants, namely, *Aralia elata* (Miq.) Seem, *Polygonum multiflorum* Thunberg, *Artemisia asiatica*, *Platycodon grandiflorum* (Jacq.) A. Dc., *Polygonum cuspidatum* S. et Z., *Magnolia obovata* Thunb, significantly inhibited preadipocyte differentiation. Additionally, 4 plant extracts were also found to have antioxidant activities in DPPH radical scavenging assay. Taken together, these results show that 6 plant extracts suppress the differentiation of preadipocytes, suggesting the potential use of 6 plant extracts as anti-obesity agents.

Keywords: *anti-obesity, preadipocyte, differentiation, DPPH radical scavenging assay, oil red-O staining method*

1. Introduction

Obesity is a chronic metabolic disorder that results from the imbalance between energy intake and energy expenditure. It is characterized by enlarged fat mass and elevated lipid concentration in blood[1,2]. The amount of fat mass is increased when the number and/or size of adipocytes are multiplied by proliferation and differentiation. Differentiated adipocyte stores fatty acids (FAs) in the form of triglycerides (TGs) in their cytoplasm, with an involvement of various enzymes such as strearoyl-CoA desaturase-1 (SCD-1) and fatty acid synthase (FAS). This overall lipid synthetic process is called lipogenesis[3]. Adiposity has been shown to have a strong linear correlation with elevated plasma levels of FA. If blood FA levels are elevated for prolonged periods by excessive energy intake, TG can be accumulated in non-adipose tissues including liver and muscle, which can lead to pathological consequences such as the development of fatty liver or ketosis[4]. To prevent this undesirable situation, the body employs an FA-oxidation mechanism that allows the FA to break down into CO_2 and ketone body, and be released into blood. In this lipolytic process, many

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enzymes are involved[5].

Many attempts have been made to correct the metabolic disparity of the obesity condition, producing a number of reagents including Sibutramine (appetite suppressor), Orlistat (gastrointestinal lipid uptake inhibitor), and Fibrates (PPAR alpha agonists)[6,7]. However, administration of these drugs is known to often cause undesirable side effects such as a dry mouth, anorexia, constipation, insomnia, dizziness, and nausea[8]. Therefore, there has been high demand for therapeutically potent, and yet safe, anti-obesity reagents.

A number of preadipocyte cell lines have been used for studying obesity, including the following: 3T3-L1 [9,10], 3T3-F442A[11], Ob17[12], and PFC6[13]. Green *et al.* have clonally isolated a cell line of mouse fibroblasts (3T3-L1 cells) that can differentiate into adipocytes. This cell line offers an excellent model system for the study of differentiation processes[10]. During exponential growth, 3T3-L1 cells are morphologically indistinguishable from murine fibroblasts. Following growth arrest, they differentiate into adipocytes under appropriate culture conditions. The most efficient means to trigger the differentiation is to treat the confluent cultures of 3T3-L1 fibroblasts with a combination of insulin, dexamethasone, and 3-isobutyl-1-methylxanthine (IBMX)[14].

In this study, we have demonstrated that among 31 wild plants of Jeju island, treatment with 6 plant extracts inhibits differentiation of 3T3-L1 preadipocytes.

2. Materials and Methods

2.1. Cell Culture Treatment

3T3-L1 preadipocytes (A.T.C.C., Manassas, VA, U.S.A.) were cultured in Dulbecco's modified Eagle's medium (DMEM, WelGENE, Korea) with 10 % (v/v) fetal bovine serum (FBS), 1 % (v/v) penicillin/ streptomycin (10,000 units/mL penicillin, 10,000 μg/mL streptomycin in 0.85 % saline) at 37 ℃ in 95 % air, 5 % CO_2. To induce differentiation, 2-day postconfluent 3T3-L1 preadipocytes (day 0) were stimulated for 72 h with 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone and 10 μg/mL insulin (MDI) added to DMEM/10 % FBS culture medium. From day 3 on, cells were incubated with DMEM, 10 % serum, 10 μg/mL insulin, which was changed every 3 days thereafter until analysis. Plant extracts were reconstituted as 10 mg/mL stock solutions in DMSO.

