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Effect of rosella-based kombucha tea on the lipid profile on hyperlipidemic rats (*Rattus norvegicus*)

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A B S T R A C T

The purpose of this study was to determine the lipid profile of white rats (*Rattus norvegicus*) with high-fat feed after fermentation of rosella kombucha with various concentrations. This study used 20 white male rats (*Rattus norvegicus*) aged two months, with rosella kombucha treatment fermented for five days at 25oC per oral. This study used Completely Randomized Design with four treatments (for 21 days) and four replications, namely: P0 = Control, rats were only given food and drink as usual (with the same type of rat feed). P1 = 1 ml of pork oil/day for 14 days + 1.8 ml of rosella kombucha 50% in the morning and evening for seven days. P2 = 1 ml of pork oil/day for 14 days + 1.8 ml of 75% rosella kombucha morning and afternoon for seven days. P3 = 1 ml of pork oil/day for 14 days + 1.8 ml of 100% kombucha rosella morning and afternoon for seven days. The variables measured were levels of LDL (Low-Density Lipoprotein) and HDL (High-Density Lipoprotein). The data obtained were analyzed using ANOVA followed by Duncan Test with 95% confidence level using SPSS 16.0 software. The results showed that administration of rosella kombucha did not have a significant effect on LDL and HDL levels of white rats (*Rattus norvegicus*). Based on the results of the study, it can be concluded that the 5-day fermentation of kombucha rosella with various concentrations did not affect LDL and HDL levels.

Keywords: Rattus norvegicus, Kombucha rosella tea, LDL (Low-Density Lipoprotein) and HDL (High-Density Lipoprotein.

I. INTRODUCTION

Hyperlipidemia has been known as a pathological condition due to abnormalities of blood lipid metabolism which is characterized by increased total cholesterol, triglycerides, *low-density lipoprotein* (LDL) and decreased *high-density lipoprotein* or HDL levels (Heryani, 2016). Hyperlipidemia has been known as one of the factors that can trigger thickening of the blood vessel walls resulting in narrowing and hardening of the arteries called atherosclerosis (Rahayu, 2005). Atherosclerosis has a very close relationship with increased levels of LDL in the blood. Increased levels of LDL in the blood will be followed by accumulation of cholesterol esters in macrophages. High LDL levels cause high LDL in the tunica intima in blood vessels. Furthermore, LDL deposited in the tunica of the intima of the blood vessels will undergo oxidation and attract monocytes from the blood circulation so that it changes phenotypically into macrophages. Increased levels of oxidized LDL in the arterial wall are accompanied by the formation of foam cells which can develop into fat plates. (Isdadianto dan Tana, 2016).

Kombucha tea has been known as a fresh fermented beverage from sweet tea with the help of fungi and acid-forming bacteria. This drink is usually consumed by some people because it is beneficial for the health of the body (Jayabalan *et al*., 2007). Naland (2008) has stated that kombucha tea contains a variety of important nutrients, which include vitamin B (thiamine/B1, riboflavin/B2, niacin/B3, pyridoxine/B6, cyanocobalamin/B12), vitamin C, dan polyphenols. Kombucha tea plays a role in fat metabolism, so this type of drink has the potential to reduce levels of

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bad cholesterol, namely LDL and triglycerides. This drink also has the potential to increase HDL levels so that it can reduce this risk of vascular disease and coronary heart disease. One of the main elements of polyphenols ic catechins, This compound has been known to have the potential to prevent high blood pressure, reduce the accumulation of cholesterol in the blood, accelerate the disposal of cholesterol through feces, ward off free radicals, and reduce the risk of cardiovascular disease (Anugrah, 2005).

