

Potential of *Lactobacillus casei* Shirota Strain Probiotic Toward Total Cholesterol Levels and Sod Activity in Rat with High Cholesterol Diet

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ABSTRACT

Probiotic of *Lactobacillus casei* Shirota strain is lactic acid bacteria that have benefits to enhance immunity system, as an antioxidant and has the ability to decrease the cholesterol level. The aim of this study was to determine the potential of *Lactobacillus casei* Shirota strain probiotic toward total cholesterol decreasing and SOD activity increasing in rats' aorta with a high-cholesterol diet. This study used 25 male rats of *Rattus norvegicus* Wistar strain that will be divided into 5 groups. The 1st group as the negative control, and the 2nd group as positive control. The positive control group was induced by high-cholesterol diet contained quail egg yolk, pure cholesterol, cholic acid and waste oil that were given for 3 weeks. The 3rd, 4th and 5th group were treated by probiotic *Lactobacillus casei* Shirota strain with variation dose of 3.6×10^7 , 7.3×10^7 and 10.9×10^7 cells/kg bodyweight/day. The results showed that probiotic of *Lactobacillus casei* Shirota strain therapy dose of 10.9×10^7 cells/kg bodyweight/day can decrease the total blood cholesterol of rats' serum that were given with high-cholesterol diet of about 32.0%. In addition, the probiotic of *Lactobacillus casei* Shirota strain therapy also increase the activity of SOD about 50.70%.

Keywords: *Lactobacillus casei* Shirota strain, total cholesterol, superoxide dismutase

INTRODUCTION

Cardiovascular disease is caused by a disturbance in the heart and blood vessel function that are dangerous to health, the examples of cardiovascular diseases are coronary heart disease and stroke (Rastini, 2010). The cause of these disease is atherosclerosis. The main factors causing atherosclerosis is hypercholesterolemia or high cholesterol levels.

Cholesterol levels in the body can be determined by examining the total cholesterol level in the blood. The total cholesterol levels include of LDL, HDL and triglycerides. Normal total cholesterol levels in humans is <200 mg/dL. High levels of total cholesterol in the blood can be caused by high cholesterol diet. High cholesterol diet that performed continuously will cause an accumulation of LDL cholesterol in the blood vessels and oxidation of LDL cholesterol (Towil & Pramono, 2014). In addition, it will produce of free radicals or ROS (Reactive Oxygen Species) in the body.

ROS can be assailed by antioxidant of SOD (Superoxide Dismutase). The activity of SOD can be inhibited by ROS. The high of ROS in the body due to the induction of high-cholesterol diet can cause a decrease in SOD

antioxidant activity (Bayatmakoo, Rashtci-zadeh, Yaghmaei, Farhoudi, & Karimi, 2017). SOD activity test is used to determine the resistance of cells against ROS.

Probiotic of *Lactobacillus casei* Shirota strain a lactic acid bacteria that can improve the health of digestive system. Moreover, these bacteria also have the ability to boost the immune system as well as antioxidant of cholesterol reducer. Probiotic of *Lactobacillus casei* Shirota strain commonly used in fermented beverages made from milk. Sujono, Becti, Hikmawan & Yuananda (2016) goat milk yogurt that used *Lactobacillus bulgaricus* bacteria can be decreasing the levels of total cholesterol by 52,17 mg/dL. Yuniastuti (2004) Fermented milk containing *Lactobacillus casei* Shirota strain can reduce the total cholesterol level. The ability of each probiotic to decrease the cholesterol may varies, depending on the type of probiotic bacteria used.

The aim of this study is to determine the potential of *Lactobacillus casei* Shirota strain probiotic with therapeutic dose of 3.6×10^7 , 7.3×10^7 and 10.9×10^7 cells/kg bodyweight/day of male white rats (*Rattus norvegicus*) that induced by high-cholesterol diet toward total cholesterol decreasing in rats'

blood serum and SOD activity increasing in rats' aorta with a high-cholesterol diet.

