Antihiperlipidemic Activity of the Methanolic Extract of Parijoto (*Medinilla speciosa*) on the Protein Profile of Hyperlipidemic Rats

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Abstract: High-fat diets and frequent feeding contributes to the onset of hyperlipidemia, a family of disorders that is characterised by abnormally high levels of lipids in the blood. Hyperlipidemia is a major cause of atherosclerosis, which is related to conditions such as coronary heart disease (CHD), ischemic cerebrovascular disease, peripheral vascular disease and pancreatitis. Parijoto (*M. speciosa*) is an endemic plant in Asia with a distribution center in Malaysia, Indonesia and Philippines that is generally consumed by pregnant women and used to treat diarrhoea and cholesterol. The parijoto fruit contains a flavonoid compound which has been suggested to decrease the risk of coronary heart disease, inflammatory process, and atherosclerosis through their antioxidant activities. During pathological conditions, there are differences in the protein profiles that indicate the presence of protein biomarkers. Different levels of protein expression serves as a biomarker of disease progression. This study aims to determine the effects of the methanolic extract of parijoto (*M. speciosa*) on the protein profile of hyperlipidemic rats. Rats were divided into five groups: normal rats, hyperlipidemic rats, and hyperlipidemic rats that were given the methanolic extract of parijoto (*M. speciosa*) at 500 mg $\cdot$ kg^{-1}, 1000 mg $\cdot$ kg^{-1}, and 1500 mg $\cdot$ kg^{-1} body weight. Rats were terminated after the 30 day treatment of the methanolic extract of parijoto (*M. speciosa*) and their blood collected. Protein profile was determined by the Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) method. Results showed that the proteins that appeared in each group were proteins with the molecular weight 160; 144; 131; 124; 117; 110; 93; 76; 59; 52; 49; 42; 33; 25 and 14 kDa, however the(control) protein 117 kDa was not present in group I. Protein 117 kDa was presumed as the sterol regulatory element binding protein-1c (His-SREBP-1c), the transcription factor that transduces the insulin signal.

1 INTRODUCTION

Hyperlipidemia is a family of disorders that is characterised by abnormally high levels of lipid (fats) in the blood (Verma, 2016). Hyperlipidemia contributes to the occurrence of atherosclerosis, one of the factors that triggers cardiovascular disease, hypertension and coronary heart disease (Kumar, 2010). Cardiovascular disease is one of the health problems in society and is one of the leading causes of death worldwide. Based on data from the World Health Organization (WHO), it is predicted that 23.300.000.000 people will die of cardiovascular disease in 2030, while Basic Health Research (Riskesdas) in 2013 showed the prevalence of heart disease was 1.5 % on a national scale (Kemenkes RI, 2014).

Lipids are associated with blood plasma proteins and remain in a dissolved state in the blood.

Hyperlipidemia may be classified as primarily caused by specific genetic abnormalities and defects in lipid metabolism which is caused by the defect in lipoprotein lipase activity or the absence of the surface Apoprotein C-II. Secondary hyperlipidemia results from another underlying disorder that leads to alterations in plasma lipid and lipoprotein metabolism and environmental factors (Nirosha et al., 2014).

Puskas et al. (2004) reported that hyperlipidemia causes an alteration of genes expression in the heart, including procollagen type III, cofilin/destrin, tensin, transcription repressor p66, synaptic vesicle protein 2B, Hsp86, chaperonin subunit 5ε, metallothionein, glutathione S-transferase, protein kinase C inhibitor, ATP synthase subunit c, creatine kinase, chloride intracellular channel 4, NADH oxidoreductase and dehydro-genase, fibronectin receptor β chain, CD81 antigen, farnesyltransferase, calreticulin, disintegrin, p120 catenin, Smad7, etc. Some of these genes are

123

suspected to be related to cardiovascular diseases. During pathological conditions, there are differences in the protein profiles that indicate the presence of protein biomarkers. Protein biomarkers are used for the diagnosis and prognosis of various diseases. Different levels of protein expression serves as biomarkers of disease progression (Naz et al., 2009).

Many natural resources containing phytochemical components have been used as anti-hyperlipidemic drugs. The parijoto (*M. speciosa*) plant is a species endemic to Indonesia but has not been fully explored pharmacologically; it contains phytochemical components such as flavonoids, saponins and kardenolin (Tussantiet al., 2014).

