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ANTIOBESITY OF FUCOXANTHIN FROM *Sargassumechinocarpum* BY INCREASING β-OXIDATION IN ADIPOCYTE

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ABSTRACT

Fucoxanthin is a one of carotenoids that contained in brown seaweeds. This compound has been affected to lipid metabolism. The aim of this study was to evaluate the antiobesity of fucoxanthin of *Sargassumechinocarpum* on adipocyte by increasing of adiponectin and decreasing of tumor necrosis factor α expressions. Fucoxanthin was isolated from *Sargassumechinocarpum* and validated by infrared spectrophotometer. Adipocyte was obtained from pre-adipocyte cell from viscera tissue of wistar rats (*Rattusnorvegicus*) and cultured in fetal bovine serum and Dulbeccos modified eagle's medium. Adipocyte was treated with fucoxanthin and quercetin as control. Expression of adiponectin and tumor necrosis factor α of adipocyte was verified by enzyme link immunosorbent assay (ELISA) and observed by ELISA reader. The result showed that infrared spectra of *Sargassumechinocarpum*extract was equal with infrared spectra of fucoxanthin. Adipocyte cells treated with fucoxanthin showed increasing of adiponectin and decreasing of tumor necrosis factor α expression. It indicated that fucoxanthin able to enhance β-oxidation in adipocyte cells. In conclusion, our findings indicate that fucoxanthin from *Sargassumechinocarpum* able to increase adiponectin and decrease tumor necrosis factor α expression in adipocyte and it is promising to develop anantiobesitynutraceutical.

Keywords: adipocyte, adiponectin, fucoxanthin, *Sargassumechinocarpum*, tumor necrosis factor α

INTRODUCTION

Obesity is a one of enormous syndrome that affect metabolism and molecular system of human. Obesity able to induce macrophage for producing inflammation cytokines (WellenandHotamisligil 2003). These cytokines capable to decrease insulin activity (insulin resistance) and it increaseproducingmore free fatty acids. This condition provoke increasing expression of adipokine in adipocyte, such as: leptin, resistin, interleukin (IL-6), RBP4, and TNFα, and decreasing expression of adiponectin (Nadler *et al*. 2000; Soukas*et al*. 2000; Attieand Scherer 2009).

Fucoxanthin is one of carotenoids that found in brown seaweed (Dembitsky 2007; Terasaki *et al*. 2009; Miyashita 2009). This active compund have several functional effect for human body, for instance: antiproliferation of cancer (Hosokawa *et al*. 2004), antiinflamation (Shiratori *et al*.2005), antioxidant (Sachindra *et al*. 2007), antihyperglycemic (Maeda *et al*. 2007) and antiobesity (Maeda *et al*. 2005; Jeon *et al*. 2010). Study of nutrigenome show that fucoxanthin act as antiobesity by molecular mechanisms. The capacity of fucoxanthin induce

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expression of *uncoupling protein* 1 of mithocondria in white adipose tissue will increase β-oxidation and then it provoke the antiobesity effects(Miyashita 2009, Jeon *et al*. 2010).

The fucoxanthin of*Sargassumhorneri*S. *thunbergii, S. confusum, S. fushiforme, and S. patens*have been known as antiobesity compound (Miyashita 2009). *Sargassum echinocarpum*is a one of *Sargassum* sp in aquatic out the utilization of this seaweed is limited.The purposes of this study were to isolate fucoxanthin from *Sargassum echinocarpum* and evaluate it by increasing adiponectinand decreasingTNF αexpression mechanismn adipocyte

MATERIAL AND METHODS

The research was divided to two steps; the first was fucoxanthin isolation from brown seaweed (*Sargassum echinocarpum*) and the last was the evaluation of fucoxanthin on pre-adipocyte cells.

1. Extraction

Sample was dried at 40^{0}C for two days and then powdered.Powder extracted by acetone:methanol (7:3) and extracts were concentrated by rotary evaporator at 50◦C. After that 0.1 g of extract was partitioned with 200 mL of hexana and 220 mL of metanol 90% three times, and then adding 220 mL of methanol 90%, methanol 70% and finally it was added 200 mL hexana. The upper phase was obtained and macerated with 150 mL of diethyl ether for 24 hours.

Extracwas concentrated, macerated by 10 mL of benzene and eluted in column silica gel for 2-3 times by acetone:hexane (1:3). The orange eluat was collected and concentrated by rotavapor. Concentrat was diluted by acetone:hexane (1:3) and freezed immediately at -20°C for 2 days, and prior to ucoxanthin will be obtained, it was washed by cold hexane.

2. Identification

The extract was diluted to $10^{-3}$with acetone and subsequently the spectra of extract was identified by infrared and ultraviolet visible spectrophotometer.

