

Ekstrak Semanggi Air terhadap Aktivitas Enzim Lipoprotein Lipase pada Tikus Hipertrigliseridemia

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Abstrak

Hipertrigliserida atau kadar trigliserida tinggi dalam darah merupakan salah satu gangguan unsur lemak yang dikaitkan dengan peningkatan resiko penyakit kardiovaskular. Flavonoid memiliki peran penting sebagai pelindung jantung dengan meningkatkan enzim Lipoprotein Lipase sehingga dapat menurunkan resiko penyakit kardiovaskulas dengan menurunkan kadar trigliserida. Flavonoid dalam semanggi air mengandung 105.8 mg/g. Penelitian ini bertujuan untuk membuktikan bahwa ekstrak semanggi air dapat meningkatkan aktivitas enzim Lipoprotein Lipase. *Study true experimental* ini menggunakan desain *pre-post test control group* pada tikus strain Wistar. Sampel dipilih berdasarkan dengan rancangan acak lengkap yang dibagi menjadi lima kelompok yaitu, kontrol positif (n = 5) hanya diberikan diet normal, kontrol negatif (n = 5) hanya diberikan diet tinggi kolesterol dan kelompok perlakuan dengan pemberian tiga dosis ekstrak semanggi air (n = 15) dengan pemberian diet kolesterol selama 7 hari dan ekstrak semanggi air selama 14 hari. Hasil dari penelitian ini menunjukkan bahwa terjadi peningkatan aktivitas Enzim Lipoprotein Lipase sebelum dan sesudah perlakuan dari pemberian Ekstrak Semanggi Air berdasarkan *Paired T-Test*, terdapat perbedaan yang signifikan (p <0,05). Aktivitas enzim Lipoprotein Lipase pada kelompok kontrol negatif dan kelompok perlakuan ketiga menunjukkan perbedaan yang signifikan (p <0,05) berdasarkan analisis uji *MANOVA (Analisis Multivariat Varians)*. Kesimpulan dari penelitian ini adalah ekstrak semanggi air dapat mempengaruhi peningkatan aktivitas enzim Lipoprotein Lipase secara signifikan.

Kata Kunci: hipertrigliseridemia, LPL, Semanggi Air, flavonoids.

Water Clover Extract in Liprotein Lipase Enzyme Activity of the Hypertriglyceride Rats

Abstract

Hypertriglyceridemia or high blood triglyceride levels is one of the lipid fraction disorders associated with an increased risk of cardiovascular disease. Flavonoids has an important role as cardioprotection by increased Lipoprotein enzyme so that decreased risk of cardiovascular disease by lowering triglyceride levels. The flavonoids in water clover contain 105.8 mg/g of extract. This research aims to prove that Water Clover Extract can increase Lipoprotein Lipase enzymes activity. True experimental study used design pre-post test control group design in

rats. The sample was selected based on by completed randomized design sampling to be divided into five groups, the negative control (n=5) were only given normal diet, positive control (n=5) were only given high cholesterol diet and treatment groups with three doses (n=15) were given cholesterol diet during the 14 days and water clover extract that intervention during the 14 days. The results of this research can increase activity of Lipoprotein Lipase enzymes as an effect of water clover extract based on Paired T-Test, before and after treatment there that there was significant ($p. < 0.05$). Activity of Lipoprotein Lipase enzymes in the negative control group and third treatment showed a significant difference ($p.< 0.05$) based on MANOVA (Multivariate Analyses of Variance). Conclusions of this research is water clover extract affecting increase Lipoprotein Lipase enzymes activity significantly.

Keywords: *hypertiglyceridemia, LPL, Water Clover, flavonoids.*

INTRODUCTION

Hypertriglyceridemia is a condition in which the triglyceride levels raised in the blood (Abbas *et al*, 2014). High triglyceride level comes from increased production of triglyceride, or reduced triglyceride catabolism, or both. Triglyceride levels 150 mg/dL are connected with risk of cardiovascular disease. To people with severe hypertriglyceridemia associated with premature coronary heart disease, premature coronary heart disease, and mortality which is caused cardiovascular disease (Christian *et al*, 2014).

