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Anti-Obesity Effects of Red Beet Extract

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Abstract

Obesity is caused by the accumulation of triglycerides in adipocytes by the differentiation and lipid synthesis process of pre-adipocytes, and excessive accumulation of adipocytes by the activated Adipogenesis process within the differentiated cells. Therefore, inhibiting the differentiation of adipocyte cells or controlling the adipogenesis process is known as an effective treatment method for obesity. This study evaluates the inhibition of Red beet root extract on pancreatic lipase and pre-adipocyte cell differentiation. Also it evaluates the Red beet root extract activities on C/EBP-α,β, and PPAR-Υ. The experiments proved that the Red beet root extract inhibits pancreatic lipase by concentration dependency. Further, in 3T3-L1 inhabitation experiment, it was found Red beet root extract inhibited adipocyte formation. Red beet root extract also inhibits the expression of C/EBP-α, C/EBP-β, and PPAR-γ which effect the process of adipocytic differentiation. We therefore concluded that RBE has a high potential to further studies on anti-obesity effect.

Keywords: *Red beet root, Anti-obesity, Slimming, Pre-adipocyte, lipase inhibition*

1. Introduction

Westernized diet and improvement of living standards increase the number of overweight or obese patients in modern society. Obesity is a condition that excessive energy is not consumed but hyperaccumulation as triglyceride. This has emerged as a serious worldwide problem as it is known to be a cause of cardiovascular disease, diabetes and high blood pressure. [1-3] There are methods to prevent and treat obesity which include physical exercise and dietary therapy. However, improvement in life style is not simple, and medication treatment is not effective or has side effect [4, 5].

Obesity is caused due to triglyceride accumulation in fat cells during pre-adipocyte differentiation and lipid synthesis process, and excessive accumulation of fat cells from Adipogenesis process activation within differentiated cells. Therefore, inhibiting pre-adipocyte differentiation or controlling lipogenesis process is widely known to be the effective treatment for obesity [6]

Red Beet, a family of goosefoot which its origin is known to be the southern Europe, has low calories and rich in mineral and vitamins [7].

Especially, it is reported that the betalains, natural pigment mater, has remarkable free radical elimination

and also has remarkable antioxidant activity such as preventing lipid peroxidation action caused by active oxygen [8-10].

In addition, Beet extract has intracellular anti-inflammatory effects, precautionary effects for skin and lung cancer with its tumor suppression, and protective effect on liver damage by lipopolysaccharide and alcohol [11].

However, studies on slimming-related such as anti-obesity effects are very rarely done. Therefore, this study has checked the possibility of developing anti-obesity effect and slimming through *in vitro* experiment using low side effect natural material, Red Beet.

2. Material and Methods.

2.1 Manufacturing Red Beet Extract.

Red Beet Extract was made from twenty times volume of distilled water, extracted for 2 hours at 95 Celsius degree then filtered. After that, concentrated using Rotatory Vacuum Evaporator (EYELA, Japan) then freeze-dried using Freeze Dryer. (EYELA, Japan)

2.2 Cell Line and Cell Culture.

For the experiment, 3T3-L1 pre-adipocyte cell, purchased from American Type Culture Collection (ATCC) was used, and 10% of fetal bovine serum and 1% of antibiotics-antimycotic adding Dulbecco's modified Eagle's medium (DMEM, Gibco Inc., USA) were used to subculture with CO_2 cell incubator (MCO-170AIC, Panasonic) under the condition of 37.5% of CO_2.

2.3 Cell-Toxicity Experiment

MTT assay was experienced before the anti-obesity active experiment to verify the effect of the sample on cell survival, and cell treatment concentration [12]. 1×10^5 cells were attached to 48-well tissue culture plate for 24 hours, treated the samples by concentration, then incubated for 72 hours again. After incubation, MTT (5 mg/mL, Sigma, USA) was diluted with PBS, treated 120μL per well, incubated in CO_2 incubator for 4 hours, eliminated supernatant, added 100μL of dimethyl sulfoxide (Sigma, USA) in each well to dissolve formazan crystals, and then measured absorbance at 540nm using the microplate reader (SpectraMax iD3, Molecular Devices, Canada).

