Effect of time fermentation kombucha tea on lipid profile of rats (*Rattus norvegicus* L.)

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Abstract. The purpose of this study was to determine the effect of kombucha tea as drinking water by the time variation of fermentation. This study used 50 rats (*Rattus norvegicus* L.) male two months of age, with the treatment of the fermented kombucha tea for 6, 9 and 12 days at a temperature of 25°C orally. This study used a completely randomized design with 4 treatments (for 60 days) and 5 replications, namely: P0 = control, without the addition of kombucha tea, P1 = water drink + 1.8 ml in the morning and afternoon tea kombucha fermentation 6 days, P2 = water drink + 1.8 ml morning and afternoon tea kombucha fermentation 9 days, P3 = water + 1.8 ml in the morning and afternoon tea kombucha fermentation 12 days. The variables measured were cholesterol, HDL and LDL. Data were analyzed using ANOVA followed by Duncan test with a 95% confidence level using SPSS 10.0 software. The results showed that administering kombucha tea can raise levels of HDL, lowering LDL cholesterol and blood serum of white rats (*Rattus norvegicus* L.). In conclusion, kombucha tea is fermented for 6, 9 and 12 days could potentially be used as a supplement for the prevention of risk to vascular disease and coronary heart disease.

1. Introduction

Kombucha tea is a drink that is easily made by people in Indonesia. This tea has several benefits for the body, especially for stabilization of the body's metabolism. By increasing the metabolism, the over-accumulation of fat in the body's can be avoided. The tea contains chemical compounds including vitamin B (thiamine / B1, riboflavin / B2, niacin / B3, pyridoxine / B6, sianokobalanin / B12), vitamin C and polyphenols. The tea plays an important role in fat metabolism to reduce levels of bad cholesterol, the LDL and triglycerides, and increasing HDL levels, to reduce the risk of vascular disease and coronary heart disease. Catechins is one element of polyphenols can prevent high blood pressure, reduce the accumulation of cholesterol in the blood, speeding up the disposal of cholesterol through the feces, as well as free radicals. Naland reported that catechins might reduce the risk of cardiovascular disease.

Results of research conducted by Purwaning and Rahayu using kombucha tea on white rats showed a decrease in cholesterol levels. Similarly, by Winarni *et al.* stated that kombucha tea tends to lower blood cholesterol levels although not statistically proven with the object of treatment of students of the Faculty of Medicine, Sebelas Maret University, Indonesia. Furthermore, Adriani *et al.* in an experiment with ducks were used as testing animals stated that kombucha tea could lower total blood cholesterol, but it also reduces bad cholesterol (LDL) and raises good cholesterol (HDL) after consuming kombucha tea for four weeks.
Cholesterol in the blood circulation is not in a free state but is within lipoprotein particles. Lipoproteins are complex compounds between fat and protein. In blood serum, lipoprotein consists of 4 types of chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL)\(^6,7\). Chylomicrons containing 96% triglycerides, 1.7% protein, 1.75% cholesterol and 0.6% phospholipids. Low density lipoprotein (LDL) containing 10% triglycerides, 45% cholesterol, 25% protein, and 20% phospholipids. Based on the description above shows that LDL cholesterol is high enough. This means, increased levels of LDL in the blood is always accompanied by hypercholesterolemia. High density lipoprotein (HDL) containing 3% triglycerides, 18% cholesterol, 50% protein, and 30% phospholipids. HDL functions as a transporter of cholesterol to the liver to be degraded to bile acids and brought into the gallbladder\(^8\).

One or more apoproteins (protein and polypeptide) are found in each lipoprotein. HDL is the major of apoprotein A, the main LDL apoprotein is apoprotein B, which is also found in VLDL and chylomicrons. Apoprotein A receptor function as HDL and apoprotein B as the LDL receptor. Atherosclerosis is associated with a high ratio of LDL: HDL in plasma\(^9\). Apoprotein B is the main protein in atherogenic lipoprotein particles and mainly contained in the LDL particle. Each LDL particle contains one molecule of apo B. Thus the concentration of apoprotein B reflects the number of LDL particles in the body\(^10\). Atherosclerosis is associated with increased LDL. Effect of elevated levels of LDL will be followed by the accumulation of cholesterol esters in macrophages which then referred to as macrophage foam cells. High LDL levels lead to high levels of LDL intimal. Furthermore, intimal will oxidize LDL and attracts monocytes from the blood circulation as well as phenotypic change into macrophages. Increased oxidized LDL in the arterial wall is accompanied by the formation of foam cells, will develop into a plate of fat\(^11\).