The results of the research conducted by Isdadiyanto dan Tana (2016) showed that kombucha tea with 75% levels could increased HDL levels, reduce total cholesterol and LDL levels in the rat (*Rattus norvegicus*) blood serum. Purwaning (2010) dan Rahayu (2005) have also reported the results of their research that kombucha tea can reduce blood cholesterol levels in rat test animals. Further stated that cholesterol is not entirely a compound that is detrimental to the body, but cholesterol is an important compound in the body that is needed to assist the metabolic regulation process. However, high levels of cholesterol in the blood for a long time can cause atherosclerosis which will lead to coronary heart disease. Various findings of the research show that there is a real correlation between atherosclerosis of coronary artery and total cholesterol in the blood, especially LDL cholesterol. Conversely, there is a negative correlation between coronary artery atherosclerosis and HDL cholesterol levels. Patients with high LDL levels are at risk for heart disease, where as patients with high HDL levels rarely suffer from heart disease. Based on this background, a study was obtained which obtained evidence and information about the effect of various variations of the concentration of kombucha tea on HDL and LDL levels in rats fed with high-fat content (hyperlipidemia).

II. MATERIAL AND METHODS

Research has been advanced at the Biology Department Laboratory, Faculty of Mathematics and Natural Sciences, Semarang State University in March to May 2018. The test animals used in this study were 20 male rats with an age of 2 months. The treatment material used is rosella kombucha tea which has been fermented for five days. The treatment of kombucha tea in the test animals was carried out by an oral method with a tip syringe (gavage). The tools that have been used in this study include 20 individual cages equipped with feed and drink containers, measuring cups, Erlenmeyer, 2 liter fermentation containers, LDL and HDL measuring devices, and digital scales.

The research design that has been applied in this study is a completely randomized design consisting of 4 treatments with four replications, which include P0; namely control treatment (rats without kombucha tea and only given drinking water in the form of tap water). P1, P2, dan P3; that is, rats fed 1.8 ml of Roselle-based kombucha tea, in a sequence of 50%, 75%, and 100%. The treatment of kombucha tea has been given every morning and evening for seven days. Model animals namely hyperlipidemic rats were made using rats given 1 ml of oil with high cholesterol content per day for 14 days. At the end of the treatment, blood samples were taken through the ophthalmic vein and followed by measurement of LDL and HDL levels in the blood.

The variables measured in this study are LDL and HDL levels in the blood. The data obtained were then analyzed by analysis of variance (ANOVA) with a significance of 5% and continued with the Duncan test at a significance of 5%. Devices that have been used for analysis using *software SPSS 16,0* (Mattjik dan Sumertajaya, 2006).

III. RESULTS AND DISCUSSION

The results of the analysis of LDL and HDL in the blood by the influence of rosella kombucha tea are shown in Table 1.1. The results of analysis of HDL levels in the blood with ANOVA at a significance of 5% showed that supplementation of rosella kombucha tea had no significant effect on HDL levels in rat blood plasma (P>0,05). Sequentially the average HDL levels in rat blood plasma at P1, P2, P3 are 39.69; 38.61; 36.85 mg/dL. The HDL levels in the three treatments were not significantly different from the control rats that had blood plasma HDL levels of 41.16 mg/dL. HDL levels of blood plasma that have been analyzed belong to the normal category but not high enough. The results of HDL levels in the blood obtained in this study are in accordance with the results reported by Gani *et al*. (2013) which states that normal HDL levels in rat blood plasma are ≥35 mg/dL. Normal blood HDL levels are very useful in reducing the risk of atherosclerosis because HDL functions to carry cholesterol from peripheral tissues go to the liver so that it can prevent the occurrence of atherosclerosis (Hartoyo *et al.,* 2008). The treatment of kombucha tea with a fermentation time of 5 days at levels between 50-100% has not been optimal in producing

Table 1.1. The results of the analysis of the average levels of LDL and HDL in blood in rats by the influence of rosella kombucha tea.

Variable	P0	P1	P2	P3
HDL (mg/dL)	43,16 [a]± 2,93	39,69[a] ± 7,21	38,61[a]± 5,03	36,85 [a]± 1,90
LDL (mg/dL)	156,56 [a]± 13,74	162,56[a] ± 31,4	173,49[a] ± 26	142,87 [a]± 10,35
LDL/HDL	3,63[a]±4,69	4,10[a]±4,35	4,49[a]±5,17	3,88[a]±5,45

Keterangan : P0; namely control treatment (rats without kombucha tea and only given dringking water in the form of tap water). P1, P2, dan P3; that is, rats fed 1.8 ml of roselle-based kombucha tea, in a sequence of 50%, 75%, and 100%.