EXPERIMENTAL SECTION

Material and Instrumentation

Chemicals used in this study is the probiotic of *Lactobacillus casei* Shirota strain that commercial, cholic acid (Kasei Tokyo), pure cholesterol (SIGMA), oil, egg yolk of quail, SOD kit (*Biovision*), BioSiytem S.A Costa Brava 30 kit, phosphate buffer saline (PBS) solution, tris HCl, PBS-tween, 0.9% sodium chloride, ethanol and distilled water.

The tools used in this study were centrifuges, analytical balance (Mettler Toledo), incubators, sepectrophotometer UV-Vis (Thermoscientific Genesys 20), bath water, gavage needle, microhematocrit or capillary tubes, Eppendorf tubes, microtube, surgical equipment, vacutainer, mortar, freezer, refrigerator, 96 well microplate, 3 mL syringe (Terumo), cuvette, mikropipet 100 μ L and 1000 μ L (Nichipet EX), microplate reader and *Easy Touch GCU* to check the blood cholesterol level experimental animals.

Procedure

Sample Preparation

Probiotic of *Lactobacillus casei* Shirota strain sample stored in the refrigerator (storage conditions temperature 0 – 10 °C). Probiotic bacteria used were *Lactobacillus casei* Shirota strain. The number of probiotic bacteria is calculated by calculating the total lactic acid bacteria (total LAB).

Preparation of Experimental Animals

Rats used were 25 white male rats (*Rattus norvegicus*) weighing 175 -250 grams and age 2 – 3 months. Rats were divided into 5 groups: negative control (healthy), positive control (sick) that were given high-cholesterol diet for 3 weeks, and the three treatment groups were given high-cholesterol diet and treated with the probiotic of *Lactobacillus casei* Shirota strain orally with 3 dose (3.6×10^7 , 7.3×10^7 and 10.9×10^7 cells/kg bodyweight/day) variations therapy. Probiotic of *Lactobacillus casei* Shirota strain therapy carried out for 2 weeks.

The use of experimental animals which have been approved by the ethical acceptance of UB's Research Commission No. KEP-554-UB. High-cholesterol diet that were given consists of 1 g pure cholesterol, 0.02 g cholic acid, 1 g quail egg yolk, and 0.24 mL oil which

has been mixed with pure water to 3 mL. All blood's rats be taken from aortic for blood serum analysis of total cholesterol and abdominal aortic tissue for activity of SOD analysis.

Measurement total cholesterol levels of blood serum

Total cholesterol levels were determined using BioSiytem S.A Costa Brava 30 kit, with CHOD-PAP method. Each rats' blood was taken 5 mL then it was centrifuged at 3000 rpm for 10 min. Afterward, the serum were collected at microtube and it will be added cholesterol kit as much as 1 mL. Sample absorbance was measured using a spectrophotometer at a wavelength of 500 nm.

Measurement of superoxide dismutase (SOD) activity in aortic tissue

Measurement were started with SOD protein isolation from aortic tissue. Then, SOD activity is determined using SOD *Biovision* kit. Each sample of solutions were added about 20 μ L and 2nd blank, and 20 μ L of H₂O were added in 1st blank and 3rd blank. Then they were added by 200 μ L of WST (Water Soluble Tetrazolium) working solution in each sample. After that, dilution buffer was added about 20 μ L in each 2nd blank and 3rd blank. In the other hand, in each sample and 1st blank were added by 20 μ L of enzyme working solution and all of the mixture were being homogenate. Each mixture was incubated at 37°C for 20 minutes. Samples will be measured using a microplate reader with a wavelength of 450 nm.

RESULTS AND DISCUSSION

Total LAB And Total Cholesterol Levels of Blood Serum

The quantity of bacteria in probiotic *Lactobacillus strain* Shirota strain samples determined using Total LAB method which is 7.3×10^7 CFU/mL. These results are in accordance with the standards of SNI (2009) that the amount of LAB in fermented milk is at least equal to 10^7 .

The effective dose of probiotics as hypocholesterolemia treatment are vary widely, depending on the strain used. The range of probiotic used in human $10^7 - 10^{11}$ CFU/day and animals $10^7 - 10^9$ CFU/day. Some probiotics have been efficacious in inducing at the lowest levels, while some others require larger quantities to give

hypokolesterolemia effect (Ooi, Gaik, & Liong, 2010).

