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The Effect of Gembili Yogurt (*Dioscorea esculenta L.*) Intake on Lipid Profile of Hypercholesterolemic Rats

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ABSTRACT

Hypercholesterolemia is a condition in which the concentration of cholesterol in the blood exceeds the normal value. Yogurt is food product that has been shown to lower blood. The addition of inulin can support the cholesterol-lowering effect of yogurt. Gembili (Dioscorea esculenta L.) is a high inulin food ingredient. This study aims to determine the effect of giving gembili yogurt (D. esculenta L.) on the lipid profile (total cholesterol, LDL, and triglyceride) of hypercholesterolemic rats. The study was conducted on 30 male hypercholesterolemic Wistar rats which were grouped using a simple randomized system into 1 control group and 4 intervention groups. Subjects were given 1, 2, 3, and 4 ml/day of gembili yogurt for 14 days. Total cholesterol, and LDL were determined using the CHOD-PAP method and triglyceride level was determined using the GPO-PAP method. Giving gembili yogurt with doses of 1, 2, 3, and 4 ml/day within 2 weeks could significantly reduce lipid profile on hypercholesterolemic rats, the largest decrease was occurred at group with a dose of 4 ml yoghurt/day with total cholesterol levels of 100.68 mg/dl, LDL levels 28.87, and triglyceride levels 85.80 mg/dl.

Keywords: *gembili yogurt; inuin; lipid profile; hypercholesterolemia*

INTRODUCTION

Based on 2018 data, it showed an increase in the total cholesterol of the Indonesian population by 43%, an increase in triglycerides by 26% and increase in LDL by 83% (Anonim, 2019). One alternative that is safe to reduce total blood cholesterol levels is to modify the diet by consuming foods that are hypocholesterolemic. One of the products that have hypercholesterolemic properties is yogurt. Yogurt is a product obtained from pasteurized milk, then fermented with bacteria from Lactic Acid Bacteria (LAB) as a starter.

Consumption of yogurt from increases by years this can be seen from the value of increase in consumption of yogurt, in 2002-2005 the volume of yogurt increased by 70% from 1,039,279 L to 1,765,831 L (Central Bureau of Statistics, 2011). With increased consumption of yoghurt, it is necessary to improve the quality of yogurt. One of the improvemented in the quality of yofurt is the manufactured of synbiotic yogurt. Synbiotic yogurt is a combination of probiotics and prebiotics. There is a synergistic effect between probiotics and prebiotics that significantly lower cholesterol, so it can be an alternative food for hypocholesterolemic foods for people with hypercholesterolemia (Hendrati, 2014). There are many types of prebiotics, one of them is inulin. In Indonesia, there are many plants that are a source of inulin, namely from tubers. Gembili tuber (*Dioscorea esculenta L.*) is one of the plants that grows in Indonesia and contains high inulin. Istianah (2010) stated that inulin levels in gembili were 14.17%.

Hypercholesterolemia is a condition in which the concentration of cholesterol in the blood exceeds the normal value. Yogurt is food product that has been shown to lower blood. The addition of inulin can support the cholesterol-lowering effect of yogurt (Rachman, 2015). Researched by Hai-Qing Ye (2016), consumption of oat-based frozen yogurt containin fermented oats, probiotics, and inulin can reduce total cholesterol and tryglycerides in rats.

Research on giving gembili yogurt to lipid profiles has never been done. Based on this description, this study was conducted to determine how the effect of giving gembili yogurt on the reduction of lipid profile in hypercholesterolemic Wistar rats.

METHODS

Material

The materials used in this study for the manufacture of gembili yogurt were gembili tubers purchased at the Soponoyo Market, fresh cow's milk purchased at milk traders on Jl. Raya Jemursari Surabaya, and *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Bifidobacterium bifidum* were obtained at the Biology Laboratory, Faculty of Science and Technology, Airlangga University, Surabaya. Additional ingredients used were sucrose and skim milk. For testing with experimental rats, 30 Wistar rats were used and a high-cholesterol diet in the form of pure cholesterol.

Tool

The tools used in this research were scales, blender, stove, autoclave, incubator, inoculation container, colony counter, test tube, other glassware, sonde, centrifuge, cholesterol reagent kit, LDL reagent kit, triglyceride reagent kit and others.