Experimental research results by Pratiwi et al. certified that making kombucha tea has the highest alcohol content in fermentation day 12 and decreased on day 16, the content of vitamin C decreased at day 4 and rose back up to the 16th day. Total acid continued to increase until the 16th day, the pH value and total sugars decreased on day 16. Based on the above background, research must be done to obtain evidence and information on the lipid profile of white rats after administration kombucha tea as drinking water by the time variation of fermentation\(^12\).

2. Material and Method

2.1. Preparation of kombucha tea

Materials treatment kombucha tea was fermented for 6, 9 and 12 days at a temperature of 25\(^\circ\)C.

2.2. Laboratory Animals

Research conducted at the Laboratory of Biological Structure and Function of Animals Department of Biology, Faculty of Science and Mathematics University of Diponegoro, in May and July 2015. The tools used are 20 individual cages equipped with drinking and feeding, measuring cups, digital scales. Using two months age of Rattus norvegicus L. rats as many as 20 males. They were acclimatized for seven days in the laboratory condition before early treatment. The rats were handled in a good-controlled room, with the average temperature at 27\(^\circ\)C. Feed and water provided ad libitum.

2.3. Experimental Design and Treatment of Animals Procedures

This study uses a completely randomized design with 4 treatments and 5 replicates ie: P0 = control, without the addition of tea kombucha, P1 = water + 1.8 ml in the morning and afternoon tea kombucha fermentation 6 days, P2 = water + 1.8 ml morning and afternoon tea kombucha fermentation 9 days, P3 = water + 1.8 ml in the morning and afternoon tea kombucha fermentation 12 days. Tea kombucha was administered orally by using gavage, for 60 days.

2.4. The variables measured were Cholesterol, HDL and LDL.

The day after the last treatment, blood was taken from the orbital vein by hematocrit tube, and it was centrifuged so that the Serum is obtained. Cholesterol, HDL and LDL were counted by Roche/Hitachi cobas c systems automatically calculate.
2.5. Statistical analysis
Data were analyzed by ANOVA, based on completely randomized design (CRD) at the level of 95% ($\alpha = 0.05$). Overall analysis using SPSS 10.0 software.

3. Results
The result of analysis of the average cholesterol, HDL and LDL on Rats (*Rattus norvegicus* L.) male after administration of kombucha tea is fermented for 6, 9 and 12 days are presented in Table 1.

**Table 1.** The average levels of cholesterol, HDL and LDL in Rats (*Rattus norvegicus* L.) male after administration of the fermented kombucha tea for 6, 9 and 12 days

|          | Cholesterol (mg/dL) | HDL (mg/dL) | LDL (mg/dL) |
|----------|---------------------|-------------|-------------|
| P0 (n=5) | 132.16±2.23         | 82.16±1.04  | 133.86±2.66 |
| P1 (n=5) | 94.4±0.95           | 95.36±2.92  | 114.63±1.41 |
| P2 (n=5) | 88.61±3.00          | 97.26±0.75  | 113.11±0.28 |
| P3 (n=5) | 86.43±2.97          | 99.73±0.60  | 106.41±3.23 |

Note: Values are expressed as Means ± SD. Means in the same column with different superscript letters are significantly different ($P>0.05$). Numbers with different superscripts in the same row show the real difference among the treatments. P0 = control, without the addition of tea kombucha, P1 = water + 1.8 ml in the morning and afternoon tea kombucha fermentation 6 days, P2 = water + 1.8 ml in the morning and afternoon tea kombucha fermentation 9 days, P3 = 1.8 ml drinking water morning and afternoon tea kombucha fermentation 12 days.

4. Discussion
Results of the analysis of the provision of kombucha tea were significantly different results ($P <0.05$). The longer fermentation kombucha tea is provided, decreasing cholesterol levels in rats (*Rattus norvegicus* L.) compared with controls, as shown in Table 1 and Figure 1.