The intake of flavonoids is negatively correlated to coronary heart disease because of its potential as an antioxidant; protects LDL oxidation, a process involved in atherogenesis (Yang et al., 2008); and also inhibits lipase enzyme activity (Martins et al., 2010). Sa'adah et al., (2017) reported that the methanol extract of parijoto (*M. speciosa*) reduced total cholesterol, atherogenic index, and increases HDL-Cholesterol significantly (p <0.01).

This study observed the blood serum protein profiles of hyperlipidemic rats which were given the methanol extract of Parijoto. This research is expected to reveal the anti-hyperlipidemia effects of parijoto extract on proteomic levels. Differences in protein profiles that occur during pathological conditions can indicate the presence of biomarker proteins (Naz et al., 2009).

2 MATERIALS AND METHOD

Material and method in this paper will in this section.

2.1 Materials

Parijoto (*M.speciosa*) fruits were obtained from Muria Mountain, Kudus, Central Java, Indonesia; male Wistar rats (*R. norvegicus*) aged 2 months and weighing 110 g to 150 g were obtained from the experimental animal laboratory, Faculty of Pharmacy, Airlangga University.

The content of the high-lipid diet included reused cooking oil and duck yolk. The other materials used were Comfeed® (Japfa) as a basal feed for the rats; methanol; Acrylamide; TrisHCl; sterile distilled water; 10% SDS; 10% APS and Temed.

2.2 Method

Step to reach the method will explain about preparation, treatment and collection.

2.2.1 Preparation of Experimental Animals

The rats (*R. norvegicus*) were acclimated for a week, where feed and drink were given ad libitum. After one week acclimatization, the rats were weighed and divided into five groups. Each of the groups had four individual replicates.
Group I: Control without hyperlipidemia treatment
Group II: Control with Hyperlipidemia treatment
Group III:Hyperlipidemia which was given 500 mg·kg^{-1}
of *parijoto* extract
Group IV:Hyperlipidemia which was given 1000 mg·kg^{-1} of *parijoto* extract
Group V:Hyperlipidemia which was given 1500 mg·kg^{-1} of *parijoto* extract.

2.2.2 Hyperlipidemia Treatment

Rats (*R. norvegicus*) were conditioned to be hyperlipidemic following a procedure from Sa'adah (Sa'adah et al., 2017). The experimental rats were orally fed a mixture of duck yolk and reused cooking oil (ratio 2:1) at the amount of 1% of body weight (BW) for 30 days. The hyperlipidemia treatments were given to all the groups except the control group (group I).The body weight of the rats were weighed on a weekly basis.

2.2.3 The Methanolic Extract of Parijoto Treatment

The treatment of the methanolic extract of *parijoto* with a concentration of 500 mg·kg^{-1}, 1000 mg·kg^{-1}, and 1500 mg·kg^{-1} was given for 30 days after the rats were in hyperlipidemic condition. The control and hyperlipidemic rats (Groups I and II) were only given basal feed and drink by ad libitum for 30 days. The body weight of the rats were weighed weekly.

2.2.4 Blood Serum Collection

Blood was taken from the treated rats after the treatment of the methanolic extract of *parijoto*. The blood serum was centrifugally separated from the blood cells at 3000 rpm for 10 minutes. The serum blood was collected in a microtube.

2.2.5 Proteins Profile of Rats

The protein profile of the rats' (*R. norvegicus*) serum was determined by the Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) method, which consisted of several stages: preparation of the polyacrylamide gel, assembling of the chamber and the glass plate, injection of sample (serum) in the comb, the process of running SDS - PAGE, staining, and destaining of gels.

2.2.6 Standard Curves and Analysis of Protein Bands

The molecular weight of the protein sample was calculated by using a standard curve (y = ax + b). A standard curve was constructed by measuring distance marker bands of well. The marker used was the PageRuler™ Prestained Protein Ladder® (gel concentration of 10%) with a molecular weight of 10 kDa to 170 kDa. The bands distance was used as the ordinate (x axis) and abscissa (y axis) was the logarithm of marker molecular weight.

Description:

$$y = ax + b$$

x = Bands distance from well
y = logarithm of marker molecular weight

The bands of the protein sample were analyzed by comparing the marker.

2.2.7 Data Analysis

The data was analyzed descriptively, such as the presence or lack of presence of protein bands, molecular weight of protein bands, and if the protein bands were thin or thick. The analysis of the protein profile was performed only on consistent protein bands, protein bands which were present in all replications (running replications and individuals) and protein bands which have relatively the same thickness.

