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Research Article

Effect of Red Cabbage Aqueous Extract on High-density Lipoprotein (Hdl) Levels of Male White Rats Induced By Atherogenic Diet

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Abstract.
Dyslipidemia is a lipid fraction abnormality in the form of an increase in total cholesterol, LDL, and triglyceride levels and a decrease in HDL. Red Cabbage Aqueous Extract (RCE) contains antioxidants, one of which is anthocyanins, that can increase HDL levels. This research was conducted to determine the effect of RCE on HDL levels of white male rats induced by an atherogenic diet. True experimental with post-test only control group design with 15 rats divided into the standard group (KN: regular feed), negative control group (K-: atherogenic diet), and three treatment groups (P1: atherogenic-diet with RCE doses of 6.65 mg; P2: atherogenic-diet with RCE doses of 13,3 mg; and P3: atherogenic-diet with RCE doses of 26.6 mg/100grBW) was used. The HDL measurement method used spectrophotometry. *Brassica oleracea L* extract had no significant effect (Kruskal-Wallis test p = 0.465) on HDL levels of white male rats. This result also happened due to the low bioavailability of anthocyanins in aqueous extract. The linear regression test results showed that the relationship's strength was 0.031 or very weak with a linear proportional correlation, increasing the dose and increasing HD levels. Based on the results of the tests, there was no significant effect of giving Red Cabbage (*Brassica oleracea*) Aqueous Extract on HDL levels of white male rats (*Rattus norvegicus wistar strain*), which was induced by an atherogenic diet.

Keywords: anthocyanins, atherogenic diet, HDL, red cabbage aquous extract

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1. INTRODUCTION

Atherosclerosis is a chronic inflammatory disease in blood vessels that causes structural and functional damage to the three layers of coronary artery walls (1). In 2017, there were approximately 17.8 million deaths worldwide due to cardiovascular disease; three-quarters of them occurred in low-middle-income countries. Meanwhile, in 2018, the prevalence of heart disease in Indonesia (without comparing the heart disease suffered) reached 1.5% or around 1,017,290 people, and in East Java province, around 151,878 patients (2).

One of the risk factors for atherosclerosis is dyslipidemia. Dyslipidemia is a lipid metabolism disorder that causes a decrease or increase in the lipid fraction in plasma.

The prominent lipid fraction abnormalities are increased total cholesterol levels, Low-Density Lipoprotein (LDL) cholesterol, triglycerides (TG), and decreased High-Density Lipoprotein (HDL) cholesterol (3). In dyslipidemic conditions, the HDL lipid fraction has decreased apart from an increase in the LDL lipid fraction. HDL functions as the body's defense system to control fat levels in the body. HDL also acts as an endogenous antioxidant when inflammation occurs in atherosclerosis due to increased LDL levels (4).

HDL, as an antioxidant, inhibits phospholipid oxidation and reduces the minimal activation of Ox-LDL. Several HDL components contribute to this antioxidant effect, including ApoA-1 ApoA-II, ApoA-IV, ApoC-1, ApoC-II, ApoD, ApoE, ApoF, ApoJ, ApoL-1, and, ApoM, ApoA-1 are structural HDL, activation of HDL-related enzymes such as PON-1 and LCAT, and is seen in HDL's ability to neutralize free radical oxidants (4).

Red cabbage detected a higher concentration of phenolic and flavonoid compounds in aquous extract, and high quantification of anthocyanin and ascorbic acid contributed to high antioxidant activity (5). The antioxidants in red cabbage have different functions on the lipid profile in the blood. Anthocyanins perform to increase HDL levels while reducing LDL levels by inhibiting Cholesteryl Ester Transfer Protein (CETP) and stimulating the work of Reverse Cholesterol Transport (RCT) (6). Increasing ascorbic acid level in serum plasma caused a significant reduction in lipid levels (increase HDL and reduce LDL and LDL/HDL ratio) (7). The total anthocyanin in red cabbage is about 300 mg / 100g (8).

