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Effect of Polyphenol from Rambutan Peel Extract on Serum Lipidand Protein Profileof Visceral Fat on Normal and Obesity Rat Model

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Abstract: Objective of this study are toassessthe effect of polyphenol from Rambutan Peel Extract (RPE) on protein profile and serum lipid in normal and obesity rat model. Normal and obesity rat model have been treated with polyphenol from rambutan peel extract for 12 weeks. Rat were divided into 2 major groups based on their weight which normal rat and obesity model rat respectively have average weight 180-200g and 360-380g. This treatment were divided into minor groups which were placebo (without treatment), treatment with ellagic acid and polyphenol (dosage 15 mg/kg BW; 30 mg/kg BW and 60 mg/kg BW). Polyphenol were delivered by oral administration for 12 weeks. Protein profile on visceral fats wereidentified using SDS page and serum lipid wasevaluated based onbody weight, weight on visceral fat andtriglycerides. Polyphenol were significantly inhibit body mass gain on treatment polyphenol 30mg/kg BW. Mass of visceral fats were not different on treatment and non-treatment rat, however significantly different on normal and obesity rat model. Level of triglycerides were less on rat treated with polyphenol 30mg/kg BW.Protein profile characteristic normal rat and obesity-model rat were in range between117-20 kDa. The amount of band protein were found in normal rat were less than the amount of protein on obesityrat model. There was difference molecular weight at protein density 57kDa for obesity rat model which has been treated with polyphenol. Polyphenol from RPEdecreased triglyceride level and amount of protein band on induced high calorie diet rat.
Key word: protein profile, polyphenol, obesity rat model.

Introduction

Protein is a macromolecule which constructs and dominates more than half of cell structure. Proteins has some functions in cell, such as determine cell's size and structure, facilitates cell to cell communication and biochemical reaction in cell. Protein has close relation with physiological process in the body. The variance and number of protein is a key factor which have responsibility for biological process in organism. Physiologic functions of Protein are as a catalyst, carrier, messenger, etc. Protein consistedof the series combination from amino acid. Each protein has certain number and sequence of particular amino acid[1].

Protein expression is a series of complex process which involve many factors. Genetic expression process is started and being arranged since pre-transcription initiation to translation[1]. The result of gene

translation is protein. Protein expression could be affected by several factors, i.e. nutrition intake[2]. Nutrition intake would affect to expression of some particular gene in cell. Nutrition couldinfluence genome (gene), transcryptome (mRNA), proteome (protein), metabolomics (metabolite) and changes in physiologicallevel. Polyphenol is a bioactive compoundwhich could leadthe down regulation of PPARγ activity, so it would block adipogenesis [3],[4].

Protein which found at visceral fat has a function in adipogenesis processes. It also has other function such as a fatty acid carrier from intracellularinto the cells. Protein profile analysis at the fat cell is an important study to reveal biological processes such as identified expression of chararacter and certain patter. Protein profile deciphers the expression pattern of protein. It is also represents the physiological condition of organism as well. Protein profile analysis is an important knowledge inproteomic study. Identification of protein profile can be used to reveal some modification at protein expression. One particular cell are known haveone set of protein which are able to be expressed in certain time or condition, several proteins are able to undergo significant changes which known as post translation modification, this modification would give big affect toprotein[5].

This research was conducted to assess visceral fat protein profile between normal and obesity rat model after treatment with polyphenol. Protein profile was analyzed descriptively which included either the presence or absence of protein band, molecular weight and boldness of protein band. Protein profile was analyzed at consistent protein band (protein band which available at every repetition).

Material and Method

Polyphenol Preparation

Polyphenol in this research was obtained from rambutan peel extract. Rambutan peel(500 gram) was investigated and dried atroom temperature in shaded area, then grounded using mixer grinder. The powder was extracted using ethanol 70%, then dried with rotary evaporator. Five mg of polyphenol were diluted up to 10ml,then homogenized and centrifuged 10000rpm for 5 minutes. Supernatant that obtained from centrifugation have 0.5 mg/ml concentration. Furthermore polyphenol solutions stocks were provided at 15mg, 30mg, 60mg/kg BW.

Preparation of Animal

Animal model for this study were 21 daysold male rat whichobtained from animal laboratory D'wistar (Bandung). Rats housed in polypropylene cages with stainless steel covers foracclimatization under laboratory conditions for one week. Rats were divided into two major groups which were fed normal diet (63% carbohydrate, 3% fat, 13% protein, 21% vitamin and mineral - manufactured by PT Comfeed, Indonesia) and fed high calorie diet (74% carbohydrate, 6% fat, 20% protein, vitamins, minerals and fiber 1% - manufactured by PT Phokphan, Indonesia). Twelve weeks after fed with normal and high calorie diet, rats were divided into 2 groups normal and obesity model rat, respectively have body weight 180-200 g and 360-380 g. Criteria of obesity model rat is based on Lee index [6].