2.2. Oil Red-O Staining Assay

Culture dishes were washed twice in phosphate-buffered saline and fixed for 30 min in phosphate-buffered saline containing 4 % formaldehyde. After a single wash in water, cells were stained with oil red-O for 30 min. After the staining, dishes were washed twice in water and photographed. Oil red-O was prepared by diluting a stock solution (0.5 g of oil red-O (Sigma, MO, USA) in 100 mL of isopropanol) with water (3 : 2), followed by filtration.

2.3. Cytotoxicity Assay

This assay was performed after 3T3-L1 preadipocytes were induced to differentiate into adipocytes in the presence of plant extracts. The general viability of cultured cells was determined by the reduction of WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) (Dojindo Laboratories, Japan) to a highly water-soluble formazan dye. To each well, 10 mL of WST-8 solution was added. And then cells were incubated at 37 ℃ for 3 h and the absorbance was measured at 450 nm using a spectrophotometer (Power Wave, Bio-tek Inc, VT, USA). Data are presented as mean ± S.D. All values were significant ($*p < 0.05$) compared with values for control. The entire experiments were performed in triplicate and results were confirmed by three independent experiments.

2.4. DPPH Assay

1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma, MO, USA), a stable nitrogen-centered free radical, was dissolved in methanol for 5 min to give a 200 μM solution. The tested compounds were added to DPPH of equal volume in a 96-well microplate as quadruplicates, along with sample blanks and controls. The concentration (absorption) of DPPH during the 30 min observation time was measured at 540 nm. The decrease in absorption at 540 nm was correlated with the scavenging action of the tested compound. Data were presented as the mean ± S.D. Experiment was performed in duplicate and repeated three times.

Table 1. 31 Plants Extracts Used for Experiments

No.	Genus species	Plant part	Preparation used
1	*Allium monanthum* MAX	E	70 % EtOH
2	*Sedum sarmentosum* Bunge	E	〃
3	*Hypochoeris radicata*	L, ST	〃
4	*Aralia elata* (Miq.) Seem	R. B	〃
5	*Polygonum multiflorum* Thunberg	R	〃
6	*Sargassum fulvellum*	R	〃
7	*Persicaria hydropiper* (L.) Spach *var. hydropipe*	E	80 % MeOH
8	*Oenanthe javanica*	E	70 % EtOH
9	*Mentha arvensis*	L, ST	〃
10	*Plantago asiatica* L.	E	〃
11	*Duchesnea chrysantha* (Zoll. et Morr.) Miq	E	〃
12	*Centella asiatica* (L.) Urbain	E	〃
13	*Selaginella involvens* (sw) Spring	E	〃
14	*Allium odorum*	E	〃
15	*Brassica oleracea*	E	〃
16	*Amaranthus magostanus*	E	〃
17	*Cirsium japonicum* var. ussuriense	R	〃
18	*Pinus densiflora* Siebold & Zucc	L	〃
19	*Artemisia asiatica*	E	〃
20	*Platycodon grandiflorum* (Jacq.) A. Dc	R	〃
21	*Achyranthes japonica* N	R	〃
22	*Althaea rosea*	E	〃
23	*Polygonum cuspidatum* S. et Z	E	〃
24	*Magnolia obovata* Thunb	B	〃
25	*Elsholtzia splendens* Nakai	E	〃
26	*Corydalis ochotensis*	E	〃
27	*Cocculus trilobus* (Thunb.) DC	E	〃
28	*Gynostemma pentaphyllum* (Thunb.) Makino	E	〃
29	*Mosla punctulata* (J.F.Gmelin) Nakai	E	〃
30	*Gnaphalium affine* D. Don	E	〃
31	*Solidago serotina* Aiton	E	〃

E: entire plants, R: roots, L: leaves, ST: stems or twigs, B: barks

2.5. Statistics

The statistical significance of the data was determined by Student's t-test. $p < 0.05$ was considered significant.