organic acids. Wistiana & Zubaedah (2015) stated, fermented kombucha tea for 8-12 days can produce various kinds of organic acids; including ascorbic acid, acetic acid, glucuronic acid, gluconate acid, and antioxidants. The results of Mutiara's (2014) research show that to produce kombucha tea with high antioxidant content requires a minimum fermentation time of 8 days. The more organic acids and antioxidants produced in kombucha tea will increasingly influence organoleptic quality, including taste, aroma, and color (Napitupulu *et al*., 2015). Organic acids and antioxidants (ascorbic acid and polyphenols) in kombucha tea will affect the increase in HDL levels in blood plasma

The results of a similar study have also been reported by Bahaudin (2008) who have stated that normal HDL levels in rat blood plasma are very useful for reducing the risk of atherosclerosis. HDL has an antiatherogenic effect which is able to carry cholesterol free from blood vessels and other tissues in the body to be taken to the liver. In the liver, cholesterol will be secreted to the bile and will then be carried to the duodenum. From the duodenum, cholesterol will be released from the body with feces (Hartoyo *et al.,* 2008; Dalimartha 2003). Moeliandari dan Wijaya (2002) stated HDL has antioxidants effects that can prevent oxidation of LDL so that cholesterol cannot stick to the walls of arteries. In addition to HDL which has the potential as an antioxidant, the antiatherosclerosis effects are thought to originate from oxidants found in kombucha tea. Falahuddin *et al*. (2017) have stated, the longer the time of fermentation in producing kombucha tea the higher the antioxidant content, especially vitamin C. This is because in the fermentation process, bacteria *Acetobacter xylinum* will produce vitamin C. D-Glucose will be reduced to D-sorbitol. In the early stages of fermentation, the D-sorbitol compound will change shape to L-sorbosa in the presence of an enzyme produced by bacteria *Acetobacter xylinum*. Alcohol groups from sugar compounds can be oxidized by bacteria in the presence of oxygen. L-Sorbosa which has undergone further fermentation will be converted to ascorbic acid (vitamin C). Roselle-based kombucha supplements as in this study have an important role to play in improving the health quality and body defense system of test animals.

Kombucha tea can improve organ function. The active content of roselle-based kombucha tea will be distributed throughout the body system and processed through metabolism so that it is useful to support cholesterol metabolism, especially in maintaining HDL levels to remain normal or high (Naland, 2004). Normal or high HDL with small molecular characteristics compared to other lipoproteins will easily pass through vascular endothelial cells and enter into the intima portion of the blood vessel wall to transport the cholesterol collected in the macrophage (Harini dan Astirin, 2009). High HDL levels can prevent cell damage (cells injury) in the walls of blood vessels, especially endothelial cells followed by the deposition of lymphocytes and monocytes, macrophage formation, lipid deposition, smooth muscle proliferation and extracellular matrix synthesis (Harini dan Astirin, 2009). The higher the HDL level, the more the potential to prevent atherosclerosis.

The results of LDL analysis with ANOVA at 5% significance showed that the supplement of roselle kombucha tea had no significant effect on the LDL levels of rat blood plasma ($P>0.05$). Sequentially the average LDL levels of rat blood plasma at P1, P2, and P3 are 162.56; 173.49; and 142.87 mg/dL; not significantly different from control rats that had LDL levels as much as 156.56 mg/dL. LDL levels in this study belong to the high category. The results of this study are in accordance with the results of research conducted by Gani *et al*. (2013) who reported that LDL levels in rat blood plasma were ≥69,3 mg/dL, including the high criteria. The results of this study as in Table 1 show that LDL levels in both control rats or treatment belong to the high category. Normal HDL levels allow high levels of LDL in blood plasma. This condition is related to the main component of lipoprotein and LDL and HDL receptors. Mayes and Botham (2003) has stated that LDL contains apoprotein B (protein and polypeptide) and has the main receptor namely

apoprotein B, while HDL contains apoprotein A and has the main receptor apoprotein A. Furthermore, the condition of test animals such as the results of this study can be determined based on LDL with HDL. The higher the LDL/HDL ratio, the higher the risk of atherosclerosis, and vice versa the lower the LDL/HDL ratio, the better the condition of the test animal (Isdadiyanto, 2015).