Treatment

Normal and obesity rat models were dividedinto minor groups: normal-non treatment (Normal-NT), normal-placebo (Normal-P), normal and treated with Ellagic Acid (Normal-EA), normal and treated with Polyphenol 15mg/kg BW (Normal-RPE15), normal and treated with polyphenol 30mg/kg BW (Normal-RPE30), normal and treated with polyphenol 60mg/kgBW (Normal-RPE60), obesity non treatment (Obese-NT), obesity-placebo (Obese-P), Obesity and treated with Ellagic Acid (Obese-EA), Obesity and treated with Polyphenol 15mg/kg BW (Obese-RPE15), normal and treated with polyphenol 30mg/kg BW (Obese-RPE30), obesity and treated with polyphenol 60mg/kgBW (obese-RPE60). The treatment wasgiven for 12 weeks orally, once every 2 days. Normal and obesity model rats were fed normal and high calorie diet during treatment.

The Polyphenol effect on the body weight, visceral fats mass and level of triglycerides.

The body weight gain was recorded once every one week. The body weight gain was analyzed by regression analysis for body weight increasement report. Twelve weeks after treatment, rats were dissected under light ether anesthesia. Blood was collected into dry clean centrifuge tubes and serum was separated by centrifuging 3000rpm for 10 min. Serum sample were kept frozen for triglycerides analysispreparation. At the

end of the experiment, visceral fats were taken from the dorsal side.Visceral fat were washed in Phosphate Buffered Saline, then followed by protein isolation preparation.

Running SDS-Page

Protein were separated using discontinuous SDS-PAGE (12,5% for separating gel and 3% for stacking gel). The electrophoresis method by Laemli[7]. Protein fromvisceralfat sample were addedTris-CL and 20µL Reducing Sample Buffer with 1:1 comparison. Sample were heated about 5 minutes at 100°C and located into well ±30µL electrophoresis.Protein running were conducted at constant electrical current 20mA until tracking dye reach 0,5 cm upper the gel. Protein band distributions wereidentified by dye CoomasieBriliant Blue (CBBR 250). Each band from electrophoresiswas measured bymolecular weight.

Determination of protein molecular weight

Every protein weight wasmeasured by regression analysis between marker protein relative mobility (protein marker) and logarithm of protein marker. Relative protein mobility wasmeasured with comparing the distance of protein migrationbetween first line marker and tracking dye.

Protein Profile Analysis

Protein profile were qualitatively analyzed using SDS-PAGE and quantitatively analyzed using Gel Doc (Bio-Rad). Protein band density were analyzedusingQuantity One software.Protein band data which found at visceral protein fat was statistically analyzed(Student T-Test).

Ethical Clearence

The study was approved by committee of Brawijaya University Research Ethics Committee as a member of National Research Ethics Committee of Republic Indonesia.

Result

Evaluation of Body weight gain, visceral fat mass and level of triglycerides

Polyphenol treatment caused reduction on body weight gain compared to non-treatment and normal-placebo and obesity ratmodel on all concentration. Obese rats consumed significantly higher caloriediet compare than normal rat.Calorie intake on normal and obesity rat were shown differently after treated with polyphenol, calories intake were not affected by all treatments. Weight gain regression analysis results both on normal and obesity rat model were shown that polyphenol 30mg/kg BWhas the smallest effect in treatment rat models(Figure.1).