3. Results and Discussion

3.1. Effect of Plant Extracts on Differentiation of Preadipocytes

We assayed water-soluble extracts from various plants for anti-obesity activities. For the ease of screening procedure, oil red-O staining assay was employed in this study. 3T3-L1 preadipocytes were treated with 10 μg/mL plant extracts, and the inhibitory effect on adipocyte differentiation was tested by photospectrometrical quantification of oil red-O stain bound to the lipid droplets. Among 31 plants, we found that 6 plants, including *Aralia elata* (Miq.) Seem, *Polygonum multiflorum* Thunberg, *Artemisia asiatica*, *Platycodon grandiflorum* (Jacq.) A. Dc., *Polygonum cuspidatum* S. et Z., *Magnolia obovata*. Thunb. As shown in Figure 1 and Figure 2, plant extracts inhibited adipocyte differentiation. Among 6 plants with anti-obesity activity, *Platycodon grandiflorum* (Jacq.) A. Dc., *Polygonum cuspidatum* S. et Z, showed more potent activity than others. This result suggests the possibility that tested plant extracts may be involved in blocking the differentiation of preadipocytes. However, there remain the possibility that the inhibition of adipocyte differentiation was induced by a cytotoxic effect of each plant extracts. To confirm this result, we performed an cytotoxicity assay in 3T3-L1 preadipocytes. According to the results of this assay, each plant extracts showed no cytotoxic effects at the tested

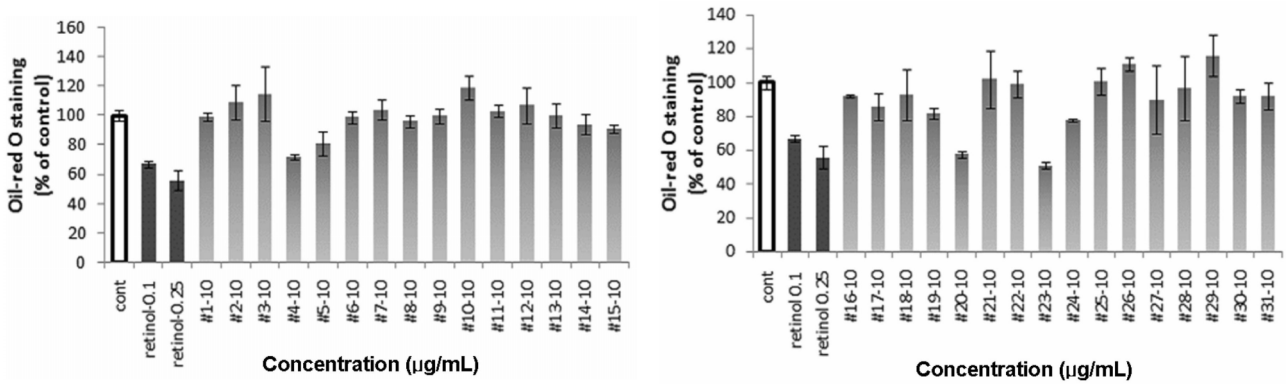

Figure 1. Effect of plant extracts on adipocyte differentiation. To determine the effect of plant extracts on adipocyte differentiation, 3T3-L1 preadipocytes were differentiated into adipocytes in the presence or absence of the indicated concentrations of plant extracts and analyzed 14 days after the induction of differentiation. Data are expressed as mean ± S.D. $*p < 0.05$ compared with untreated control. Results were confirmed by three independent experiments.

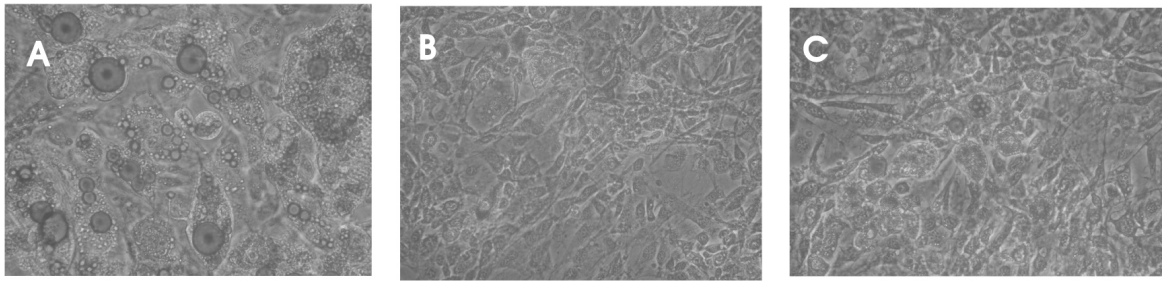

Figure 2. Lipid accumulation of 3T3-L1 preadipocytes cultured in the absence or presence of plant extracts. Preadipocytes were incubated with (a) 0 (b) 10 μg/mL *Platycodon grandiflorum* (Jacq.) (c) 10 μg/mL *Polygonum cuspidatum* S. et Z. for 14 days after initiation of differentiation protocol. Cellular lipid was stained with oil red-O.