The results showed that probiotic of *Lactobacillus casei* Shirota strain therapy can reduce the total cholesterol level. The results of statistical analysis were determined using One Way ANOVA, and exhibited that probiotic therapy with *Lactobacillus casei* Shirota strain with variation dose of 3.6×10^7 , 7.3×10^7 and 10.9×10^7 cells/kg bodyweight/day can decrease the total cholesterol levels by based on its extremely significant differences between each treatments ($p < 0.01$). The results of the total cholesterol level can be seen in **Figure 1**.

Positive control group had a higher total cholesterol levels than the other groups of 81,2 mg/dL and an increasing of 69.8% toward a negative control. Total cholesterol levels in positive control group showed that rats had hypercholesterolemia, because of the total cholesterol levels above the normal range in

rats'. Harini & Astirin (2009) the normal total cholesterol levels in *Rattus norvegicus* Wistar strain rats is 10 – 54 mg/dL. This result indicates that the induction of high-cholesterol diet with the feed that contain of of pure cholesterol, cholic acid, quail egg yolk and oil (*jelanta*) can increase total cholesterol levels of rats.

Increased levels of total cholesterol is influenced by the content of saturated fatty acids and cholesterol that very high in a quail egg yolk and used oil and it completed with the addition of pure cholesterol. The content of cholesterol in eggs quail is 3640 mg/100g (Dewi, 2009). The used oil (*jelantah*) have highest total cholesterol levels among coconut oil, *curah* oil (oil without label) and lard oil, which is 86.20 mg/dL (Bogoriani & Ketut, 2015). Feeding 1% cholesterol for 2 months can increase blood serum cholesterol level of hypercholesterolemia group of rats by 90% (Wresdiyati, Astawan & hastanti, 2006).

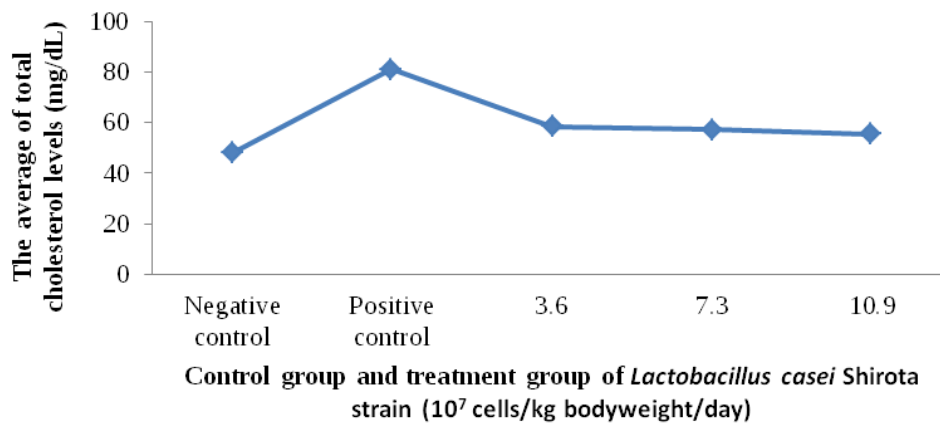


Figure 1. The result of total cholesterol levels after treatment by probiotic of *Lactobacillus casei* Shirota strain

