The effect of polyphenol to visceral fat profile protein at obesity model rat

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1ARTICLE INFO

Article history:
Received
Accepted
Available online

Keywords:
Polyphenol
Obesity model rat
Profil protein

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ABSTRACT

Objective: To assess visceral fat profile protein between normal and obesity model rat.

Material and Methods: Normal and obesity rat model have been treated with polyphenol from rambutan peel extract for 12 weeks. Rat were divided into 2 major group based on their weight which normal rat and obesity model rat respectively have average weight 180-200 gram and 360-380 gram. This treatment were divided into minor group which are placebo (without treatment), treatment with ellagic acid and treatment with polyphenol (dosage 15 mg/kg body weight; 30 mg/kg body weight and 60 mg/kg body weight. Rat were sacrificed after 12 weeks treatment to assess visceral fat profile protein and continue with running SDS PAGE12.5 %. Protein molecular weight sample were calculated with regression analysis between marker protein mobility and logarithm from marker. Band protein analysis were analyze qualitative with SDS PAGE meanwhile quantitative analysis with Gel Doc (Bio Rad). Density band protein were analyze were using Quantity One software and confirmed with Western Blotting.

Result: Protein profile characteristic normal rat and obesity-model rat were in range between117-20 k Da. The amount of band protein were found in normal rat were less than the amount of protein at obesity-model rat.

Conclusion: There were difference molecular weight at protein density 57 k Da for obesity model rat which has been treated with rambutan peel extract.

1. Introduction

Protein is a macromolecule which consisted more than half of cell structure. Protein determine cell’s size and structure, major component from cell communication between cells and as biochemical reaction in cell. Protein is a molecule with long chain which consist of amino acid and combined into peptide chain. Protein function has closely related into physiological process in the body. The variant and number of protein is a key factor which have responsibility for biological process in organism. Protein function physiological function is as a catalisator, carrier, messenger , etc. all protein consist of the series combination from amino acid. Each protein has number and sequence of particular amino acid [1,2].

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. The result of gene translation is Protein. Protein expression could be affected by several factors, i.e. nutrition intake [5]. Nutrition intake would affect cells to express particular gene. Nutrition could affect genome (gene), transcriptome (mRNA), proteom (protein), metabolom (metabolite) and changes in physiological level. Polyphenol is a bioactive ingredient and lead down regulation for PPARγ activity so it would block adipogenesis activity [8,4].

Protein which provided at visceral fat has a function in adipogenesis processes. It also has other function such as a carrier for fatty acid from intracellular cellin to the cells. Profile protein analysis at the fat is a important study to reveal
biological process which want to be examined. Protein profile decipher expression pattern at protein level. It also be able to analyze physiological in organism as well. Protein profile analysis ia an important knowledge to study proteomic. Identification of protein profile are used to reveal same modification at protein expression. One particular cell are known having one set protein which are able to expressed in certain time or condition, several proteins are able to undergo significant changes which known as post translation modification, this modification has great affect to certain protein itself [3].

Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis (SDS-PAGE) is a gel electrophoresis technique which use polyacrilamide to separate protein based on molecular weigh. It is an effective method to analyze of protein profile. This is also often used to determine of protein molecular weigh as well. Gel acrylamide has benefit compare than other gel since it has higher separation, does not react with other sample and does not make matrix with sample itself, so they are not impede with sample movement which able to facilitate to separate protein completely[7].

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

2. Materials and Methods

2.1 Polyphenol Preparation

Polyphenol in this research were obtained from rambutan peel extract. Five mg polyphenol were diluted up to 10ml and then homogenized. Centrifuged with 10000rpm for 5 minutes. Supernatant from centrifugation have 0.5 mg/ml concentration. Furthermore polyphenol stock solution were provided at 15mg, 30mg, 60mg/kgBB.

2.2 Preparation of Animal

Subject were 12 weeks old male obesity rat and normal rat. Rats were obtained from animal laboratory D’wistar (Bandung). Rats were divided into two major group which were normal rat and obesity model rat that have average weigh 180-200g and 360-380g respectively. Furthermore this group were divided into sub minor group.

2.3 Running SDS-Page

Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis (SDS-PAGE). SDS-PAGE are using discontinuous for gel 12.5% and stacking gel 3%. The electrophoresis method by Laemli (Hemmes, 2002). Protein fat visceral sample were added Tris-CL and 20µL RSB with 1:1 comparison. Sample were heated about 5 minutes at 100°C and located into well ±30µL. electrophoresis Running were conducted at constant current 20mA until tracking dye reach 0.5 cm upper the gel. Protein band distribution were known by dye gel using Coomasie Brilliant Blue (CBBR 250). Each band from electrophoresis would be counted by this molecular weight.

2.4 Determination of protein molecular weight

Protein sample molecular weight were counted by regression analysis between marker protein relative mobility (protein marker) and logarithm from marker (which already known of molecular weight). Relative protein mobility were counted with compare of distant migration protein from first line marker up to migration distant between tracking dye.