The results of the analysis of the ratio of LDL/HDL with ANOVA at a significance of 5% have shown that kombucha tea supplements made from rosella did not significantly affect the ratio of LDL/HDL in rats (P>0,05). Sequently the ratio of LDL/HDL rats at P1, P2, and P3 is 4.10; 4.49; dan 3.88; not significantly different from control rats which had an LDL/HDL ratio of 3.63. The LDL/HDL ratio in this study included in the not high category even though it was found to have a high increase in LDL in blood plasma. The results of research conducted by Isdadiyanto (2015) show that the ratio of normal LDL/HDL in non-hyperlipidemic control rats is between 3,08 - 3,42 and or below 8,63. The high LDL/HDL ratio is evidence that the supplement of rosella-based kombucha tea is a safe category. This means that the supplement of rosella-based kombucha tea with a fermentation time of 5 days does not have the potential to trigger atherosclerosis.

Rosella kombucha tea contains medium chain fatty acids (MCFA). MCFA are more polar (faster-releasing H ions) than long chain fatty acids (LCFA). The solubility properties of MCFA in water that are higher than LCFA make it easier for these compounds to be transported into the liver directly through the portal vein. The existence of MCFA is the results of absorption that takes place in the small intestine without involving pancreatic lipases. The prominent characteristic of MCFA is that they undergo a metabolic process faster to be converted into energy. This MCFA does not enter the cholesterol cycle and is not buried as fat in the body's tissues (Dayrit 2003). According to Enig (2001), MCFA contained in rosella-based kombucha tea is able to burn fat from other sources and is quickly able to convert it into energy, so it increases metabolism. In this study, the effect of rosella-based kombucha tea with MCFA content caused an increase in HDL levels in rat blood.HDL function is known to transport cholesterol from peripheral tissues to the liver, get rid of excessive cholesterol and inhibit atheroma plaque formation so that high levels of HDL in the blood will prevent the risk of atherosclerosis (Toth, 2005). Suhartatik *et al*. (2009) stated that rosella-based kombucha tea with a fermentation time of 5 days was effective in reducing cholesterol levels and preventing atherosclerosis in hyperlipidemic rats compared to black tea and placebo treatment.

Other active ingredients found in rosella kombucha tea are ascorbic acid (vitamin C) and polyphenols (flavonoids). Both of these active ingredients have been known as antioxidants which have a beneficial effect on the function of endothelial cells, namely reducing LDL oxidation and increasing *nitric oxide* (NO) production (Vita, 2005). LDL oxidation will induce an inflammatory response by producing leukocytes and cytokines in endothelial cells. Flavonoids reduce LDL oxidation and prevent inflammation in endothelial cells. NO is an endogenous vasodilator that has an anti-atherosclerotic ability. Sunarno dan Djaelani (2018) has stated, that polyphenol compounds are involved in increasing protein synthesis in the body. Availability of increased protein (enzymatic or non-enzymatic) affects the metabolic process more efficiently and effectively. Further stated, the active compounds in the form of flavonoid can increase levels of neurotransmitters such as dopamine, norepinephrine, epinephrine, and serotonin which function to ensure the availability of energy needed to maintain cellular integrity (Sunarno, 2018). Polyphenols will prevent LDL oxidation so the formation of *reactive oxygen species* (ROS) can be stopped so that atherosclerosis does not occur.

The overall analysis of HDL, LDL, and LDL/HDL ratio showed that the test animals were still in the normal category. This means that the use of rosella-based kombucha tea with a concentration range of 50-100% is still safe to use as a feed supplement. The use of rosella-based kombucha tea with this concentration range has not been able to increase HDL levels or significantly reduce LDL levels in blood plasma of test animals.

The conclusion is that the 5-day fermented rosella-based kombucha tea with a concentration of 50-100% cannot reduce LDL levels and cannot increase HDL levels in blood plasma in hyperlipidemic rats.

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