Methods

Make gembili yogurt begins with making gembil tuber filtrate. Gembili is peeled using a knife and made sure there is no more skin attached to the tuber. The peeled bulbs are washed with clean running water until the dirt is gone. Cleaned gembili is cut into small pieces using a knife to facilitate the next process. The bulbs are mashed using a blender with the addition of 1:3 water until smooth. The smooth gembili is filtered using a filter cloth. The gembili tuber filtrate obtained was precipitated for 1 hour at room temperature and filtered. After 1 hour, the gembili tuber filtrate was pasteurized at 70°C for 15 minutes and cooled. For the preparation of yoghurt, fresh cow's milk was pasteurized at 70°C for 15 minutes and added with 5% (b/v) skim milk and 8% (b/v) sucrose. The heated cow's milk was mixed with gembili tuber filtrate in the ratio of 50:50 and then cooled. The cooled cow's milk was inoculated with 5% (v/v) Lactobacillus bulgaricus, Streptococcus thermophilus, and Bifidobacterium bifidum starters and incubated at 27°C for 18 hours.

Tests with lab rats were conducted with 30 Wistar rats were kept in individual cage for adaptation period. Rats were fed a diet high in cholesterol in the form of pure cholesterol for 1 week. Rats that have been given for 1 week (week 0) checked for total cholesterol, low density lipoprotein, and trygliceride levels. The 30 rats divided into 5 groups were given a different diet consisting of 24% casein, 0.3% DL-Methionie, 61% cornstarch, 1% vitamin mix, 3.5% mineral mix, 0.2% choline chloride, alpha cell 5%, and corn oil 5% with 1 control group, and 4 treatments with doses of yogurt gembili 1 ml, 2 ml, 3 ml, and 4 ml. Mice fed with gembili yogurt and feed were checked for cholesterol levels at weeks 1 and 2 (total cholesterol, low density lipoprotein, and trygliceride).

In this study, a nested completely randomized design (CRD) was used with groups: feeding time (0,1, 2 weeks) and yogurt treatment factor at 4 levels (0,1, 2, 3, 4 ml) with 6 replications.The data obtained were tested by ANOVA.

The method for measuring total cholesterol and LDL levels was determined enzymatically colorimetrically using the CHOD-PAP (Cholesterol Oxidase – Para Aminophenazone)

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method, while serum triglyceride or TG levels were determined enzymatically colorimetrically using the GPO-PAP (Glycerol Phosphate Oxidase ± Para Aminophenazone) method.

RESULT AND DISCUSSIONS

Total cholesterol

Blood sampling was carried out in all treatment groups after the acclimatization stage, the high-cholesterol feeding stage, and the intervention stage. Relationship between intake yogurt and total cholesterol levels in hypercholesterolemic rats can be seen on Figure 1.

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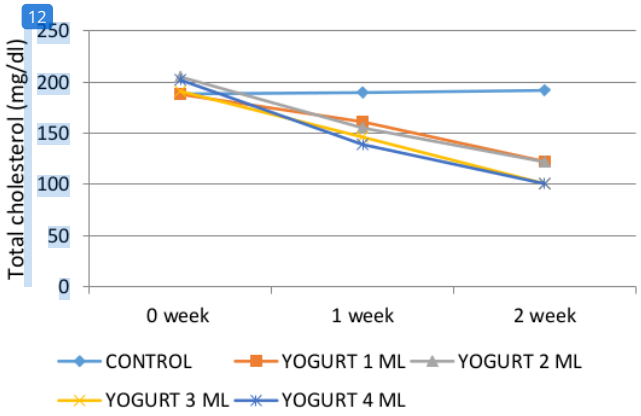

Figure 1. The Relationship Between Intake Yogurt And Total Cholesterol Levels in Hypercholesterolemic Rats

Figure 1. shows that in the control there was an increase in total blood cholesterol, while with 1, 2, 3, and 4 ml yogurt there was decrease in total blood cholesterol. Yogurt is a probiotic which in its fermentation use lactic acid bacteria. The consumption of probiotics which contain lactic acid bacteria become a natural way for lowering cholesterol levels (Baroutkoub et al, 2010). The decrease in total cholesterol levels was influenced by the presence of inulin, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* found in gembili yoghurt. Inulin is known to increase the viscosity in the gastrointestinal tract and increase the thickness of the lining of the small intestine, thereby preventing the absorption of cholesterol and increasing its excretion through feces. This causes cholesterol catabolism and has a hypocholesterolemic effect (Lay, 2010).