National Health and Nutrition Examination Survey (NHANES) (1999–2004), 33% of 6000 participants had serum triglycerides of at least 150 mg/dl (37% for men and 30% for women) (Ford, 2009). According Carol (2013) triglyceride levels in the US 2009-2012 in woman increased by 14,7% at age 20-39 years, 23% at age 40-59 years and 21,5% at age over 60 years. While in men increased by 25,1% at the age of 20-

39 years, 34,9% at age 40-59 years and 28,7% at more than 60 years. Based of Healthy Basic Research in 2013, the population of Indonesia experienced an increased of TG in high and very high category in men as much as 14,7% and woman 10,2% (Risksedas, 2013)

Triglycerides are ester compound compose of glicerol and three fatty acid molecules. Triglycerides are stored in the body lacks glucose supply (Hartono, 2006; Jae-Kwang, 2014). TG which is rich chylomicron and VLDL remnants which penetrate arterial wall, contribute atherosclerotic lesions and their metabolic remnants which is associated with risk of CVD (Sacks, 2000). For the lates of 20 years plant base therapies have been increasing rapidly in worldwide. Because this therapy has a minimal effect and no side effects at all. Plants containing antioxidants such as isoflavones, phytosterols, saponin, fibers, polyphenols, ascorbic acid and flavonoids

which influence instrumental in fat metabolism (Visavadiya, 2011). Water Clover is alternative of plant-based therapy which is a popular food in Surabaya it is pecel semanggi. Water clover contains bioactive components are alkaloids, steroids, saponin, carbohydrates, reducing sugar, amino acid, essentials oils and flavonoids (Nurjanah, 2012; Tiyaningsih, 2007). Based on the results of antioxidant screening in the pharmaceutical laboratory of Airlangga University was found that water clover extract contains flavonoid was 10,71 mg / g of dry extract.

The mechanism action of the flavonoid in decreasing triglycerides by increasing the lipoprotein lipase (LPL) enzyme through the activation of PPAR (Peroxisome Proliferator Activated Enzyme) which is the sub family of intracellular isoform receptors (also functions as transcription factors) inducing the proliferation of peroxisomes (Fan. C, 2006). Another flavonoids, water clover also contains saponins and steroids. Saponins form complex compounds that are not soluble with cholesterol, therefore inhibiting cholesterol absorption in the intestine. In addition, saponins may inhibit the reabsorption of bile acids (synthesized from cholesterol) by the small intestine so that bile acids are directly excreted with feces resulting in a decrease in the amount

of bile acids (Hakim, 2010). Research based on Roslizawaty, et al., 2016: 79) that is by giving ant flavor extract containing flavonoid significantly ($P < 0,01$) to activity of LPL serum of male white rat which given high triglyceride feed.

Based on this background, then conducted in depth study water clover can increased Lipoprotein Lipase enzyme in Rat fed a high triglyceride diet. This study is expected to be an effective solution without causing side effects for patients with hypertriglyceride.

SUBJECT AND METHODS

The type of this research used true experimental, with pre-posttest control group design. Subject of this study is rats Strain Wistar. The number of samples used were five per treatment group (normal diet; high cholesterol diet; high cholesterol diet and Water Clover extract with three doses). High cholesterol diet were given to rats are quail egg yolk, cattle brain and glucose which are mixed together to form emulsion. This diet were given by oral gavage for each rats as much as 2 cc in 1,8 grams per day for 14 days. The doses of Water Clover extract were given are 16,63 mg/kg, 33,264 mg/kg and 66,528 mg/kg. Water Clover extract were given by oral gavage for 14 days after the high cholesterol diet is given. At the last treatment (29th days), the rats blood were

taken to measure activity of Lipoprotein Lipase after treatment. Statistical analysis used Paired Sample T-Test to compare Lipoprotein Lipase enzyme before and after given high triglyceride diet and Water Clover extract. The difference of tLipoprotein Lipase in five group used MANOVA (Multivariate Analyses of Variance).