2.4 Inhibition Measurement of Pancreatic Lipase Activity

First, put 0.25 M Tris (pH 7.7), 250 mM CaCl2, 5 mM 4-nitrophenyl dodecanoate (PNPD) to 1.5 ml EP-tube, waited to react for 5 minutes at 37 °C, mixed Beet Extract from each concentration with lipase which is dissolved in 0.25 M Tris-buffer, waited to react for another 10 minutes at 37°C, and lastly added 20% sodium dodecyl sulfate (SDS) to end the reaction. The reacted solution was centrifuged at 15,000rpm, 4°C for 20 minutes, and added supernatant to dispense in 94-well plate. For the last step, eliminated the absorbance of the initial sample from the absorbance of the sample using microplate reader, waited to be reacted at 412nm for 10 minutes, and the value was calculated in percentage basis in comparison to control.

2.5 Adipogenesis Induction

The products which are used to induce cell differentiation, such as MDI including 0.5 mM IBMX, 1μM dexamethasone and 1μg/ml insulin, were purchased from Sigma (USA). The induction of adipocytic

differentiation was carried out by moving 3T3-L1 fat bulb cells 2×10^5 cells/ml into 6 well-plate until the cell density reached close to 95%, Then it was put to be incubated for another 48 hours after changing medium. All after, replaced it to differentiated induction medium containing MDI, then Red Beet Extracts were treated by concentration level for 8 days having 2-day interval followed by treatment of insulin and samples. The samples which were not treated but induced differentiation, were put into negative control group.

2.6 Oil Red O Assay

After eliminating the cell culture medium, washed twice using PBS (WelGENE Inc., Korea) and treated 4% formaldehyde (Sigma, USA) to react for 20 minutes at room temperature. Then, washed twice, a minute each using ddH20 and 60% of isopropanol, treated Oil Red O Solution for 10 minutes and dyed, and checked the adipocytic differentiation by using microscope (CKX53, Olympus). In order to quantitively analyze the fat globule formation, isopropanol was treated and Oil Red O was retrieved, removed to 96 well-plate and the absorbance was measured at 520nm using microplate reader.

2.7 Real-time PCR

The cell line 3T3-L1 were put into 6-well plate in the size of 5×10^5 cells/well and attached it for 24 hours, then it was treated in the same manner as adipogenesis induction. After this treatment the cells were yield, RNA was separated accordance with NucleoSpin RNA (MN, Germany) Kit protocol, then cDNA was synthesized using ReverTra Ace RT-Kit (Toyoba, Japan) to use as a template for Real time PCR. The primer for the experiment is listed in Table 1.

Table 1. C/EBP-α,β, PPAR-ϒ , GAPDH primer

Primer		sequence
C/EBPα	Forward	TGTGCACGTCTATGCTAAACC
	Reverse	CCGTTAGTGAAGAGTCTCAGT
C/EBPβ	Forward	GTTTCGGGAGTTGATGCAAT
	Reverse	AACAACCCCGCAGGAACAT
PPARϒ	Forward	CGCTGATGCACTGCCTATGA
	Reverse	TGCGAGTGGTCTTCCATCAC
GAPDH	Forward	ACCACAGTCCATGCCATCAC
	Reverse	TCCACCACCCTGTTGCTGTA

