Hypocholesterolemic Effect of Beet Root Extract (*beta vulgaris*) in Rats

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**Abstract**

Dyslipidemia is a disorder of lipid metabolism that causes narrowing of blood vessels. Risk factors for coronary heart disease are characterized by an increase in cholesterol. Thus, prevention needs to be done by changing a balanced diet that is eating one fruit and vegetable, one with beet root that has a high antioxidant content. The purpose of this study was to examine the activity of beet root extract on rat cholesterol levels. This research method is experimental with post test only randomized control group design. Treatment at a dose of 100 mg / kg, 200 mg / kg combined with 0.18 mg / kg simvastatin. The sample in this study was Wistar strain male rats aged 2-3 months with a weight of 100-200 grams, the technique of random sampling using the formula Federer as many as 5 animals / group and divided into 7 treatment groups. Beet root extract and atherogenic feed were given for 35 days. Cholesterol levels were examined by the CHOD-PAP (Cholesterol Oxidase-Peroxidase Aminoantipyrine Phenol) method using a clinicon 4010 photometer. Data were analyzed using the Kruskall Wallis and Mann Whitney tests. The effect of beet root extract activity on reducing cholesterol levels at a single dose of 100 mg/kg BW of 17.40% (p-value = 0.67), single dose 200 mg / kg BW of 21.59% (p-value = 0.009), combination dose of 100 mg / kgbb of 7.10% (p value = 0.009), a combined dose of 200 mg / kgbb of 18.65% (p value = 0.347). Beet root extract is effective in reducing rat cholesterol at a dose of 200 mg/kg BW.

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INTRODUCTION

Dyslipidemia is a disorder caused by changes in environmental factors and lipid profile metabolism, which is characterized by increased levels of cholesterol, triglycerides, LDL (Low Density Lipoprotein) and decreased levels of HDL (High Density Lipoprotein). A long increase in cholesterol levels results in thickening of the arteries with the risk of narrowing of the arteries causing coronary heart disease (CHD) (Budiman & Rosmariana Sihombing, 2015).

WHO (World Health Organization) In 2013, cardiovascular disease was the first rank of Non-Communicable Disease (NCD) and the biggest cause of death in the world at 17.1 million deaths per year (46% of total deaths due to NCD) (Esaningsih, Yuniastuti & Handayani, 2018). Early deaths caused by heart disease occur in the range of 4% in high-income countries to 42% in low-income countries (Kemenkes RI, 2014).

The prevalence of dyslipidemia in Indonesia in 2007 was 14% and increased by 25-28% in 2013, followed by the prevalence of coronary heart disease (CHD) in Indonesia based on Riskesdas in 2007 as much as 7.2%, in 2013 as much as 0.5% and years 2018 reached 1.5% (Husnah, 2017).

Behavior of Indonesian are consume fatty foods, cholesterol and fried foods> 1 time per day as much as 40.7% in Central Java the highest of 60.3% (Risksdas, 2013). With such a diet, Indonesian population has a total cholesterol level disturbance of 35.9% (Hayudanti, Kusumastuty & Tritisari, 2016). High-fat food intake causes LDL to be unable to carry cholesterol into the tissues so that it stays circulating in blood vessels and eventually sticks to blood vessel endothelium (Tate S, 2007; Pirillo, Norata & Catapano, 2013).

Prevention of dyslipidemia can be done by regulating a balanced diet, physical activity and eating fruits and vegetables that have high antioxidant content that can work by stabilizing free radicals in the body that exceeds the body's capacity to be neutralized (Ravichandran et al., 2013). These antioxidant groups include polyphenols, flavonoids and betalain (Olumese, F.E. & Oboh, 2016). The mechanism of action of antioxidants such as flavonoids to reduce cholesterol levels by inhibiting the absorption of cholesterol in the intestine and increase the reaction of the formation of bile acids from cholesterol to then be excreted through feces (Yokozawa, 2002; Yuliana & Ardiaria, 2016).

Nutritional content of beet root extract has been widely studied and is known to have anticancer, antidiabetic and anti-hypercholesterolemic effects. Based on research by Canadanovic et al in 2011, Rabeh naem in 2014 and Attia et al in 2013 showed beet extract per 100 grams containing 380 mg betalains, polyphenols 218.7 mg and flavonoids 269.70 mg (Canadanovic-Brunet et al., 2011; Rabeh & Ibrahim, 2014). Beet tubers are rich in phytochemical compounds, namely ascorbic acid, carotenoids, phenols, flavonoids and betalain which have high antioxidant activity (May et al., 2016). Polyphenols, flavonoids and Betalain in beet root are antioxidants that play a role in preventing discipidemia which is a major factor causing coronary heart disease (CHD) (Ravichandran et al., 2013).