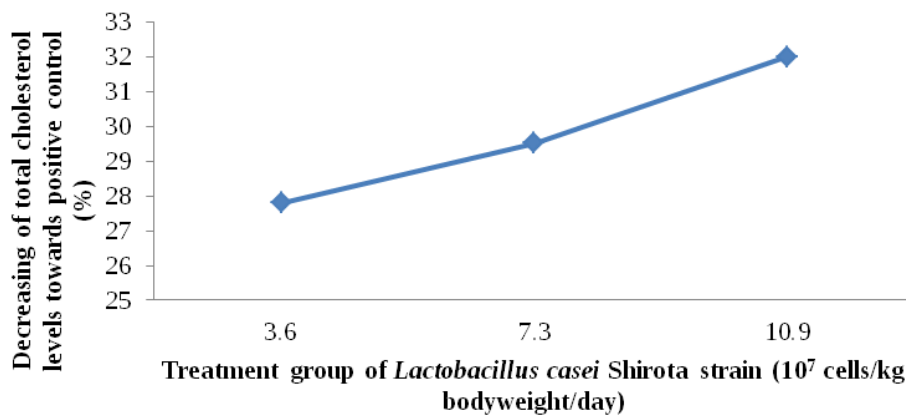


Figure 2. Decreasing of total cholesterol levels after treatment by probiotic of *Lactobacillus casei* Shirota strain

Therapy group that were treated with probiotic of *Lactobacillus casei* Shirota strain 10.9×10^7 cells/kg bodyweight/day had the best total cholesterol levels which is 55,2 mg/dL with a decreasing of 32.0%. Decreasing of total cholesterol levels can be seen in **Figure 2**. This results showed that the probiotic of *Lactobacillus casei* Shirota strain can decrease the total cholesterol levels. This is in accordance with Yuniastuti (2004) that the provision of fermented milk of *Lactobacillus casei* Shirota strain capable to lowering total cholesterol, triglyceride levels and LDL cholesterol levels significantly. Kumar, Grover, & Kumar (2010) showed that, supplementation of the diet with *L. plantarum* Lp91 can significant reduction in plasma total cholesterol 23.26%.

Probiotic bacteria can reduce the total cholesterol levels of blood serum in a variety of ways, include increasing the excretion of bile acids, with deconjugating the bile acids by BSH (Bile Salt Hydrolase) enzyme from probiotic mechanism (Yuliana, 2012). In the other hand, cholesterol assimilation is done by the probiotic directly. In the mechanism of cholesterol assimilation, lactic acid bacteria will take cholesterol from the intestine and incorporate with cellular membrane of bacteria, so the bacteria will more resistant to lysis (Yuniastuti, 2004). Cholesterol was bound to bacterial cells and this was a result of the chemical and structural properties of their cell wall peptidoglycans (Kimoto-Nira et al., 2007). The decreasing of cholesterol absorbed in the intestine bring in on the decreasing of blood cholesterol levels.

Lactobacillus acidophilus were able to assimilate cholesterol by 48,45 $\mu\text{g/mL}$ (Tamaro-duschesneau et al., 2014). In addition, another mechanism of cholesterol reduction by bacteria can be done by converting cholesterol into coprostanol. Lye, Rusul, & Liong (2010) the results showed that cholesterol levels were decreased in medium due to fermentation by probiotic bacteria followed by increased coprostanol. Some of these mechanisms may work together to lowering cholesterol levels.

Superoxide dismutase (SOD) activity in aortic tissue

The statistical analysis results with One Way ANOVA showed that probiotic therapy with *Lactobacillus casei* Shirota strain with dose variation of 3.6×10^7 , 7.3×10^7 and 10.9×10^7 cells/kg bodyweight/day can increase the activity of SOD by showed extremely significant differences among the treatments ($p < 0.01$). The results of SOD activity in rats' aorta can be seen in **Figure 3**.

In Group positive control, the rats experienced a decreasing activity of SOD by 38.16% compared to the negative control group. The low activity of SOD in the positive control group is due to the high-cholesterol diet which has an effect on the increasing of free radicals in the body exceed the body's capacity to avoid them. The high production of ROS in the body can caused a decreasing of SOD. Dianita, Jantan, Jalil, & Amran (2016) suggested that the hypercholesterolemic status has triggered oxidative stress as indicated by a significant decrease of serum SOD.