2.1 Culture of Pre-adipocyte cells

Pre-adipocyte cells were isolated from adipose tissue of white rats viscera (*Rattusnorvegicus*). Cells were washed dPBSand chopped to smaller and then washed in DMEM *(dulbecco's modified eagle's medium)*containing 10% FBS *(fetal bovine serum)*, 100 U/ mL of penicillin, and 100 µg/mL of streptomycin at 37°C. During washing it was aerated by 95% of air and 5% of CO_2. Chopped adipose tissue was mixed with type I collagen in DMEM and it was incubated on water bath at 37◦C for 60 minutes. After that it was centrifuged at 1500 rpm for 7 minutes and residue was dissolved DMEM for two times. Finally it was added DMEM and FBS, divided and incubated.Each two days, media were changed until the adipocyte cell grew.

2.2 Fucoxanthin assay

Culture cells were washed with PBS, tripsin EDTA 0.25% solution, incubated at 37°C, added with media and FBS serum, homogenized and finally incubated at 37°C and CO_2 5%. The culture cells were treated with several concentration of fucoxanthin, i.e.: 0, 25, 50, 75, and 100 µM in DMSO.

2.3 Determination of Adiponectin and TNF α(Indra 2005)

Adiponectin was measured by elisa (enzyme linked immunosorbent assay) method. Adipocyte was incubated in C buffer (1:4000) at 4°C for a night and washed with PBS Tween 0.2% for six times. Cells was added primer antibody (1:4000) and incubated and shaked at room temperature for 1-2 hours. Subsequently it washed with PBST 0.2% for six times, mixed secondary antibody with biotin anti rabbit labelled (1:8000) and incubated for one hour. Afterward it was rinsed PBST 0.2%for six times, added SA-HRP (1:8000) enzyme and incubated and agitated at room temperature for one hour. Later it was washed PBST for six times and added TMB substrate for 15-20 minutes until it colored blue. If it was yellowish, it was stopped by HCl 1 N for 15 minutes and finally it was observed by *Elisa Reader*at 450-492 nm.

3. Data analysis

This experimental design used the complete random design and each treatment was repeated three times. Data was evaluated by analysis of variance and the difference among treatments were determined by the least significance difference. The probability level of this research was α - 1%.

RESULTS AND DISCUSSION

1. Identification of extract

Based on infra-redspectra of *S.echinocarpum* extract indicated that extract arranged of several group function of fucoxanthin identifier, i.e.: 3481.90, 3000-2858, 1724, 1652 and 1247 nm.

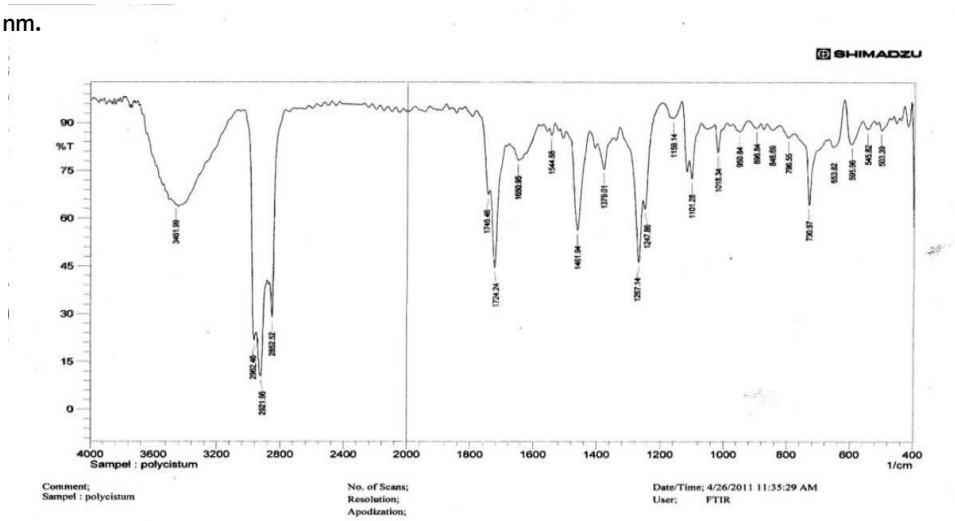

Figure 1. Infra-red spectra of from *S. echinocarpum*

These spectra were as same as with infra-red spectra of fucoxanthin that determined by Hauganet *al*. (1992), where 3481,90 nm showed OH group, 3000-2858 nm was CH group, 1724 nm was C=O as acetat group, 1652 nm was conjugated C=O groupedand 1247 nm was CO asetat group, respectively. Infra-red spectra of brown seaweed sample showed at Figure 1

2. Adiponectin

The result showed that adiponectin level of adipocyte among treatments were highly significant ($p < 0.01$). Decreasing of adiponectin level in adipocyte treated fucoxanthin showed at Figure 2.