3 RESULTS AND DISCUSSION

Sa'adah et al. (2017 & 2018) reported that rats that were given high-lipid diets for 30 days showed significant increases of total cholesterol, LDL-C, TG levels and atherogenic index value (p<0.01) at approximately 184.06 mg · dL^{-1}, 80.11 mg ·

dL^{-1},130.25 mg ·dL^{-1}, and 5.7 respectively; the HDL-C level also decreased significantly (p<0.01) after the intake of the lipid-rich diet from 55.99 mg · dL^{-1}to 27.72 mg · dL^{-1}.

Lipids are associated with blood plasma proteins and remain in a dissolved state in the blood. Primary hyperlipidemia is caused by specific genetic abnormalities (Nirosha et al., 2014). Previous studies have reported that hyperlipidemia causes alteration of gene expression in the heart and some of these genes have been suspected to be related to cardiovascular diseases (Puskas et al., 2004).The differences in gene expression during pathological conditions can indicate the presence of biomarker proteins (Naz et al., 2009). An intake of the methanolic extract of parijoto reduced total cholesterol, LDL-C levels, atherogenic index values, and increased the HDL-C level significantly (p<0.01) (Sa'adah et al., 2017). The lipid level is associated with blood plasma proteins; when the lipid level of the blood serum decreases, it may alter the serum protein profile of the hyperlipidemic rats.

3.1 Consistency of the Serum Protein Profile of Rats

The marker used in the method was the PageRuler™ Prestained Protein Ladder® which contains proteins with molecular weights of 170, 130, 100, 70, 55, 40, 35, 25, 15 and 10 kDa. Various proteins were obtained from the results of the SDS-PAGE of blood serum of the rats (*R. norvegicus*). The results of running and individual replications showed consistent bands, protein bands which were present on all replicates (running and individual replications) with relatively the same thickness (Figure 1).

The consistency of blood serum protein bands is affected by the physiological condition of individual rats, such as feed intake or specific immune responses to the pathogen. The rats in all groups were given Comfeed® (Japfa) as the basal feed and were located in the same condition. It was presumed that the rats had no differences physiologically. Protein band consistency describes the protein profile differences of treatment groups and controls (Sa'adah et al., 2016).
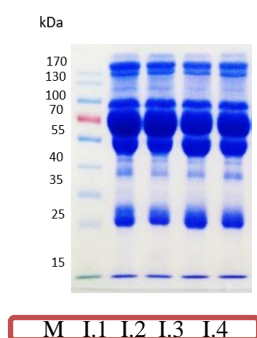
Figure 1: Blood serum protein profile of control rats using the SDS-PAGE method

3.2 Analysis of Serum Protein Profile of Rats

The proteins that appeared in each group were proteins with the molecular weights of 160; 144; 131; 124; 117; 110; 93; 76; 59; 52; 49; 42; 33; 25 and 14 kDa, however protein 117 kDa was not present in group I (control) (Figure 2). Protein 117 kDa was presumed as the sterol regulatory element binding protein-1c (His-SREBP-1c), the transcription factor that transduces the insulin signal (FoufelleandFerre, 2007).

The sterol regulatory element-binding protein-1c (SREBP-1c) plays a major role in hepatic lipogenic gene expression (Botolin and Jump, 2003). SREBP-1c is one of the major isoforms of SREBP expressed in mammalian liver (Khesht and Hassanabadi, 2012). Overexpression of active SREBP-1c in the liver is accompanied by increases in lipogenic enzymes levels (Hansmannel et al., 2006). Dif et al. (2006) also reported that the SREBPs are transcription factors which have been shown to regulate gene expression of several enzymes implicated in cholesterol, lipid and glucose metabolism. The SREBP-1c promoter was activated by insulin and can also be induced by the activation of the nuclear receptors LXRs that have been implicated in the control of lipid and cholesterol metabolism (Schultz et al., 2000).