The mean of activity of Lipoprotein Lipase in five groups before and after treatment in the negative control group were 55.82 (pretest) and 57.58 (posttest), the positive controls (high cholesterol diet) were 56.38 (pretest) and 191.78 (posttest), the first treatment (16,63 mg) were 58.14 (pretest) and 55.22 (posttest), the second treatment (33,264 mg) were 54,68 (pretest) and 51,50 (posttest), and the third treatment (66,528 mg) were 57,60 (pretest) and 47,46 (posttest).

RESULT

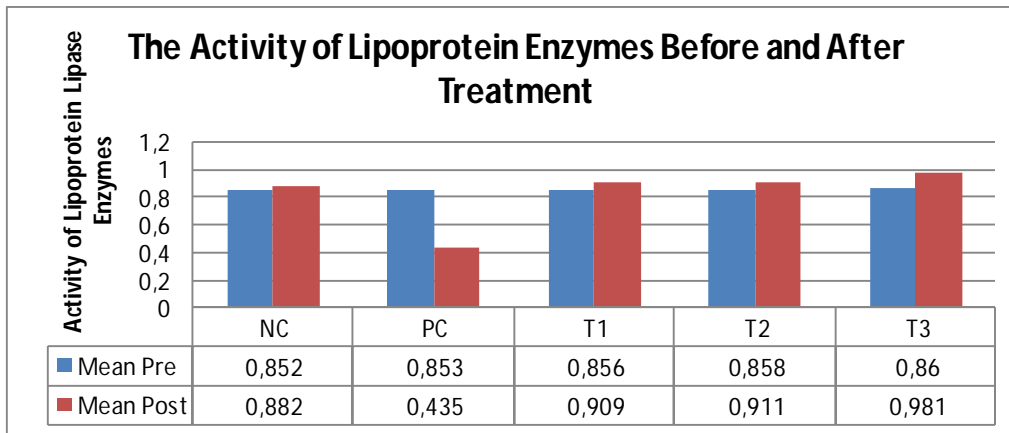
Activity of Lipoprotein Lipase Enzyme Bivariate Analysis

Tabel 1. The Mean Difference Activity of Lipoprotein Lipase Before and After Treatment of Rats Fed High Cholesterol Diet

Group	n	Pretest	Posttest	p.
		Mean ± SD (mg/dl)	Mean ± SD (mg/dl)	
NC (Normal Diet)	5	0.852 ± 0.020	0.882 ± 0.021	0.106
PC (High Cholesterol Diet)	5	0.853 ± 0.026	0.435 ± 0.051	0.000
T1 (16,63 mg extract)	5	0.856 ± 0.018	0.909 ± 0.041	0.048
T2(33,264 mg extract)	5	0.858 ± 0.017	0.911 ± 0.018	0.027
P3 (66,528 mg extract)	5	0.860 ± 0.040	0.981 ± 0.013	0.031

Note: NC : Negative Control; PC : Positive Control; P1 : Doses 1; P2 : Doses 2; P3 : Doses 3

The mean difference activity of Lipoprotein Lipase in each group before and after treatment can be see in **Figure 1.** below.



Note: NC : Negative Control; PC : Positive Control; P1 : Doses 1; P2 : Doses 2; P3 : Doses 3
Figure 1. Graph Activity of Lipoprotein Lipase Before and After Treatment Among Groups

Based on the statistical analysis showed that there were differences activity of Lipoprotein Lipase in the positive control (high cholesterol diet group), first treatment, second treatment, and third treatment before and after treatment significant ($p < 0.05$). However, in the control positive (normal diet group), there was no significant difference ($p > 0.05$).

Activity of Lipoprotein Lipase Changes Analysis

The significance value obtained was 0.000 ($p < 0.05$) on MANOVA test. Accordingly, there are differences in triglyceride levels in among groups (negative control, positive control, first treatment, second treatment, and third treatment). Then, to know the different groups significantly based on p . value can be seen in the following Table 2.