2. Materials and methods

This study was a true experiment using a post-test-only control group design for 21 days in the Biomedical Laboratory of the Faculty of Medicine, University of Muhammadiyah Malang. The samples used were 15 white male rats (Rattus norvegicus Wistar strain) aged 4-5 months, 200-250 grams body weight, a health condition characterized by active (nocturnal) movement, clear eyes, and thick hair. The rats were divided into 5 groups, namely, normal control, negative control, and treatment groups one, two, and three with doses of 6.65 mg / 100grBW, 13.3 mg / 100grBB and 26.6 mg / 100grBW respectively.

Red cabbage is obtained from a supermarket in Malang, and then it is extracted using water (8) and given orally with a stomach feeding tube before being given an atherogenic diet. The atherogenic diet used in this treatment with lard mixed with AD II pellets with a ratio of 1: 9 as much as 40 grams/rat/day (9).

Then before surgery, rats fasted for 8-12 hours. Then, surgery was performed, and blood was drawn from the right ventricle. The blood was left to stand for 15 minutes, then centrifuged for 20 minutes at 3000 rpm to get the serum. The serum was examined by spectrophotometric method to determine the HDL absorption rate. Then the absorption value is entered into the following formula to get the HDL value:

$$C = \frac{A\ Sample}{A\ Standard}\ x\ C\ Standard\ (200mg/dL)$$

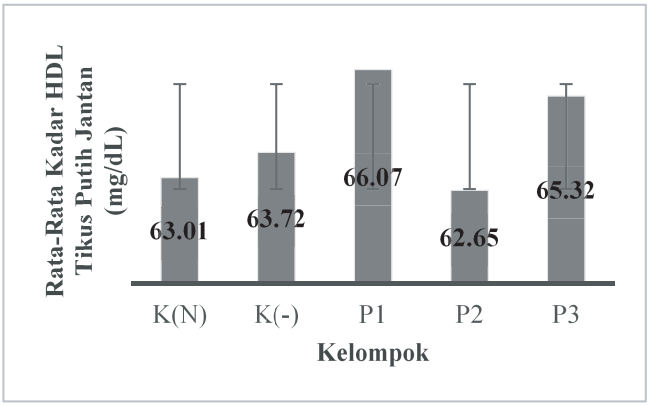
Note:

C = HDL cholesterol levels (mg/dL)

A = Absorption

3. Results

The effect of RCE on blood HDL levels in rats induced by an atherogenic diet showed in Figure 1. There were differences in HDL levels between rats given RCE compared to the standard and negative control groups.



Notes :
K(N): standard AD II feed 40 grams/rat/day
K(-): lard mixed with AD II pellets with a ratio of 1: 9 as much as 40 grams/rat/day
P1 : atherogenic diet + RCE 6.65 mg/100 grams BW/day
P2 : atherogenic diet + RCE 13.3 mg/100 grams BW/day
P3 : atherogenic diet + RCE 26.6 mg/100 grams BW/day

Figure 1: Average HDL levels measurement.

Notes :

K(N): standard AD II feed 40 grams/rat/day

K(-): lard mixed with AD II pellets with a ratio of 1: 9 as much as 40 grams/rat/day

P1 : atherogenic diet + RCE 6.65 mg/100 grams BW/day

P2 : atherogenic diet + RCE 13.3 mg/100 grams BW/day

P3 : atherogenic diet + RCE 26.6 mg/100 grams BW/day

In this study, the results of the measurement of the largest HDL levels were group P1 (atherogenic diet + RCE 6.65 mg / 100g BW / day) of 66.07 mg / dL followed by group P3 (atherogenic diet + RCE 26.6 mg / 100 g BW / day) of 65.32 mg / dL, and group P2 (Atherogenic diet + RCE 13.3 mg / 100 g BW / day) was 62.65 mg / dL. A graph of the mean HDL levels in the five groups is presented in Figure 1.