3. Result and Study

3.1 Cell-Toxicity Experiment

Cell-toxicity of Red Beet Extracts were measured using MTT. As shown in Figure 1, cell survival was 98% at the maximum concentration of 500ug/ml, and 99.5% at 100ug/ml. The test was executed in the range between 100ug/ml ~ 500ug/ml, confirming Red Beet Extracts do not show cell-toxicity was not found up to
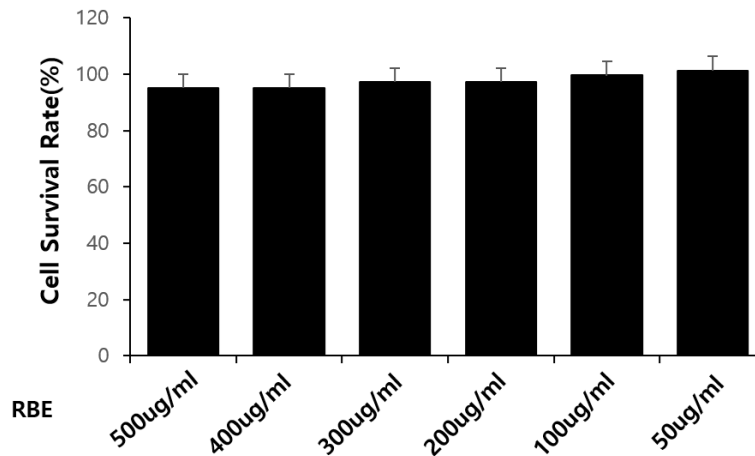
concentration of 500ug/ml.

Figure 1. Cell Survival rate in 3T3-L1

3.2 Pancreatic Lipase Activity Inhibition

Pancreatic lipase secreted from the pancreas works for hydrolysis of triglyceride into glycerol and fatty acid help the fatty acid to enter easily into the cell. The entered fatty acids are converted into triglyceride and accumulated if not consumed by energy metabolism. Inhibiting pancreatic lipase activity can expect the obesity prevention effects by blocking the entering of fatty acid and thus inhibit the accumulation of triglycerides [13].

It is studied that Red Beet Extracts were able to inhibit 8% of lipase activity at concentration of 100ug/ml, and 38% at 500ug/ml. As shown in Figure 2, it was confirmed that the inhibitory activity of lipase was dependent manner.
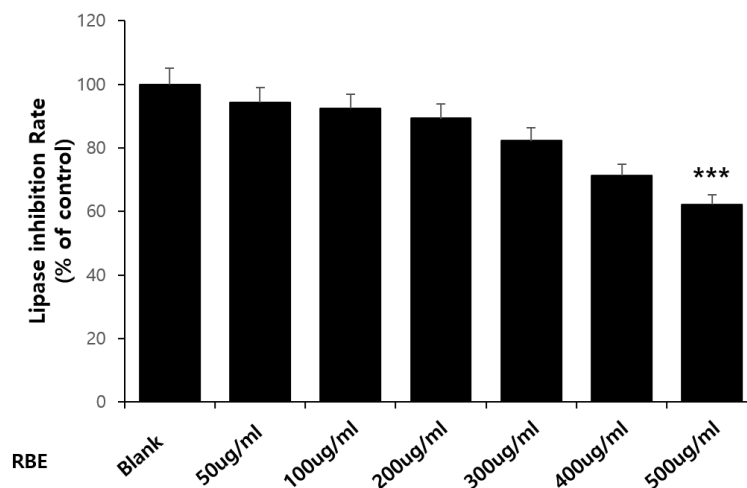

Figure 2. Pancreatic Lipase Inhibition Rate by Red Beet Extracts

3.3 Formation of adipocyte (Oil Red O Assay)

Oil Red O staining experiment was executed to check the effect of Red Beet Extracts on cell differentiation inhibition of preadipocyte cells and 3T3-L1 cells, and on fat globule formation inhibition. The result showed that Red Beet Extracts inhibited the differentiation of 3T3-L1 cells, and significantly reduced the number of fat globules dyed in Oil Red O in Figure 3(a).