In addition, yogurt can bind cholesterol. Inulin is fermented in the colon by LAB to produce short chain fatty acids such as butyric acid and propionic acid. These short chain fatty acids can affect cholesterol metabolism in the liver. Propionic acid was identified to reduce cholesterol levels by inhibiting the action of the enzyme hydroxy-β methyl glutamyl CoA (HMG-CoA) reductase which plays a role in cholesterol synthesis. In addition, propionic acid can also inhibit the incorporation of acetate into plasma cholesterol by way of propionic acid competing with acetic acid transporters to the hepatocyte cells. This will result in a decrease in cholesterol synthesis because acetate is a precursor in the formation of cholesterol (Beylot, 2005).

The mechanism of cholesterol reduction by LAB includes the mechanism of cholesterol assimilation and cholesterol transformation into coprostanol. Cholesterol assimilation occurs

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through the mechanism of cholesterol uptake by lactic acid bacteria which then the cholesterol will be incorporated with the bacterial cell membrane, causing cholesterol to not be absorbed. Lactic acid bacteria can also convert cholesterol into coprostanol compounds, this is due to the cholesterol redutase enzyme produced by LAB. Coprostanol is not absorbed by the small intestine, but is directly excreted with feces (Baroutkoub et al, 2010).

LDL (Low Density Lipoprotein)

Intake yogurt gembili on hypercholesterolemic rats has a significant effect on LDL level. Relationship between intake yogurt and LDL level in hypercholesterolemic rats can be seen on Figure 2.

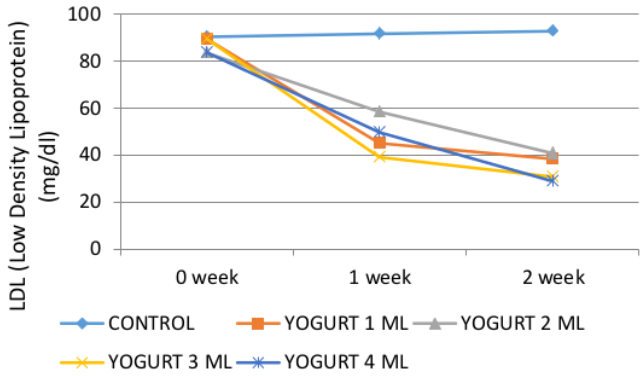

Figure 2. The Relationship Between Intake Yogurt and LDL Level in Hypercholesterolemic Rats

Figure 2. shows an increase in LDL in the control, whereas with 1, 2, 3, and 4 ml yoghurt there was a decrease in LDL. Inulin as a prebiotic and lactic acid bacteria *Streptococcus thermophiles and Lactobacillus bulgaricus* found in synbiotic yogurt affect the reduction of LDL cholesterol level (Zainudin et al, 2008). The mechanism of LDL cholesterol lowering by is the hypotriglyceride effect of inulin through inhibition of lipogenic enzyme activity in the liver resulting in reduced triglyceride synthesis. Decreased triglyceride production leads to decreased VLDL secretion. Decreased synthesis and secretion of VLDL in the liver has an impact on decreasing serum LDL cholesterol levels, because triglycerides are broken down, VLDL will form into IDL (intermediate density lipoprotein) / residual VLDL. IDL / residual VLDL can be absorbed by the liver directly through LDL receptors or can be converted into LDL (Karlina, 2013).

Yogurt is a probiotic that uses lactic acid bacteria in its fermentation. Consumption of probiotics containing lactic acid bacteria will be a natural method to reduce blood cholesterol levels (Karlina, 2013). The mechanism of probiotics in reducing LDL is that inulin undergoes a fermentation process by probiotics to produce short chain fatty acids in the form of propionic acid. Propionic acid reduces cholesterol synthesis in the liver by inhibiting the action of the HMGCoA reductase enzyme. HMGCoA reductase enzyme plays a role in the synthesis of cholesterol in the liver. Decrease of cholesterol production inhibit the synthesis and secretion of VLDL. With synthesis and secretion of VLDL can reduce LDL levels because LDL is a product of VLDL metabolism (Akoma et al, 2000).

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Triglyceride

Intake yogurt gembili on hypercholesterolemic rats has a significant effect on trigliceride level. Relationship between intake yogurt and trigliceride level in hypercholesterolemic rats can be seen on Figure 3.