The benefits of consuming beet root (beta vulgaris) are the content of betalains and phenolic compounds in beetroot can reduce lipid oxidative damage thereby reducing the risk of dyslipidemia, reducing the risk of coronary heart disease (CHD), nitrate content can reduce the risk of hypertension, useful as an anti-inflammatory or inflammation, iron content in beet root can also prevent anemia (Kumar & Bhaumik, 2016; Olumese, FE & Oboh, 2016).

Beet tubers have many benefits and can be consumed starting from the root, fruit to the leaves, however, if consumed in excess can cause some side effects that are not good for the body, among others, can cause the color of the urine red purple, commonly called beeturia, acid content oxalate can cause kidney stones, high
levels of nitrite cause stomach disorders, can cause allergies in the form of rashes, itching, swelling and even fever and harmful to the liver and pancreas, the presence of iron, magnesium, copper and phosphorus if consumed in excess then the metal that enters the body will accumulate causing liver damage (Domínguez et al., 2018; J. Kapadia et al., 2012; Putri et al., 2016).

The purpose of this study was to examine the activity of beet root (Beta vulgaris) extracts against cholesterol levels as a preventive measure for dyslipidemia at a single dose of 100mg / kgBB, 200mg / kgBB and a combination dose of simvastatin 0.18mg / kgBB.

METHODS

This research is an experimental research with posttest only randomized control group design. The study was conducted at the Mathematics and Natural Sciences Laboratory of Semarang State University from 18 April to 20 May 2019. The population in this study were male Wistar strain rats with age 2-3 months with a body weight of 100-200 grams. The sample size in this study was calculated using the Federer formula and obtained 5 tails in each group with random sampling techniques.

Beet Root Extraction

Beet root extraction was done using remaseration method. Beet root was peeled, thinly cut fruit flesh and then dried for 5 days. The dried beet root was immersed in 96% ethanol for 3 days, filtered and evaporated with rotary evaporator for 4.5 hours.

Animal and Experimental Design

Male rats weighing 100-200 grams were acclimatized for 7 days to get used to the experimental environment.

Rats are grouped into 7 groups:
1. Group 1 (normal), given food and drink adlibitum.
2. Group 2 (negative control, atherogenic feed).
3. Group 3 (positive control, atherogenic feed and simvastatin 0.18mg / kg BW orally).
4. Group 4 (single dose treatment group, atherogenic feeding and beet root extract 100mg / kg BW orally).
5. Group 5 (single dose treatment group, atherogenic feeding and beet root extract 200mg / kg BW orally).
6. Group 6 (combination treatment group, atherogenic feeding and beet root extract 100mg / kg BW orally + simvastatin 0.18mg / kg BW orally).
7. Group 7 (combination treatment group, atherogenic feeding and beet root extract 200mg / kg BW orally + simvastatin 0.18mg / kg BW orally).

The composition of atherogenic feed given to rats is 5% duck egg yolk and 10% goat fat added with standard AIN feed. Atherogenic feeding is carried out by means of a round that is given on the hour. Giving beet root extract is done by way of a given round at 9 am. Simvastatin was given by disonde given at 3 pm. Atherogenic feed, beet root extract and simvastatin are given every day for 35 days.

Serum Collection

Blood is drawn through the plexus orbitalis 1-2 ml with microhematocrit tubes. The blood is allowed to sit for 30 minutes and centrifuged for 15 minutes at 3000 rpm to obtain serum.

Cholesterol Analysis

Cholesterol were analyzed using CHOD-PAP method uses Human reagents with a clinicon 4010 photometer. The results were analyzed using the Kruskal Wallis and Man Whitney tests.