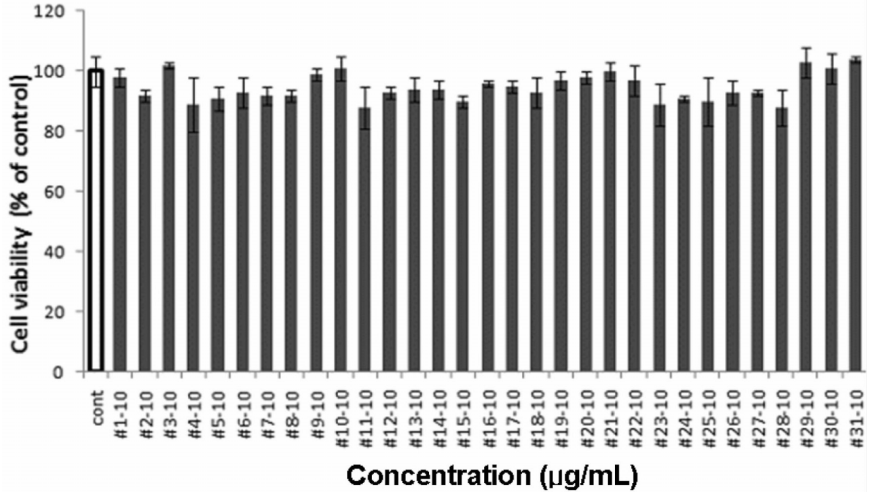

Figure 3. Cytotoxicity of plant extracts against 3T3-L1 preadipocytes. This cytotoxic assay was performed after the incubation of 3T3-L1 preadipocytes in the presence or absence of indicated concentrations of plant extracts, for 14 days at 37 ℃ in a 5 % CO_2 atmosphere. Cellular cytotoxicity was determined according to the protocol described in materials and methods and was expressed as the mean ± S.D. All values were significant ($*p < 0.05$) compared with values for untreated control.

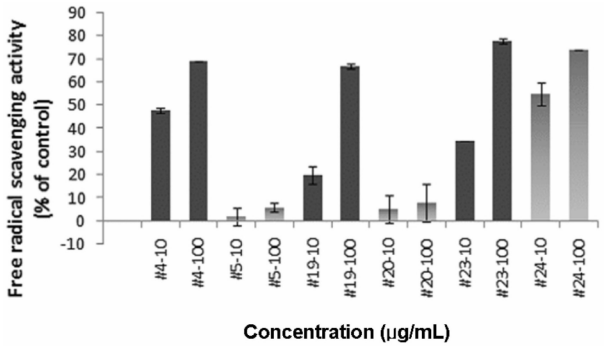

Figure 4. *In vitro* anti-oxidative activities of plant extracts using DPPH radical scavenging assay. Data are expressed as mean ± S.D. $*p < 0.05$ compared with a control. Results were confirmed by the experiment which was repeated three times in triplicate.

concentrations (Figure 3).

3.2. Antioxidant Effect of Plant Extracts

Until now, we found that 6 plants have anti-obesity effect on 3T3-L1 preadipocytes. In order to investigate anti-oxidative effects of plant extracts, we carried out *in vitro* test for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The DPPH test showed that tested plant extracts have significant anti-oxidative activities (Figure 4).

4. Conclusions

In order to screen plant extracts having anti-obesity activity, oil red-O staining assay was introduced. In these experiments, 6 plants were found to have inhibitory effect on 3T3-L1 adipogenesis.

These data suggest that 6 plant extracts can be introduced as an anti-obesity ingredient.

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