The adiponectin concentration of adipocyte treated by 100 and 150 mM of fucoxanthin showed the higher than other treatments. It mean that using fucoxanthin at these concentrations able to decrease expression of inflammation cytokine and consequently increase β-oxidation mechanism in adipocyte.

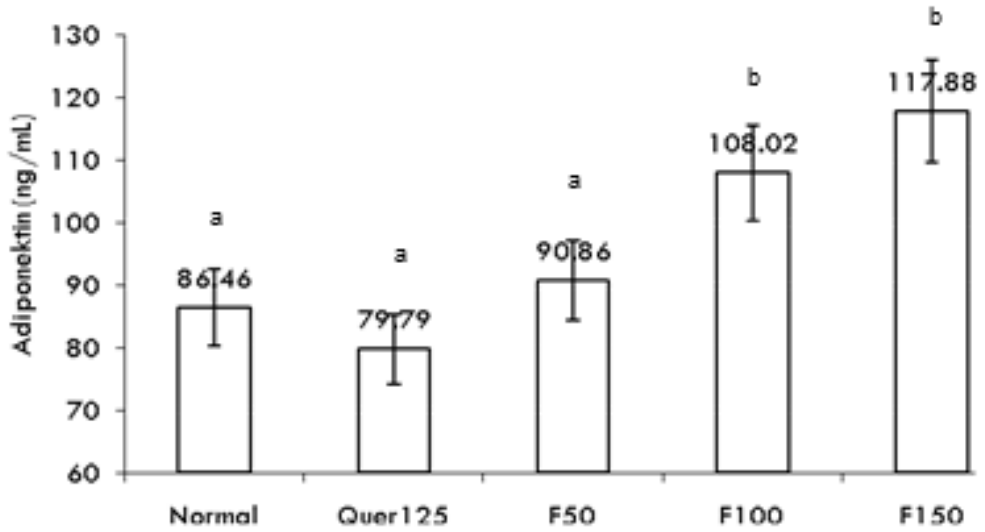

Figure2. Adiponectin level of adipocyte treated several concentration of fucoxanthin

The same result have been reported by Hosokawa *et al*. (2010) that fucoxanthin able to increase adiponectinexpression on obesity and diabetes animal model. The increasing of this cytokine was probably acute activation *uncouplingprotein* 1 (UCP 1) in mitochondria by fucoxanthin and therefore decreasing lipolysis of adipocyte. The decreasing of this activity affected on free fatty acid that released by adipocyte. Afterwards the diminishing of it increased adiponectin expression (Fruhbecke*t al*, 2001; Maeda *et al*. 2005; Maeda *et al*. 2007; Miyashita 2009, Attie and Scherer 2009; and Jeone*t al*. 2010).

3. Tumor necrocis factor α (TNF α)

Data showed that TNF αlevel of adipocyte among treatments were highly significant ($p < 0,01$), mainly between 0 and 100 mM ($p = 0,000$). Decreasing TNF αof adipocyte causedby several fucoxanthin treatments showed at Figure 3.

Expression of tumor necrocis factor αin adipocyte treated 100 mMof fucoxanthin showed the lowest concentration (Figure 3). This result indicated that expression of inflammation cytokine, such as, tumor necrocis factor α affected by fucoxanthin. Declining expression of tumor necrocis factor α influenced the capability of adipocyte tissue to perform of β-oxidation