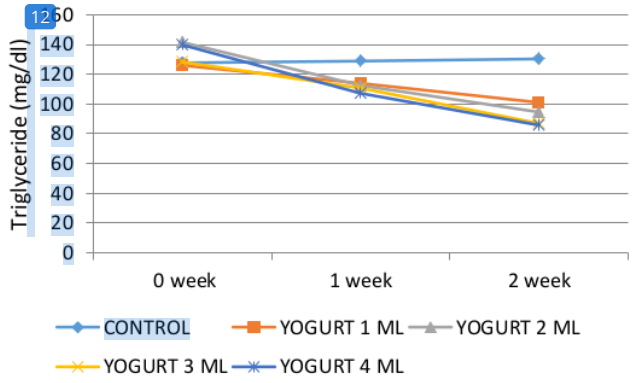

Figure 3. The Relationship Between Intake Yogurt and Triglyceride Level in Hypercholesterolemic Rats.

Figure 3. shows that in the control there was an increase in triglycerides, while with the administration of 1, 2, 3, and 4 ml yogurt there was a decrease in triglycerides. The decrease in triglyceride occurs due to the presence of inulin and lactic acid bacteria found in gembili yogurt. Another study conducted on rats stated that the influence of inulin can inhibit lipogenic enzymes that synthesize fatty acids in the liver so can reducing trygliceride levels (Kaur, 2012). The mechanism of reducing triglycerides by inulin as a prebiotic is by inhibiting the activity of lipogenic enzymes in synthesizing triglycerides in the liver. Lipogenic enzymes consist of acetyl coenzyme A (coA), malic enzyme, ATP citrate lyase, and fatty acid synthase. In fatty acid synthase, inulin inhibits the expression of mRNA genes in regulating the activity of the fatty acid synthase enzyme so that it can inhibit the formation of triglycerides in the liver (Nasar et al, 2013).

Previous research states that the administration of 4 ml of synbiotic yogurt for 2 weeks in rats can significantly reduce triglycerides by 70.10 mg/dl (Regie, 2014). The mechanism of reducing triglyceride levels by probiotics is that lactic acid bacteria (LAB) ferment inulin into short-chain fatty acids such as butyric acid and propionate. Furthermore, propionate competes with acetic acid transporters to hepatocyte cells. Propionate has a role in inhibiting the process of lipogenesis in the liver while acetate acts as a substrate for lipogenesis. By inhibiting the process of lipogenesis, the triglyceride level can decrease (Letexier D, 2002). In addition to propionate, probiotics are also able to modify the gene expression of peroxisome proliferator receptor (PPAR) in regulating triglyceride balance in adipose tissue. ppar is one of the receptors of nuclear receptor and is also a target gene of hemeostatic energy and adipogenesis (Zhang and Zhang, 2013).

Two studies that fed either oligofructose (20 g/d) or inulin (14 g/d) observed no effect on fasting total, LDL or HDL cholesterol, or serum triglycerides. Two other studies that fed inulin either in a breakfast cereal (9 g/d) or as a powdered addition to beverages and meals (10 g/d) reported similar reductions in fasting triglycerides (−27 and −19%, respectively). In one of these studies, total and LDL cholesterol concentrations were also modestly reduced (5

and 7%, respectively). Because animal studies have identified inhibition of hepatic fatty acid synthesis as the major site of action for the triglyceride-lowering effects of inulin, and because this pathway is relatively inactive in humans unless a high carbohydrate diet is fed, future attempts to demonstrate lipid-lowering effects of inulin should consider the nature of the background diet as a determinant of response (Christine, 2009).

CONCLUSION

Based on the results of research on the effect of intake yogurt gembili with doses of 1 ml, 2 ml, 3 ml, and 4 ml/day for 2 weeks on lipid profiles in hypercholesterolemic rats, it can be concluded that there are a significant interaction with total cholesterol, LDL, and triglyceride levels on intake yogurt with different doses in hypercholesterolemic rats. The largest reduction occurred at group with a dose of 4 ml yogurt/day was the best treatment with total cholesterol levels of 100.68 mg/dl, LDL levels 28.87, and triglyceride levels 85.80 mg/dl. It is necessary to conduct clinical trials of gembili yogurt consumption on humans to see if it has the same effect as the rat study.

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