Table 2. The Comparison activity of Lipoprotein Lipase Among Groups

Group	NC	PC	P1	P2	P3
CN (Normal Diet)	-	0.000	1.000	1.000	0.001
PC (High Cholesterol Diet)	0.000	-	0.000	0.000	0.000
P1 (16,63 mg extract)	1.000	0.000	-	1.000	0.023
P2 (33,264 mg extract)	1.000	0.000	1.000	-	0.029
P3 (66,528 mg extract)	0.001	0.000	0.023	0.029	-

Note: NC : Negative Control; PC : Positive Control; P1 : Doses 1; P2 : Doses 2; P3 : Doses 3

The activity of Lipoprotein Lipase in the normal diet group compared with the high cholesterol group and the third treatment showed a difference of significant ($p < 0.05$). The activity of Lipoprotein Lipase in the high cholesterol diet and first treatment showed a significant difference ($p < 0.05$).

DISCUSSION

The activity of Lipoprotein Lipase before and after treatment is significant ($p < 0.05$), except on normal diet group because its just fed standard food for rats. The difference mean of five groups are increased. The activity of Lipoprotein Lipase decrease in high cholesterol diet group because the rats feds hypercholesterolemia diet everyday. In this research, hypercholesterolemia diet use quail egg yolk, cattle brain and pure glucose. Water clover extract can increased

The activity of Lipoprotein Lipase with flavonoids.

Flavonoids are known to increase the activation and inhibition of PPAR (Peroxisome Proliferator Activated Enzyme) which is a subregister of the receptor hormone) PPAR is aided by PPRE (Proliferator response hormon promoter element) assisted by DNA to achieve domain bindings. Flavonoid compounds have the potential as an antioxidant that can release hydrogen atoms in the hydroxyl group which causes free radical activity to be reduced also has the function of increasing LPL cofactor by increasing PPAR- γ which can increase LPL enzyme activity. PPAR γ is a nuclear hormone receptor in the form of heterodimers with retinoid X receptors and binds to location (Fan. C, 2006). Lipoprotein lipase (LpL) is the main enzyme responsible for the conversion of lipoprotein into the triglycerides free fatty acids and monogliferids. It allows them uptake to muscle and adipose. LpL has an enzymatic action to hydrolyze triglycerides and phospholipids. Inside the bloodstream, that's important enzymes needed to clear kilomikron. In the study (Olivecrona, 2016), the early stages of catabolism triacylglycerol-rich lipoproteins (TRLs) is TRLs entered in the endothelium vascular requires LPL and glycosyl phosphatidyl

inositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1), transporter endothelial Lipoprotein Lipase which serves to binds the enzyme Lipoprotein Lipase with lipoprotein to make the process of lipolysis.

In the process of lipolysis, Lipoprotein Lipase enzyme is required to interact with proteins (GPIHBP1) resulting in any triglyceride-rich lipoproteins compete in the process of lipolysis and liver uptake. In addition, triglycerides are transported through the body by all classes of lipoproteins such as VLDL and chylomicrons, which resulted in a decrease in the catabolism of chylomicrons and VLDL by turning them into LDL. LDL-rich LDL triglyceride will be transformed into a small and dense (Goldberg and Merkel, 2001). Very low-density lipoprotein (VLDL) transporting TG can be hydrolyzed to glycerol fatty acid (lipolysis) which is absorbed by muscles and other tissues into energy and energy reserves stored by adipose tissue. Flavonoids also have properties to inhibit fatty acid synthase (FAS) which is a key enzyme in fat metabolism that can reduce the formation of triglycerides (Tian, 2011). Thus, any triglyceride-rich lipoprotein has competition between lipolysis and partial liver removal lipoprotein is hydrolyzed. LpL will hydrolyze triglycerides and

phospholipids in other circulating lipoproteins, LDL and HDL. Therefore That will convert the LDL-rich triglycerides into a smaller, more dense LDL (Goldber and Merkel, 2001).

CONCLUSION

Water Clover extract that can effect activity of lipoprotein lipase enzymes

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