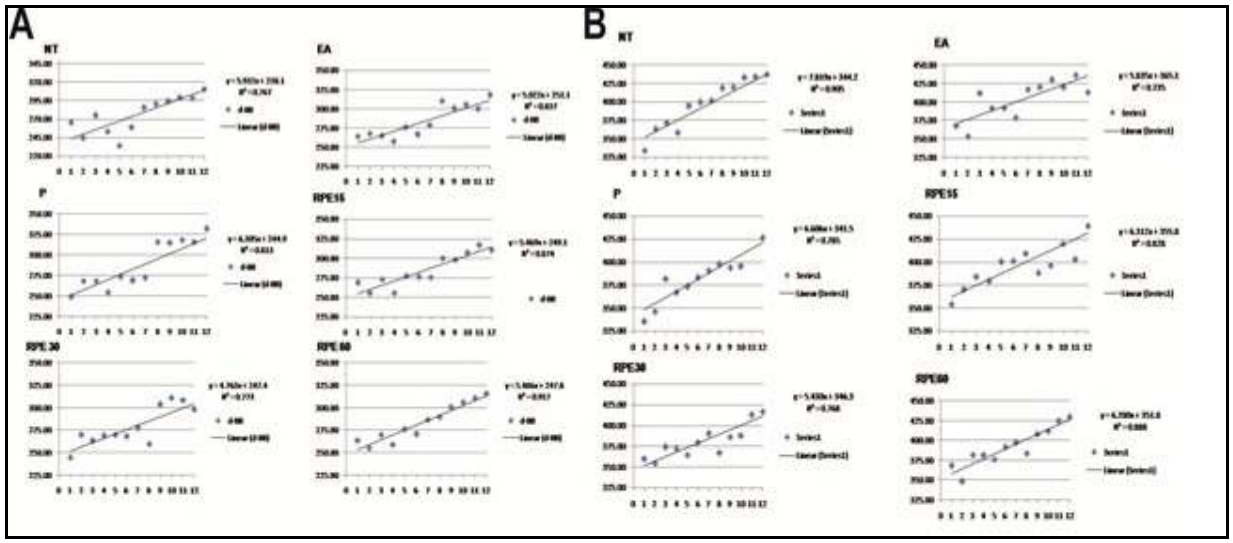

Figure 1.Weight gain on rat treated polyphenols for 12 weeks. A. Normal Rat; B. Obesity Model rat. NT: Non-treatment; P: Placebo; EA: ellagic acid, RPE 15: treated polypheol 15mg/kg BW; RPE30: treated polyphenol 30mg/kg BW; RPE60: treated polyphenol 60mg/kg BW. The red line shows the smallest change in body weight was found in the group of normal and obesity model rat treated with acid and polyphenols elagat 30mg/kg BW.

There were significantly differences in fat mass between normal and obesity rat model. Mass of visceral fat on obesity rat model was 2-fold heavier than normal. Therapy with polyphenols 30mg / kg BW were shown the lowestmass of visceral fat in both normal and obesity model rat (Table1)

Table 1. Weight of visceral fat at the end treatment on normal and obesity model rat.NT: Non-Treatment; P: Placebo; EA: Ellagic Acid; RPE15: Polyphenol 15mg/kg BW; RPE30: Polyphenol 30mg/kg BW; RPE60: Polyphenol 60mg/kg BW

Weight of Visceral Fat (g)	Non Treatment	Placebo	EA	RPE15	RPE30	RPE60
Normal	29.40 ± 0.71a	29.04 ± 2.59 a	28.98 ± 4.21 a	29.04 ± 2.59a	26.62 ± 4.48 a	29.00 ± 2.27a
Obesity	55.05 ± 9.35b	53.16 ±13.44b	52.54 ±11.52b	53.16 ± 13.22b	46.50 ± 4.81b	51.32 ± 4.59b

Note: notification a,bshowed significant different (p<0,05)

Levels of triglycerides wereindicating less on all treated polyphenol rat. Obesity rat modelhas higher level of triglycerides than normal rat.The mass of visceral fat probably correlate with the level of triglycerides, increasing of triglyceridewill make the increasing of visceral fat (Table 2).

Table 2. Level of triglycerides on normal and obesity model rat treated polyphenol.NT: Non-Treatment; P: Placebo; EA: Ellagic Acid; RPE15: Polyphenol 15mg/kg BW; RPE30: Polyphenol 30mg/kg BW; RPE60: Polyphenol 60mg/kg BW

Level of Triglycerides on serum (mg/dl)	Non Treatment	Placebo	EA	RPE15	RPE30	RPE60
Normal	78.04 ± 21.16bc	68.94 ± 13.81ab	61.04 ± 10.81bc	46.50 ± 12.05ab	45.12 ± 5.12a	55.18 ± 16.15ab
Obesity	114.50 ± 20.42d	121.78 ± 8.57d	76.64 ± 18.72ab	43.54 ± 4.54a	42.26 ± 20.73 a	65.15 ± 11.95a

Note: notification a,bshowed significant different (p<0,05)

Profile Protein Analysis

There were 4 identified protein bandson normal body weight ratin non-treatment, placebo and polyphenol treatment rat. However only 2 band protein were on normal body weigh rat which identified on ellagic acid treatment. Five band protein were found on obesity rat modelon non-treatment, placebo, and polyphenol treatment rat. Meanwhile, obesity rat model which treated with ellagic acid were shown only 3 bands. Molecular weight of protein on normal rat and obesity rat model wereinrange 117-20 kDa.