A large number of studies have demonstrated that SREBP-1c is tightly regulated by nutritional and hormonal status, especially at the transcriptional level, in various tissues. Feeding a high carbohydrate diet increases SREBP-1c mRNA and protein, whereas they are markedly decreased upon fasting (Gosmain et al., 2005).Therefore, the protein 117 kDa appeared in the groups of hyperlipidemic rat groups and does not appear in the control rat group.
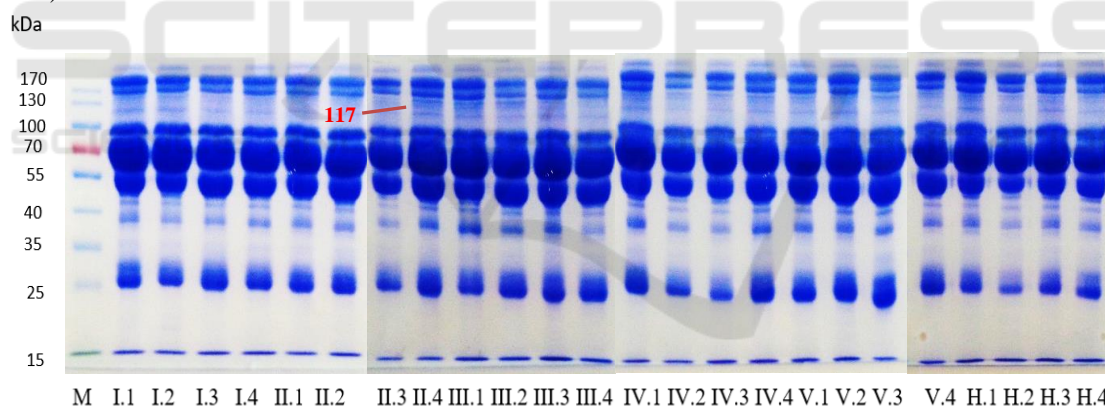

Figure 2: Blood serum protein profile of rats with the SDS-PAGE method. Protein 117 kDa appeared in the hyperlipidemic rat groups (thin protein bands) and did not appear in the control rat group

The number of individual repetition (n) = 4
Group I   : Control without hyperlipidemia treatment
Group II   : Hyperlipidemia control
Group III   : Hyperlipidemia which were given 500 mg/kg of methanolic extract of Parijoto
Group IV   : Hyperlipidemia which was given 1000 mg/kg of methanolic extract of Parijoto
Group V   : Hyperlipidemia which was given 1500 mg/kg of methanolic extract of Parijoto
Group H   : After hyperlipidemia treatment for 30 days

The hyperlipidemic rat groups were orally given a mixture of duck yolk and reused cooking oil for 30 days. Duck yolk is a food that contains high fat (35.80 % to 37.25 %), cholesterol (38.15 mg · g^{-1}) and triacylglycerols (591 mg · g^{-1}) (Ganesan et al., 2014). In addition, duck yolk has a composition of 31.85 %

saturated fatty acid (SFA), 52.49 % monounsaturated fatty acid (MUFA) and 15.66 % polyunsaturated fatty acid (PUFA) (Polat et al., 2013).

Due to the high-lipid diet and frequent feeding, the TG levels may be elevated all day long (Sahade et al., 2013). Rats that were given a high-lipid diet for 30 days experienced an increase in total cholesterol, LDL-C, TG levels and atherogenic index value, while also showing significant decreases in the HDL-C level ($p < 0.01$) (Sa'adah et al., 2017). The increased serum levels in the lipid-rich lipoproteins (LDL-C and VLDL-C) indicate that more cholesterol and triglyceride were transported from the liver to the extra-hepatic tissues to be taken up by those tissues (Adekunle et al., 2013). This process involved the enzymes that implicate in cholesterol, lipid and glucose metabolism. Therefore, the high-lipid diet was presumed to increase the SREBP-1c mRNA and protein; hence this protein appeared in the hyperlipidemic rat groups, despite the thin protein bands.

However, the SREBP-1c protein is actually found in all individuals, because this protein plays a major role in regulating the gene expression of several enzymes implicated in cholesterol, lipid and glucose metabolism (Dif et al., 2006).

4 CONCLUSION

The proteins that appeared in each group were proteins with the molecular weight of 160; 144; 131; 124; 117; 110; 93; 76; 59; 52; 49; 42; 33; 25 and 14 kDa, however the protein 117 kDa was not present in group I (control). Protein 117 kDa was presumed as the sterol regulatory element binding protein-1c (His-SREBP-1c), the transcription factor that transduces the insulin signal. A high-lipid diet was presumed to increase the SREBP-1c mRNA and protein, so this protein appeared in the hyperlipidemic rat groups, despite the thin protein bands.

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