Before data management was carried out to determine the difference between the two variables, a normality test was used to determine whether the data had a normal distribution. The normality test obtained p = 0.001 so that the distribution of the HDL mean data was not expected, even with the data transformation. So data management uses the Kruskal-Wallis alternative test. The Kruskal-Wallis test resulted in a sig value of 0.465> p (0.05), which indicated that there was no effect of giving RCE on HDL levels of white male rats (Rattus norvegicus Wistar strain). Thus, these data cannot be subjected to the Mann-Whitney post hoc test. The influence and strength of the relationship between the administration of RCE and HDL levels were carried out by linear regression. Based on the results of the linear regression test, it was found that the effect of RCE on male white HDL levels was 0.1% (R2 = 0.001), and the strength of the relationship was indicated by R = 0.031, which means that it has a very weak correlation between RCE and HDL levels of white male rats.

4. Discussion

The Red Cabbage Aquous Extract (RCE) has many phytochemical compounds, mainly phenolic, linked to antioxidant capacity and hypolipidemic effect. The extraction method in red cabbage will affect the content of phytochemical content. Furthermore, a significantly higher concentration of phenolics and flavonoid compounds in the aqueous than in the hydroethanolic one. The antioxidant capacity of the aqueous extract was also superior to the hydroethanolic (5). The anthocyanin content in aqueous extracts is significantly higher than in red cabbage juice (8). Ascorbic acid in RCE is also contributed to antioxidant activity (5).

This study showed that red cabbage extract (Brassica oleracea) increased HDL levels in male Wistar rats induced by an atherogenic diet. Administration of RCE for each treatment group at sequential doses of 6.65 mg / 100grBW, 13.3 mg / 100grBW, and 26.6 mg / 100grBW for 21 days could increase HDL levels in rats when compared to the opposing group given an atherogenic diet without RCE. This study supported previous

studies in rabbits fed with red cabbage (leaves and juice) for twelve weeks. Both groups in the study raised HDL concentration to a minor extent (10).

Red cabbage contains chemicals such as anthocyanins and ascorbic acid, which have antioxidant properties. Anthocyanins stimulate Reverse Cholesterol Transport (RCT) and form HDL by activating the Liver X Receptor (LXR). Anthocyanins also affect PON1. Anthocyanins are involved in increasing Apo-A1 production (10). This increase in production will align with the increase in LCAT activity. LCAT will convert free cholesterol taken up by discoid HDL into cholesterol esters, forming a spherical HDL (11). The serum HDL concentration increased significantly more in the subject with hypercholesterolemia that was given anthocyanin supplementation for 24 weeks (12).

Ascorbic acid, a water-soluble antioxidant, is essential in lipid regulation, increases HDL levels, and protects against LDL oxidation. The increase in HDL concentration in this study was also consistent with the previous study in patients (children) on hemodialysis, which gave supplementary Vitamin C (ascorbic acid) for twelve weeks (7).

Figure 1 shows that the HDL levels in the group receiving RCE 6.65 mg/100grBW were higher than the other two groups that were given higher doses. This condition occurred due to external factors such as physical activity or activity from rats. Active rats will cause an increase in HDL levels due to an increase in hepatic ABCA1 experience. The liver performs its function of regulating lipids through many receptors, one of which is LXR. LXR stimulates reverse cholesterol transport by increasing ABCA1 gene expression (13).

The stability of anthocyanin in RCE is affected by several factors, such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvent, enzymes, flavonoids, proteins, and metallic ions (14).

Although the graph (Figure 1) shows an increase in HDL levels, statistically, the increase between treatment groups did not have a significant difference. This result can be caused by the low bioavailability of anthocyanins in RCE. The low bioavailability of anthocyanins makes the absorption of these compounds into the circulatory system low and a high excretion rate of anthocyanins in urine and feces, thus reducing the efficacy of anthocyanins (15). Anthocyanins can increase HDL by stimulating HDL production on LXR and stimulating the RCT process(10).

5. Conclusion

In this study, it can be concluded that extract of red cabbage (Brassica oleracea) can increase HDL levels of white male rats (Rattus norvegicus Wistar strain) induced by an atherogenic diet, although statistically there was no significant difference.

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