**Figure 1.** Histogram average cholesterol levels (mg/dL) in each treatment
Description: P0 = control, without the addition of kombucha tea, P1 = water +
Kombucha tea contain metabolites include Niacin or vitamin (vitamin B3) which is also called nicotinic acid, works in the body as a coenzyme in the form of Nicotinamide adenine dinucleotide (NAD) and Nicotinamide adenine dinucleotide phosphate (NADP). This coenzyme is the acceptor of a hydrogen bond with hydrogen atoms in the form of coenzyme dehydrogenase which is a catalyst in oxidation-reduction reactions. NAD + functions as a coenzyme in the oxidation reaction of alcohol in the following reaction: \( \text{CH}_3\text{CH}_2\text{OH} \leftrightarrow \text{CH}_3\text{CHO} + \text{NAD}^+ + \text{NADH} + \text{H}^+ \). This reaction will generate \( \text{H}^+ \) ions, and then it will run well on a low pH (acidic)\(^{14}\). Nicotinamide is a component of almost all substances electron carrier in living cells (NAD + / NADH; NADP + / NADPH) and function in many metabolic pathways, especially in: (1) anaerobic glycolysis, (2) oxidative phosphorylation Kreb Cycle and (3) synthesis β-oxidation of fatty acids\(^{15}\). Decreased levels of cholesterol by niacin can be done by inhibiting changes in fat tissue, reduce the collection of free fatty acids by the liver and increase spending cholesterol by the liver through the bile. Niacin plays a role in stimulating the formation of the hormone prostaglandin I2 the hormone that prevents aggregation of platelets so as to minimize the risk of atherosclerosis\(^3\).

The results showed that the relationship with total cholesterol LDL is directly proportional. This happens because the 45% is in the form of LDL cholesterol\(^8\). That is if your total cholesterol LDL down then also fell. This occurs because of the inhibition or disruption of the process of absorption of cholesterol in the small intestine and increasing bile acid excretion in the feces. Bile acids are end products of metabolism of cholesterol. With the high excretion of bile acids, the more cholesterol is converted into bile acids to emulsification of fat, so that the serum total and LDL cholesterol decreased. Mechanism of increased levels of HDL because of the influence of tea kombucha likely caused by the binding of bile acids by metabolites (contained in tea kombucha) in the small intestine that causes increased excretion of bile acid fecal, resulting in decreased absorption of fat and cholesterol, it causes cholesterol in the liver low so cholesterol to produce bile acids less. This condition stimulates the synthesis of HDL in the liver to meet the shortage of cholesterol. As a result, lower serum LDL than HDL serum. Along with a decrease in serum cholesterol levels in the blood of the results of the analysis of HDL levels in treatment giving kombucha tea is fermented for 6, 9 and 12 days showed significant differences to the control. However, in treatment provision kombucha fermented tea 6 and 9 days and 9 and 12 days showed no real difference, then the treatment giving kombucha fermented tea 6 and 12 showed significant differences as shown in Table 1 and Figure 2.
Figure 2. Histogram average HDL (mg / dL) in each treatment

Description: P0 = control, without the addition of kombucha tea, P1 = water + 1.8 ml morning and afternoon tea kombucha fermentation 6 days, P2 = water + 1.8 ml morning and afternoon tea kombucha fermentation 9 days, P3 = Water 1.8 ml morning and afternoon tea kombucha fermentation 12 days.

Results of the analysis of the levels of LDL in the treatment giving kombucha tea is fermented for 6, 9 and 12 days showed significant differences to the control. However, in treatment provision kombucha fermented tea 6 and 9 days showed no real difference, then the treatment giving kombucha tea fermented 6 and 12 days and 9 and 12 showed significant differences as shown in Table 1 and Figure 3.
Figure 3. Histogram average LDL levels (mg / dL) in each treatment
Description: P0 = control, without the addition of kombucha tea, P1 = water + 1.8 ml morning and afternoon tea kombucha fermentation 6 days, P2 = water + 1.8 ml morning and afternoon tea kombucha fermentation 9 days, P3 = water 1.8 ml morning and afternoon tea kombucha fermentation 12 days.

Diseases of the arteries can occur with elevated levels of LDL and VLDL cholesterol in the blood (hypercholesterolemia). The increase in cholesterol levels can occur when there is an interruption formation of cholesterol in the liver or intestine\(^{16}\). High LDL cholesterol levels will trigger the accumulation of cholesterol in the blood vessel cells, which led to the emergence of atherosclerosis and plaque formation in the blood vessel walls\(^{17}\). Atherosclerosis is associated with increased LDL. Effect of elevated levels of LDL will be followed by the accumulation of cholesterol esters in macrophages which then referred to as macrophage foam cells. High LDL levels lead to high levels of LDL intimal.

Furthermore, intimal will oxidize LDL and attracts monocytes from the blood circulation as well as phenotypic change into macrophages. Increased oxidized LDL in the arterial wall is accompanied by the formation of foam cells, will develop into a plate of fat\(^{11}\). In conclusion, kombucha tea is fermented for 6, 9 and 12 days could potentially be used as a supplement for the prevention of risk to vascular disease and coronary heart disease.

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