2.5 Protein Profile Analysis

Protein profile analysis were counted qualitative by SDS PAGE meanwhile quantitively analysis were using Gel Doc (Bio-Rad). Protein band density were shown at gel were analyze with Quantity One software. Protein band data which found at visceral protein fat with analyze. (student T-Test)

2.6 Ethical Clearent

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

Characterization with SDS-PAGE 12.5\% at visceral fat for normal rat and placebo rat were identified 4 band protein range from 117 kDa-20kDa. Protein bands in normal rat treated with ellagic acid identified two protein bands that 76kDa and 20kDa. Normal rat which has been treated with polyphenol dose 15mg/kg BW, 30mg/kg BW and 60mg/kg BW, were identified with 4 bands protein range from 89kDa-20kDa. Characterization of proteins for obesity rat without treatment and placebo obesity rat were identified 5 bands protein range from 166kDa-20kDa. Protein bands of obesity rat which has been treated with elagic acid were found 3 bands protein range from 90kDa-28kDa. Obesity model rat which treated with polyphenol 15mg/kg BW, 30mg/kg BW and 60mg/kg BW were found 5 protein bands range 166kDa-20kDa. Several protein band were found with different density by using software quantitie-one. Higher protein density were found from protein separating visceral-fat obesity model rat without treatment. i.e 90kDa, 57kDa and 20kDa. Higher density for obesity model rat which has been treated with elagic acid were found at molecular weight 57kDa & 20kDa, as well as obesity model rat which has been treated with polyphenol 15mg/kg BW, 30mg/kg BW and 60mg/kg BW dosage. Higher density for normal rat and placebo were found at protein with molecular weight 57and 20kDa as well as normal rat protein which has been treated with several dosase. On the contrary, normal rat which has been treated with elagic acid were shown low density at molecular weight 97kDa, 57kDa and 20kDa.

Immunoblotting were conducted based on protein profile charactoristic data which has been found are affected by polyphenol. Immunoblotting were focused on protein with spescific molecular weight which predicted have significant adipogenesis process. Immuno blotting with primer antibody PPARγ obesity model rat without treatment and placebo rat were shown high density. Lower density were found at rat which has been treated as follow RPE 60mg/kg BW, 30mg/kg BW and 15mg/kg BW. Lowest density were found at obesity model rat which has been treated with several dosages polyphenol. Density comparison for obesity model rat and normal rat are shown at figure 2.

There are major difference protein profile characterization between normal rat and obesity model rat which has been treated with polyphenol at protein 97kDa, 57kDa and 20kDa molecular weight. The difference were shown with different density for each treatment. We can conclude that polyphenol have affects toward visceral fat profile protein at rat.

4. Discussion

Protein expression is a complex process which involved 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 [9].

Nutrition which enter the body would lead cell has to express particular genes, there were 5 protein band from profile protein fat visceral SDS page. Result which has been treated with polyphenol. meanwhile there were 2- 4 protein band at obesity model rat which has been treated with polyphenol. This result shown that protein profile were affect with polyphenol. polyphenol would inhibited protein expression with particular molecular weight 57 KDa at obesity model rat. Protein density at obesity model rat for which has been treated with polyphenol were lower than obesity model rat which has been receive non treatment & placebo. This result are consistent with Yun experiment (2010) who explain several plant phytochemical have decrease PPARγ activity, specifically at protein with molecular weight 57 Da. Wang et al. (2009) are also explain that molecule which come from plant derivation such as genestein, epigalochathecin & capsaicin are also able to inhibit PPARγ.

PPARγ is adipogenesis key gene, would trigger gene expression and lead new adipocyte, such as AGPAT, LPL, Glut and FABP4 [8, 6, 10]. Inhibition of PPARγ make down regulation of activity MAPKs. Polyphenol from rambutan peel are reported having potency as a potent candidate phytopharmaca agent to prevent obesity. RPE affect igf and igf-r1R and lead expression of ERK-2. The lowest protein density expression were on obesity model rat which has been treated with EA. These was caused by EA function to inhibit protein expression with molecular weight terse.
EA has capacity directly enter into cell membrane and able to able to bind with PPARγ receptor. Wang et al (2013) reported EA are effective to

There are no significant different between normal and rat which has been treated with polyphenol. There are 2 protein for rat which has been treated with EA. These was suspected 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. These result indicate that polyphenol which has been given to obesity model rat has potency to inhibit protein at 57kDa. Polyphenol from RPE contain several agents which are able work either sinergically and antagonist as well. Those convenience benefit that polyphenol which delivered at obesity model rat has better potency rather than EA.

5. Conclusion

Profile protein at normal and obesity model rat treated RPE around 117 kDa-20kDa. Amount of band protein at normal rat less than obesity model rat. Band protein at normal and obese rat treated with RPE less than non treatment. The highest density of proteins found in obese rat non treatment and decrease at obese rat treated with RPE.

Acknowledgment

This study is supported by DGHE Ministry of Education and Culture RI. We would like to thanks to Animal Laboratory of Brawijaya decrease adipogenesis process direct by bind C/EBPα. C/EBPα are gene which directly affect PPARγ expression. University for providing the research analysis.

Conflict of Interest

The authors declare that there are no conflicts of interest

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