RESULTS AND DISCUSSION

Body Weight

The body weight of rats in all groups are slightly increased during treatment for 35 days.
The Effect of Beet Root Extract (BRE) in Hypercholesterolemic Rats

Table 1. Results of Average Cholesterol Levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol ± SD (mg / dl)</th>
<th>p-value</th>
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<tr>
<td>Normal</td>
<td>51.060 ±</td>
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<td>Negative Control</td>
<td>70.660 ±</td>
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<td>Positive Control</td>
<td>0.7301</td>
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<td>BRE 100 mg / kg BW</td>
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<td>BW</td>
<td>58.360 ±</td>
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<td>BRE 200 mg/kg BW</td>
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<td>BRE + simvastatin 0.18 mg</td>
<td>1.6149 ±</td>
<td>p=0.000</td>
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<td>BW + simvastatin 0.18 mg</td>
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<td>BRE 200 mg/kg BW</td>
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<td>BW + simvastatin 0.18 mg</td>
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Table 1. Shows the results of a descriptive analysis of the mean values in each treatment group. Atherogenic feeding in group 2 (K-) showed an increase in cholesterol levels as much as 38.38% higher than the baseline group. Giving simvastatin in group 3 (K+) caused a decrease in cholesterol levels by 17.15%. Group 4 (P1) a single dose of 100 mg / kg reduced cholesterol levels by 17.40% while in group 5 (P2) a single dose of 200 mg / kg reduced cholesterol levels by 7.10% while a dose of 200 mg / kg in group 7 (P4) was 18.65%.

The results of statistical tests for normality of cholesterol levels in the seven treatment groups showed an abnormal data distribution with a value of $\alpha > 0.05$. Then the homogeneity test was performed with a Lavene test and $p = 0.006 < \alpha 0.005$ was obtained which showed the variance of the data was not homogeneous. Continued non-parametric test with Kruskall Wallis obtained $p = 0.000 < \alpha 0.05$ meaning that there is an influence of beetroot extract activity on cholesterol levels. To find out the differences in each group followed by the Mann Whitney test.

Table 2. Mann Whitney Analysis for cholesterol level

* Note: significant: $< 0.05$

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Table 2. shows the differences in cholesterol levels between one group and another group. Where in group 1 (baseline) compared with all groups showed a difference with the value $\alpha < 0.05$. Group 2 (K-) compared with group 6 (P3) in giving EUB 100 mg / kg combined with simvastatin 0.18 mg / kg showed no significant difference with $p = 0.076 > \alpha 0.05$. Group 3 (K+) compared with group 4 (P1) in the administration of single dose beet root extract 100mg / kg showed no significant difference seen from the value of $p = 0.673 > \alpha 0.05$.

Beet root Extract Effective in Preventing Dyslipidemia

Dyslipidemia is one of the health problems that occur in society. The prevalence of dyslipidemia in Indonesia was 14% in 2007 and increased by 25-28% in 2013 (Husnah, 2017). Based on the results of this study indicate that by giving atherogenic food for 35 days in the negative control group (K-) can increase...
cholesterol levels higher than the baseline / normal group which is only given standard feed. Atherogenic feeding in the negative control group (K-) showed an increase in cholesterol levels as much as 38.38%.

In line with the research of Gani et al. (2013) that regular consumption of high fat foods can cause an increase in blood cholesterol levels (Gani, Momuat & Pitoi, 2013). The process of formation of complex cholesterol through the hydroxy methylglutaril CoA reductase (HMG-CoA) which plays an important role in converting HMG-CoA to mevalonate, resulting in decreased cholesterol synthesis in the liver (Lairin Djala, Lyrawati & Soeharto, 2016)

The process of cholesterol formation in the body through the enzyme hydroxy-methylglutaryl-CoA reductase (HMG-CoA reductase) which converts HMG-CoA to mevalonate (Lairin Djala, Lyrawati & Soeharto, 2016). Cholesterol is produced by the liver about 80% of the body and the rest comes from dietary food consumed daily, normal cholesterol levels are <200 mg / dl (Lucius, 2013). If the increase in cholesterol levels exceeds the normal value, it will cause lipid metabolism abnormalities which results in dyslipidemia (Tia, Sistiyono & Hendarta, 2014). Dyslipidemia is a risk factor for coronary heart disease (CHD) which is a predictor of cardiovascular morbidity and mortality (Chen et al., 2018; Azam et al., 2018). Dyslipidemia can be prevented by controlling risk factors, one of which is by eating a diet that is consuming vegetables and fruits that are rich in antioxidants as protective against reactive oxygen (Yu et al., 2014).

According to J.M.’s research Canadanovic beet root extract (Beta vulgaris) is 10 vegetables with high antioxidant capacity containing 50-60 µmol / g phenolic compounds (Canadanovic-Brunet et al., 2011). Phenolic compounds have an important role as an antioxidant to free radicals that can cause cancer and cardiovascular disease (Biondo et al., 2014). In this study the content of beet root extract (Beta vulgaris) was tested by phytochemical tests using the Harbome and Folin-ciocalteu methods conducted at the IBL Laboratory of the Faculty of Medicine, Unissula Semarang.