Several protein bandswere found with different density by quantity-one software. There were 3 bands which consistentlyappeared in normal and obesity rat model. Protein density 90kDa on normal, obesity treated EA, and RPE were significantly decreased compare with control and placebo rat ($p<0.05$). Rat treated with RPE30 both of normal and obesity were found have the lowest density band at 57kDa. Band at 20kDa the lower band were shown on rat treated EA and RPE30 compare than both of normal and obesity control which treated with RPE15, RPE60.

Protein densities were found from protein separating visceral-fat obesity model rat without treatment. i.e. 90kDa, 57kDa and 20kDa. Higher density for obesity rat modelwhich has been treated with ellagic acid were found at molecular weight 57kDa & 20kDa,as well as obesity rat model which has been treated with polyphenol 15mg/kg BW, 30mg/ kg BW and 60mg/ kg BWdosage. Higher density for normal rat and placebo were found at protein with molecular weight 57and 20kDa as well as found at normal protein which has been treated with several dosage. On the contrary, normal rat which has been treated with ellagic acid were shown low density at molecular weight 97kDa,57kDaand 20kDa.

Immunoblotting were conducted based on protein profile characteristic data which has been found are affected by polyphenol. Immunoblotting were focused on protein withspesific molecular weight which predicted hassignificant adipogenesis process.Immunoblotting with primary antibody PPARγ obesity rat modelwithout treatment and placebo rat were shown high density band. Lower density bandwere found at rat which has been treated with variation dosages: RPE 60mg/kg BW, 30mg/kg BW and 15mg/kg BW.Lowest density band were found at obesity rat modelwhich has been treated with several dosages of polyphenol. Band density comparison for obesity rat modeland normal rat are shown at Figure 2. There issignificantly difference protein profile characterization between normal rat and obesity rat modelwhich has been treated with polyphenol at 97kDa,57kDa and 20kDa molecular weight. The differences were shown with different density for each treatment. We can conclude that polyphenol have affects toward visceral fat profile protein at rat.

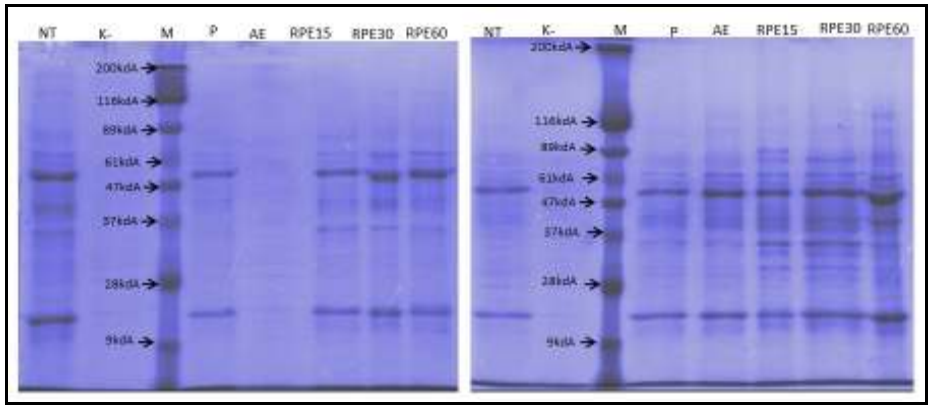

Figure 2. Profile protein in normal and obesity rat model treated polyphenol by SDS-Page 12,5% concentration 1µg protein/ml. A. Profile protein in normal rat; B. Profile protein in obesity rat model (NT: Non-Treatment; P: Placebo; M: Marker; EA: Ellagic Acid; RPE15: Polyphenol 15mg/kgBW; RPE30: Polyphenol 30mg/kgBW; RPE60: Polyphenol 60mg/kgBW)