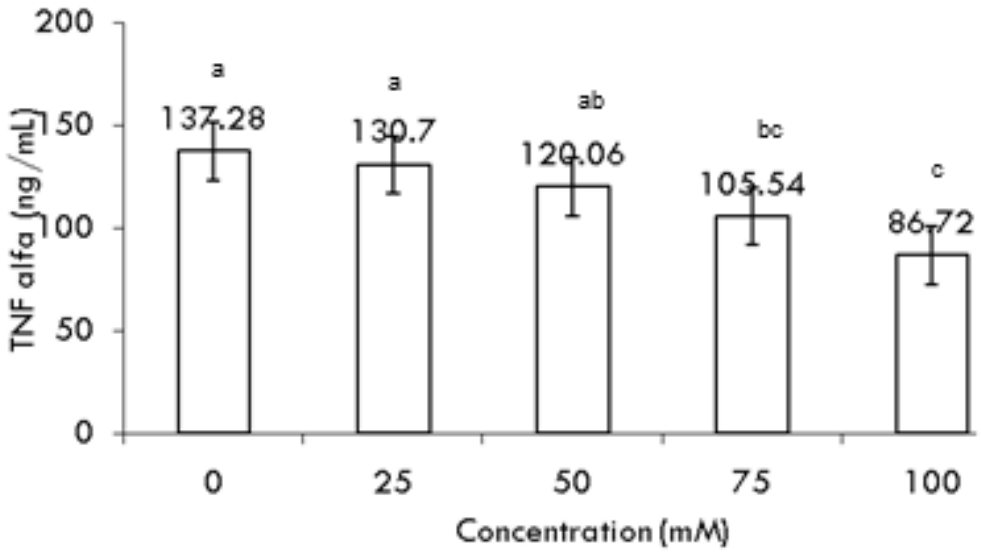

Figure 3. TNF αof adipocyte was treated several fucoxanthin concentration

Based on Hosokawa *et al*. (2010) that fucoxanthin was able to reduce tumor necrocis factor α expression on obesity and diabetes animal model. Similar to effect fucoxantin to adiponectin expression that this compound increased uncoupling protein 1 of mitochondria. The increasing of this activity affected the decomposition lipid to free fatty acid in adipocyte (Fruhbeck*et al*, 2001; Maeda *et al*. 2005; Maeda *et al*. 2007; Miyashita 2009, Attie and Scherer 2009; and Jeon*et al*. 2010).

CONCLUSION

Fucoxanthin of *Sargassumechinocarpum* able to increase adiponectin and decrease tumor necrocis factor α expression in adipocyte and it is potentially as antiobesitynutraceutical.

REFERENCES

Attie AD, Scherer PE. 2009. Adipocyte metabolism and obesity. *J Lipid Res* 50: S395–S399.

Dembitsky VM,Maoka T. 2007. Allenic and cumulenic lipids. *Prog Lipid Res* 46: 328–375.

Fruhbeck G,Gómez-Ambrosi J, Muruzábal FJ, Burrell MA. 2001. The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. *Am J PhysiolRegulIntegr Comp Physiol* 287: R112-119

Haugan JA, Aakermann T, Liaaen-Jensen S. 1992. Isolation of Fucoxanthin and Peridinin. *Method Enzymol* 213: 231-250.

Hosokawa M, Miyashita T, Nishikawa S, Emi S, Tsukui T, Beppu F, Okada T, Miyashita K. 2010. Fucoxanthin regulates adipocytokine mRNA expression in white adipose tissue of diabetic/obese KK-Ay mice. Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato, Hakodate, Hokkaido 041-8611, Japan.

Indra MR. 2005. *FisiologiKardiovaskuler*. Malang: PenerbitLaboratoriumIlmuFaalFakultasKedokteranUniversitasBrawijaya.

Jeon SM, Kim HJ, Woo MN, Lee MK, Shin YC, Park YB, Choi MS. 2010. Fucoxanthin-rich seaweed extract suppresses body weight gain and improves lipid metabolism in high-fat-fed C57BL/6J mice. *Biotech J* 5: 961–969

Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K, 2005. Fucoxanthin from edible seaweed, *Undariapinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *BiochemBiophys ResCommun*332: 392–397.

Maeda H, Hosokawa M, Sashima T, Miyashita K. 2007. Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-Ay mice. *J Agric Food Chem* 55: 7701-7706.

Miyashita K, 2009. The Carotenoid Fucoxanthin from Brown Seaweed Affects Obesity. *Lipid Technol* 21: 186-190.

Nadler ST, Stoehr JP, Schueler KL, Tanimoto G, Yandell BS, Attie AD. 2000. The expression of adipogenic genes is decreased in obesity and diabetes mellitus. *ProcNatlAcadSci*97: 11371–11376.

Shiratori K, Ohgami K, Ilieva I, Jin XH, Koyama Y, Miyashita K, Yoshida K, Kase S, Ohno S. 2005, Effects of fucoxanthin on lipopolysaccharide-induced inflammation *in vitro* and *in vivo*. *Exp Eye Res*81: 422–428.

Soukas A, Cohen P, Socci ND, Friedman JM. 2000. Leptin specific patterns of gene expression in white adipose tissue. *Genes Dev* 14: 963–980.

Terasaki M, Hirose A, Narayan B, Baba Y, Kawagoe C, Yasui H, Saga N, Hosokawa M, Miyashita K. 2009. Evaluation of recoverable functional lipid components of several brown seaweeds of Japan with special reference to fucoxanthin and fucosterol contents. *J Phycol* 45: 974-980.

Wellen KE, Hotamisligil GS. 2003. Obesity-Induced Inflammatory Changes in Adipose Tissue. *J Clin Invest* 112/12: 1785-1788.