Based on the results of this study in the four treatment groups given beet root extract at doses of 100 mg / kg, 200 mg / kg and simvastatin 0.18 mg / kg showed significantly different results. In the combination treatment group showed the results of higher cholesterol levels when compared to the single dose treatment group, this difference was shown by the value α <0.05. The high cholesterol level in the combination treatment group of beet root extract with simvastatin was due to several factors including: time of administration of simvastatin, side effects and effectiveness of the drug combined with herbs (Wulandari, Susilowati & Amelya, 2017). Previous research by Mukuntha et al. 2016 when giving statin drugs (lovastatin) was given at 3 pm, while in this study time was given simvastatin at 3 pm and beet root extract (EUB) was given at 9 am, the results showed it could be inhibits an increase in cholesterol levels even though the increase is not optimal when compared to the single dose treatment group which shows a more maximum reduction in cholesterol levels.

Simvastatin is one of the statin drugs that is effective for reducing cholesterol levels which works by inhibiting the enzyme activity of HMG CoA Reductase (Hydroxy Methylglutarin Coenzyme A) so that cholesterol production in the liver will decrease (Hardianto, 2014). Simvastatin drug class has a short half-life of 2 hours so that the most optimal time to consume it at night before going to sleep because when the body is resting is very high cholesterol synthesis (Irma Rosita, Retnosari Andrajati, 2014). Based on the survey results, millions of people use herbal medicines together with synthetic drugs without doctor’s recommendation who think that herbal medicines can reduce the side effects of synthetic drugs taken together can increase the effectiveness of treatment (Wulandari, Susilowati & Amelya, 2017). In the 2010 Isbandiyah study, administering herbs with chemical drugs simultaneously could lead to a buildup of statins in the body and risk of liver
damage, kidney failure and an increase in blood sugar levels. The content of active substances that are in chemical drugs are generally more quickly absorbed by the body compared to herbal medicines which are slower absorbed by the body and are binding substances from chemical drugs.

This study shows the results of the most effective reduction in cholesterol levels in beet root extract doses of 200 mg / kg body weight. Decreased cholesterol levels in rats that have been fed atherogenic diets due to beet root extracts contain phenolic acids such as p-coumaric, protocatechuic, ferulic, vanilic, p-hydroxybenzoic, and syringic acid (Kavalcová et al., 2015). Beet tubers have a betasianin content of 186.90 mg / 100g and antioxidant activity of 53.71% (Yuliana & Ardiaria, 2016). Flavonoids in beetroot have the effect of improving lipid profile by increasing lipoprotein lipase activity so as to reduce lipid levels. Flavonoids act as scavenger free radicals that have hydroxyl groups (OH-) in the aromatic ring and stop the lipid peroxidation chain reaction by protecting cells and chemicals in the body. The mechanism of action of antioxidants such as flavonoids can reduce plasma cholesterol levels by inhibiting the absorption of cholesterol in the intestine and increasing the reaction of bile acid formation from cholesterol to then be excreted through feces (Yuliana & Ardiaria, 2016).

The mechanism of action of flavonoids in absorbing cholesterol in the intestine acts as an inhibitor of the HMG-CoA reductase enzyme, where when cholesterol is transported from the intestine to the liver, HMG-CoA reductase converts acetyl-CoA to mevalonate in cholesterol synthesis will be inhibited so that cholesterol synthesis products by the liver will be reduced Yuliana & Ardiaria, 2016). The content of antioxidant flavonoids can inhibit the activity of the pancreatic lipase enzyme and increase fat excretion through feces, as a result the inhibited fat absorption by the liver which results in the formation of cholesterol in the blood is also inhibited. Flavonoids also play a role in changing two monoglycerides and two free fatty acids so that they can enter the blood circulation to the liver to be removed (Artha, Mustika & Sulistyawati, 2017).

**CONCLUSIONS AND SUGGESTIONS**

Beet root extract of 200 mg / kg BW was effective in reducing cholesterol levels of rats fed atherogenic feed for 35 days.

Further research needs to be done about the use of beet root extract in the form of functional drinks so that it can be used as a prevention of hypercholesterolemia.

**ACKNOWLEDGEMENT**

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