With Red Beet Extract 200ug/ml, lipid storage amount was decreased around 19% compare to control, and decreased around 38% with 500ug/ml in Figure 3(b).
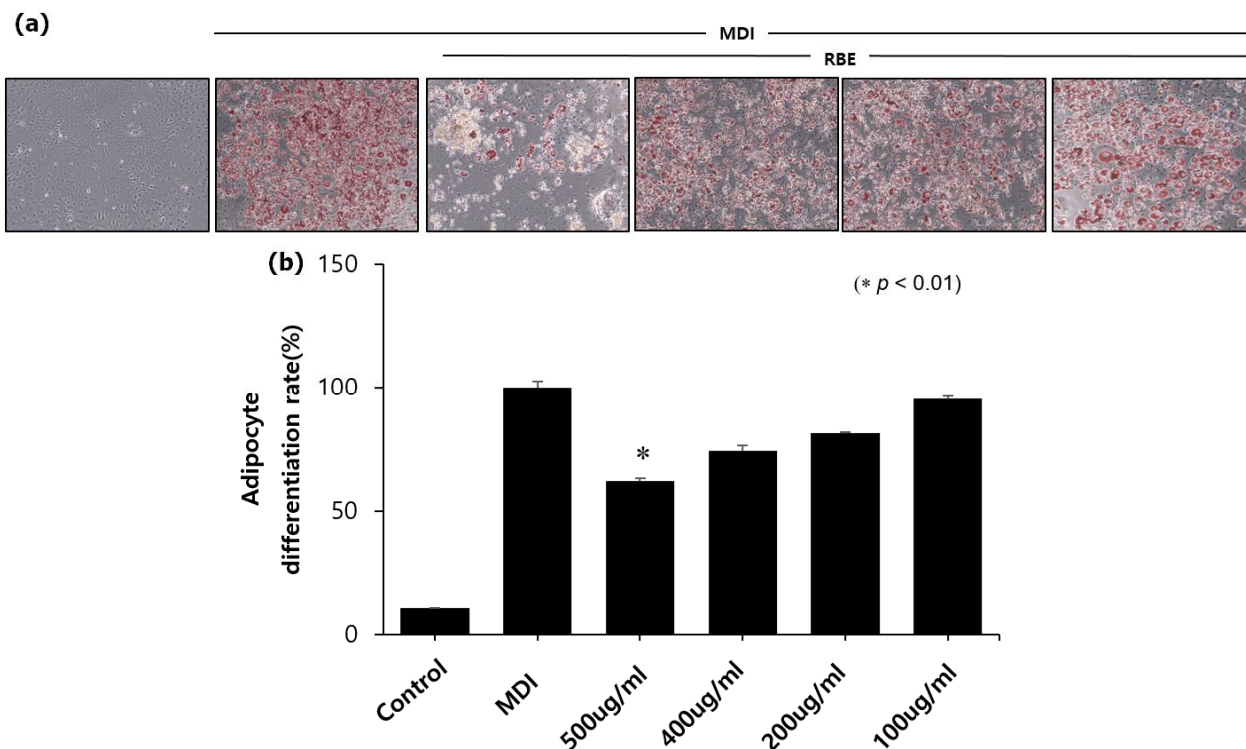

Figure 3. Inhibition of adipocyte differentiation in MDI-stimulated 3T3-L1 cells

3.4 Verification of Real-Time PCR Gene Expression relating Adipogenesis

3T3-L1 preadipocyte cell begins differentiation from preadipocyte cell to adipocyte when C/EBP-β and C/EBP-δ are stimulated by insulin, dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX) and so on [14]. During this, C/EBP-β and C/EBP-δ function individually or simultaneously to induce peroxisome proliferator-activated receptor γ (PPARγ) and C/EBP-α [15]. It is known that C/EPB-α and PPARγ express in the early stage of differentiation, and induce the expression of various adipocytes and specific genes in the late stage with the increased amount of total expression [16]. Expression amount change of C/EBP-αβ, PPAR-Υ and genes are used as a significant indicator for identifying the adipocyte differentiation control as these are the main factor in entire adipocyte differentiation process [17,18].