Discussion

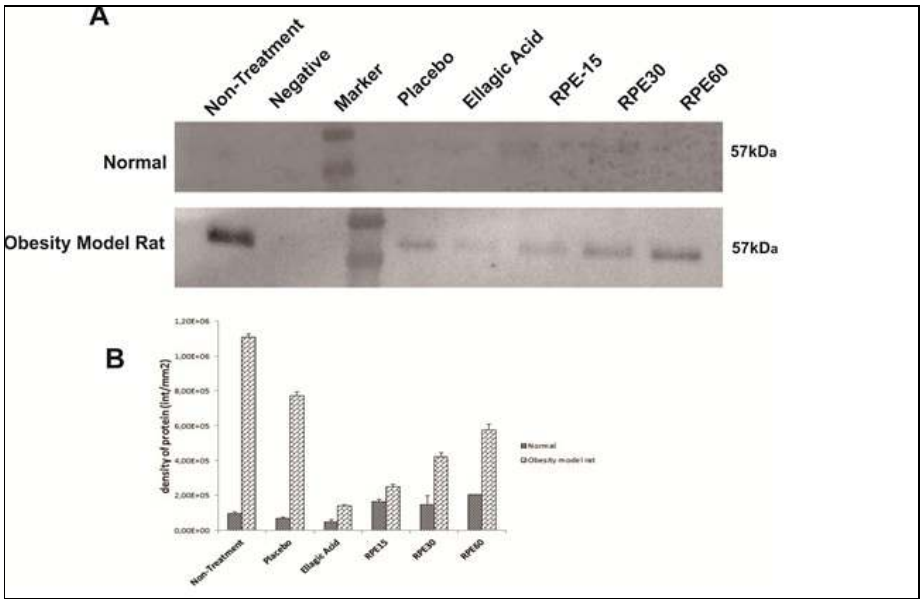

Figure 3. Expression of protein in normal and obesity rat model with specific marker 57kDa used Western Blotting method. A. Expression protein in normal and obesity rat model. B. Graphic of density protein 57kDa in normal and obesity model rat.

The present result clearly demonstrated that the body mass and level of triglycerides on all obesity model rat treated polyphenol are decreased. The decreation of body weight in obesity rat modelmaybe caused by active compound of polyphenol such as ellagic acid, coraligiin and geraniin[8,9]. Ellagic acid is active compound which usually used to avoid obesity[10,11]. Polyphenol in rambutan peel extract may have an impact on

carbohydrates metabolism through the inhibition of alpha amylase and alpha glycosidaseenzyme activity[12]. Rambutan peel extract compounds had been used as potential enzyme fatty acid synthase inhibitor[13]. These enzymes have role in adipogenesis process. Inhibition of these enzymes may prevent the expression of protein in adipocyte cell. Protein expression is a complex process which involved in transcription to translation processes. Protein expression were affected by several signal which affected by several factor from external environment such as nutrition,drugs, infection & other factors[14].

Protein band in obesity rat modelnon-treatment and placebo were 5 bands, meanwhile there were 4 protein bands in obesity rat model which treated with polyphenol. These phenomenon had been found in normal rat.Polyphenol would inhibit protein expression with particular molecular weight 57kDa at obesity rat model. Protein density at obesity rat model for which has been treated with polyphenol was lower than obesity rat modelwhich has been received non treatment & placebo. This result are consistent with many researcher that explain several plant phytochemicalwhich have decreased PPARγ activity, specifically at protein with molecular weigh 57 kDa[15,16,17]. Molecules which come from plant derivation such as genestein, epigalochathecin and capsaicin are also able to inhibit PPARγ[17]

PPARγ is adipogenesis key gene which would trigger gene expression and lead new adipocyte, such as AGPAT, LPL, Glut and FABP4[14,18,19]. Inhibition of PPARγwill make the down regulation of activity MAPKs. Polyphenol from rambutan peel are reported having potency as a potentialphytopharmaca agent candidate to prevent obesity. RPE affect igf-1and igf-1R and lead the expression of ERK1-2. The lowest protein density expression was on obesity rat model which has been treated with EA. This was caused by EA function to inhibit protein expression with molecular weight terse. EA has a directly capacity to enter into cell membrane and able to able to bind with PPARγ receptor. Ellagic acid effective to decrease adipogenesis process directly bybind C/EBPα[20]. C/EBPα isgene which directly affects PPARγ expression.

There are no significantly different between normal and rat which has been treated with polyphenol. There are 2 proteins for rat which has been treated with EA. These wassuspected that EA has effectively inhibit adipogenesis.

Polyphenol RPE which has given at normal and obesity rat model are able to decrease protein density at 57kDa. Theresult indicates that polyphenol which has been given to obesity rat model has potency to inhibit protein at57kDa. Polyphenol from RPE contain several agents which are able to work both synergic and antagonist as well. Those convenience benefit that polyphenol which treated to obesity rat model has better potency rather than EA.

Conclusion

Protein profile at normal and obesity rat model treated RPE have molecular weight around 117kDa-20kDa. The highest density of proteins found in obese rat non treatment,but it decreases at obese rat treated with polyphenol. The obesity rat model treated with polyphenol was had body mass and level of triglycerides lower compare than obesity rat model non-treatment and placebo.

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