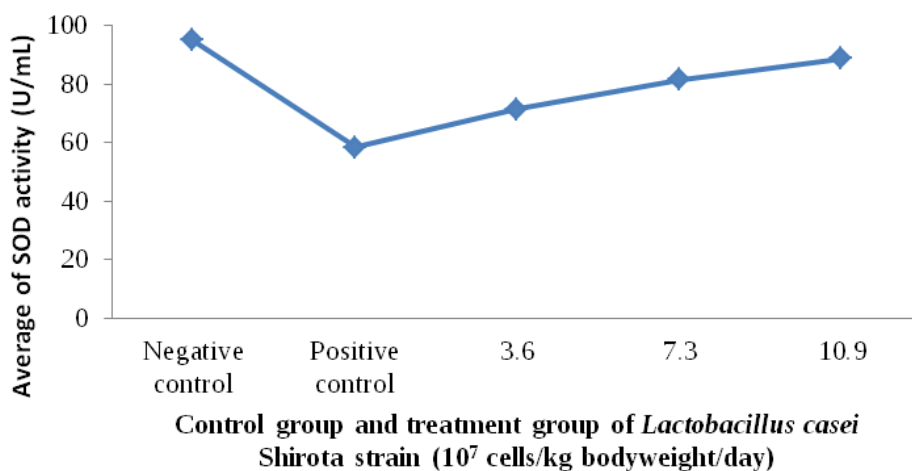


Figure 3. The result of SOD activity in rats' aorta with high cholesterol diet and after by probiotic of *Lactobacillus casei* Shirota strain

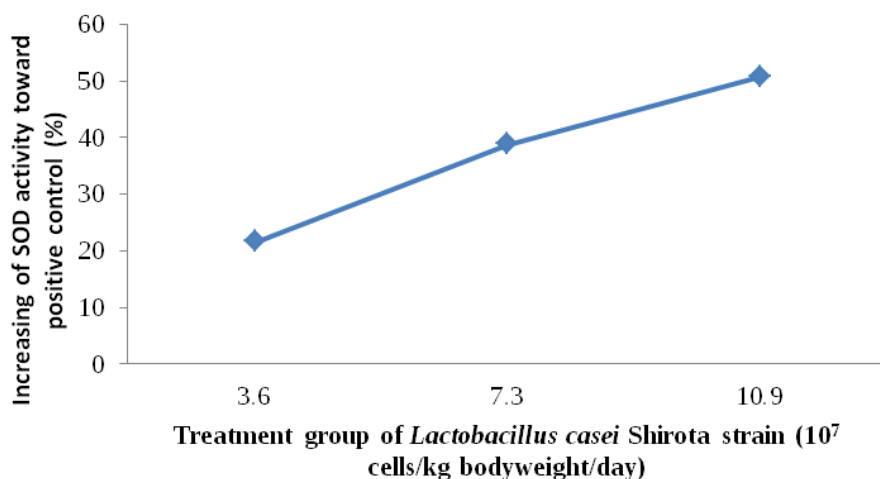


Figure 4. Increasing of SOD activity after treatment by probiotic of *Lactobacillus casei* Shirota strain

The highest increasing percentage of SOD activity is 50,70% in the treatment group with a dose of 10.9×10^7 cells/kg bodyweight/day. The result of decreasing activity of SOD can be seen in **Figure 4**. Increasing of SOD activity is due to the probiotic of *Lactobacillus casei* Shirota strain that containing bioactive peptides as an antioxidant. Sari, Kusrahayu, & Al-baarri (2014) One of the bioactive components that naturally present in milk is lactoferrin compound. The result of SDS-PAGE in skim milk showed the presence of lactoferrin compounds that is in molecular weight range of 80 kDa.

The mechanism of lactoferrin as an antioxidant protein is iron binding. Iron ion in certain circumstances can participate in the form Fenton reaction, which can generate ROS (hydroxyl radical) that are very reactive (Moradian, 2014).

The highest value of SOD activity is negative control and the lowest is positive control. The three treatment groups were slightly increased. The treatment group in 10.9×10^7 cells/kg bodyweight/day of dose therapy showed that the SOD activity had the highest value than the other treatment groups. In addition, the probiotic of *Lactobacillus casei* Shirota strain therapy can increase the activity of SOD.

CONCLUSION

Probiotic of *Lactobacillus casei* Shirota strain therapy has an ability to decrease the blood cholesterol levels and increase the SOD activity in aorta of rats that fed with a high-cholesterol diet.

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