As a result of treating the red beet extract 500ug/ml, it was confirmed in Figure 4(a) that C/EBP-α was suppressed by about 53% compared to the control group. And, in Figure 4(b), the expression of C/EBP-β was reduced by about 33%, and it was confirmed that PPAR-Υ was suppressed by about 21% as shown in Figure 4(c).
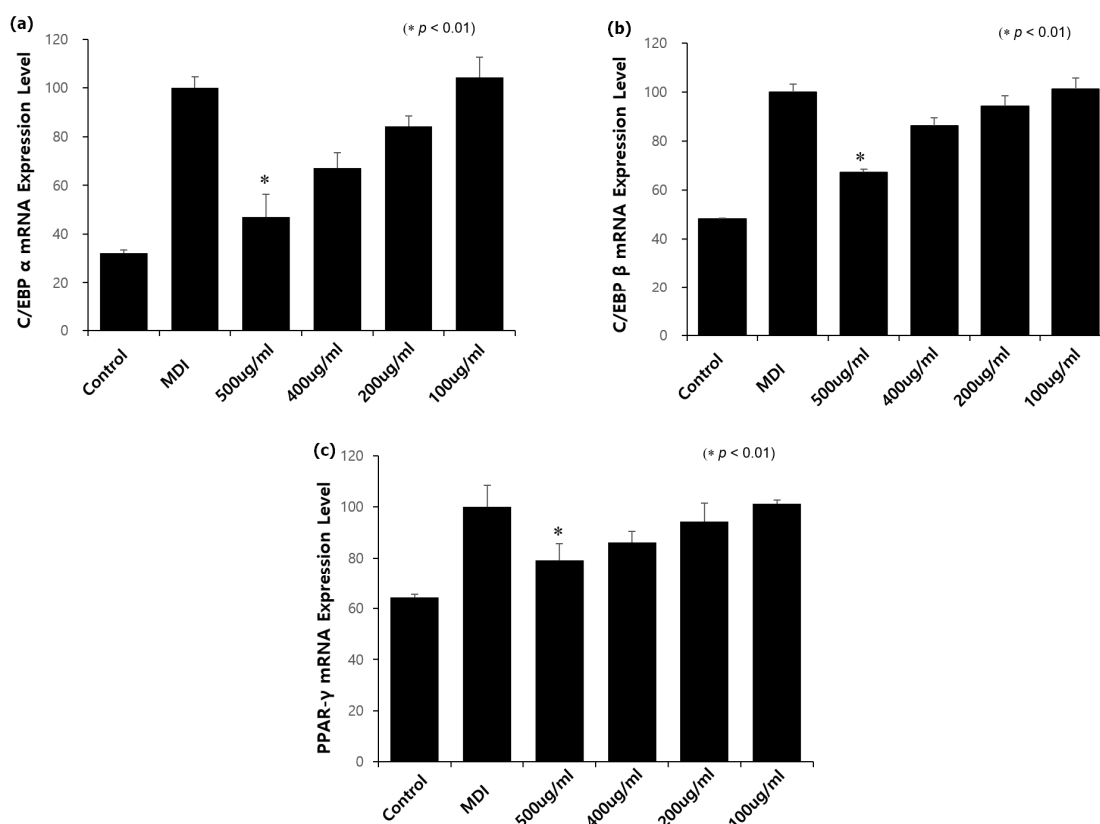
Figure 4. Adipogenesis related mRNA expression level in 3T3-L1 cells.

4. Conclusion.

This study was done to develop natural anti-obesity and slimming matter using natural extract from Red Beet. It was found that Red Beet Extracts were able to inhibit 8% of lipase activity at concentration of 100ug/ml, and 38% at 500ug/ml during pancreatic lipase activity inhibition experiment.

Also, in 3T3-L1, the preadipocyte cell, inhabitation experiment, it was found that Red Beet Extracts inhibited 38% of adipocyte formation in 500ug/ml, and in the range of 100-500ug/ml, it also inhibited adipocyte formation by concentration dependency.

Furthermore, the effect of Red Beet Extracts on the expression of C/EBP-α, β, PPAR-ϒ, the key factors at adipocytic differentiation, was studied and it was found that expression of all three factors are reduced by Red Beet Extract concentration dependency.

To sum up all the experimental results, Red Beet Extract can block the enter of fatty acid and prevent excessive accumulation of triglycerides by pancreatic lipase inhibition. In addition, by inhibiting the expression of C/EBP-α, β, PPAR-ϒ through inhibiting preadipocyte cell and fat globules, it can result in anti-obesity and slimming.

In conclusion, We suggested that red beet extracts can be used as food for obesity prevention or slimming, and can also be used as cosmetic ingredients. However, further study on single-active substance indicating anti-obesity in Red Beet Extracts is needed.

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