NUTRIGENOMIC STUDY: Igf-1 INHIBITION IN ADIPOGENESIS OF VISCERAL ADIPOSE TISSUE OF RAT USING RAMBUTAN PEEL EXTRACT

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ABSTRACT

This study observes the inhibition of rambutan peel extract to rat visceral adipose tissue adipogenesis by observing the expression of igf-1. Experiment stages consist of rambutan peel extraction, rat treatment, visceral adipose tissue protein isolation and separation, and western blot. Male wistar rats were divided into normal and obese rats. The treatments consist of control, ellagic acid, placebo, and extract at 5, 10, 15, and 20 mg/kg body weight. Decreasing body weight gain observed in obese rats with similar food intake (P>0,05). Effective dose observed at 10mg/kg, whilst at 20 mg/kg showed increasing body weight. Protein profile shows different band numbers and intensity between groups and treatments. Igf-1 expression is bound to Igfbp-1 at 36,7 kDa. Anti-obesity effect observed at 10 mg/kg extract in obese rats. Interestingly, igf-1 expression is lower at 20mg/kg group. This result suggest that obesity inhibition does not go through IR family tyrosin kinase.

Index Terms- Adipogenesis, rambutan peel extract, Igf-1, obesity.

I. INTRODUCTION

Obesity is 5th deadliest metabolic disorder which have increasing prevelancy around the world [1]. Increasing adipose tissue is one cause of the obesity. Unbalanced energy intake and expenditure will stimulate adipogenesis, which will produce mature adipose cells. These cells will store the excess energy in form of lipid, thus increase the mass and size of adipose tissue [2,3]. The process is facilitated by chemical signalling, such as insulin, growth hormone (GH), and insulin-like growth factor 1 (Igf-1) [3,4].

Plant secondary metabolites has been proved able to inhibit the adipogenesis [2]. Firdausi et al (2012) reports that black tea, which rich in catechin, able to decrease rat adipose cell by competitive inhibition at igf-1 receptor [5]. Ellagitanin substances in pomegranates are reported inhibit the pre-adipocyte differentiation [6]. Other phenols, like restrvarol, capsaicin, and curcinum, also inhibit the formation of adipose cell in vitro and in vivo [2].

Rambutan (Nephelium lappaceum L.) peel contains diverse phenolic compounds. Three major phenols are found after ethanol extraction, which are elagic acid, coraligiin and geraniin [7]. This study aims to observe the anti-obesity potency of rambutan peel by observing the effect of its extract at adipogenesis of visceral adipose tissue. The data of this study can be used as the reference for further studies of rambutan peel as the candidate of obesity therapist agent.

II. MATERIAL AND METHODS

Every protocol on animal subject (rat (Rattus norvegicus) strain Wistar) in this research has been approved by Brawijaya University Research Ethics Commission.

A. Rambutan peel extraction

Rambutan fruits were fresh picked from Blitar. The fruit’s peel were washed and sun dried. Dried peels were grinded to make rambutan peel powder. The powder was extracted in 70% ethanol for 4-5 days. Ethanol substance in the extract were removed using rotary evaporator [8]. The rambutan peel extract (RPE) was placed in sterile vials until further use.

B. Rat treatment

Three months old male rats strain wistar (D’Wistar Bandung) were divided into 2 groups based on weight; normal (+200gr), and obese (+350gr). There were 7 different oral treatments for each group (n=5): (1) control (NT); (2) placebo (P); (3) pure ellagic acid (E.A); RPE with dosage (4) 5 mg/kg of body weight (BW); (5) 10 mg/kgBW; (6) 15 mg/kgBW and; (7) 20 mg/kgBW. Treatment was administrated once in 2 days. The rats were placed under 12:12 of dark/light cycle and were fed ad libitum. Normal
rats were fed with lower calorie pellet than obese. Body weight gain and food intake were monitored periodically. After 14 weeks, the rats were sacrificed and visceral adipose tissue were isolated.

C. Protein isolation
Visceral adipose tissue were weighed 1gr and added with extraction buffer in 2:1 (v/w) ratio and then homogenized. Homogenate was centrifuged at 10,000 rpm, 10 minutes at 4°C. The second layer of supernatant were used. Protein concentration were measured using Nanodrop Spectrophotometer, and stored at -20°C until further use.

D. SDS-PAGE.
Electrophoresis were conducted using the protocols decribed by Fatchiyah et al., (2011) [9]. 12.5% acrilamide gel were used. Some of the gel were stained with CBB-R 250 and other gel were further analyzed. Every protein band was documented and its molecular weight was calculated.

E. Western blot.
SDS-PAGE gels were transfferred to nitrocellulose membrane using the protocol described by Fachiyah et al., (2011) [9]. Anti-igf-1 (H-70: sc-9013 Santa Cruz) (1:1500) were used as primary antibody and goat anti rabbit IgG AP-conjugated (1:2500) as secondary antibody with NBT-BCIP as the substrate. Any visible bands were documented and quantified using Quantity One software (Biorad).

F. Statistical analyses
Data were analyzed descriptively and statistically. Split plot ANOVA were used for statistical analyses at 95% confidence. SPSS 16.0 were used to aid the statistical analyses.

III. RESULTS AND DISCUSSION
Physiological response was represented by body weight gain (fig. 1) and food intake (data not shown). Decreasing body weight was observed at 10mg/kgBW at obese group, while increasing body weight was observed in other treatment. The highest body weight gain was observed at 20mg/kgBW. Observed food intake showed no real differences except at 5 and 10 mg/kgBW normal group. The data suggest that RPE have antiobesity effect at obese rats fed by high calorie food with the most effective dose at 10 mg/kgBW. RPE at 20 mg/kgBW gives stimulating effect on body weight. It is suggested that RPE should not administered more than 20mg/kgBW to lose weight.

Western blot analysis showed that Igf-1 band was observed at 36,7kDa and showed similar intensity. It is suggested that the igf-1 still intact with its binding protein, thus increasing the molecular weight of igf-1. Igfbp-1 may form a complex with igf-1, resulting 30-40kDa protein complex [11]. The data showed that almost every treatment in both weight group have increasing protein density compared to control. Only 15mg/kgBW showed lower density than control (fig. 2).

Fig. 1. Physiological response of rat to 14-weeks treatment; Body weight gain compared with initial weight; A. normal control; B.normal 10mg/kg RPE; C.normal weight, 20mg/kg RPE; D. obese control; E. obese 10mg/kg RPE; F.Obese 20mg/kg RPE.

Fig. 2. Protein profile and western blot of rat visceral adipose tissue. A.protein profile and western blot of normal weight; B. protein profile and western blot of obese weight; C. protein density of igf-1.
Based on physiological data, anti-obesity effect was observed at 10mg/kgBW at obese group, while stimulating effect was observed at 20mg/kgBW (fig.1). Igf-1 expression at 10mg/kgBW obese group showed higher density compared to control, but not the highest of all. The Igf-1 expression of 20mg/kg groups were slightly higher than control but still lower than the 10mg/kg of obese group (fig.2). When an organism is obese or fed by high calorie diet, the Igf-1 expression should be higher than normal at the adipose tissue [3,4]. It is suggested that the anti-obesity effect of RPE is not involved in Igf-1 signalling or does not go through IR family tyrosin kinase pathway.

Other chemical messengers are involved in adipogenesis beside of igf-1, such as bone morphogenetic protein (BMP), which will involved at preadipose differentiation using Smad signalling pathway. Tumor Growth Factor β (TGFβ) and Tumor Necrosis Factor (TNFα) are also known able to stimulate the adipogenesis [10].

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V. REFERENCES