Ability of *Lactobacillus plantarum* JR64 isolated from noni juice in lowering Cholesterol *in vivo*

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Abstract **- Recently public's attention to the importance of healthy food increases rapidly. Probiotic based food exploiting lactic acid bacteria is among the healthy food. Lactobacillus plantarum JR64 isolate from Morinda citrifolia fruit was assessed for its probiotic in-vivo by using Wistar Rat. The purpose of this research was to study the ability of probiotic Lactobacillus plantarum JR64 in lowering serum LDL (Low Density Lipoprotein) of Wistar Rat. Twenty Rats were grouped into 4, each group consisted of 5 Rats. First Group was a negative control given standard normal diet of 20 gr/day plus aquadest. Second Grup was a positive control given cholesterol normal diet 20 gr /day plus Propil Tio Urasil (PTU) 60 mg/kg body weight/day. Third Group was supplemented with normal diet 20 gr /day plus Propil Tio Urasil (PTU) 60 mg kg body weight /day and 10^{12} CFU Lactobacillus plantarum JR64. Fourth Group was the same as third Group unless the probiotic using commercial probiotic Lactobacillus bulgariccus at 10^{12} CFU. Blood samples were withdrawn for measurement of total cholesterol, triglyceride, High Density Lipoprotein (HDL), and Low Density Lipoprotein (LDL) cholesterol every week and measured by using spectrophotometer with 546 nanometers wavelength. The results show that probiotic Lactobacillus plantarum JR64 isolated from noni juice significantly ($p < 0,01$) reduce Low Density Lipoprotein (LDL) and Triglyceride in vivo and tend to reduce High Density Lipoprotein (HDL) and total cholesterol.**

[Key Words : ***Cholesterol, Low Density Lipoprotein, L. plantarum* JR64, *Noni fruit]***

I. INTRODUCTION

Recently, public attention to the importance of healthy foods increases along with the growing of knowledge society. In addition, the increasing of the community lifestyle contributes to people's demands for healthy foods. One type of healthy food products that growing rapidly is probiotic products.

Group of lactic acid bacteria (LAB) is one of the widely used probiotic cultures. Most of LAB is not pathogenic; some strains have acquired the status Gras (Generally Recognized as Safe) from the FDA. LAB able to grow in the path of intestine and can suppress the growth of pathogenic enteric bacteria. These characteristics are utilized to maintain a healthy body and considered LAB used as probiotics (Ray, 1996; Gilliland, 1989). However, not all probiotics are lactic acid bacteria. Probiotics are living microorganisms that have beneficial health effects by improving host intestinal micro flora balance.

LAB as probiotics needs requirements, namely permanent microorganisms of the human digestive track. grow and survive on the food before consumption, survive even though through the digestive tract, resistant to stomach acid, resistant to some antibiotics, resistant to lisosym, grown in the intestine and have attachment ability to the human intestinal cell ephitel, beneficial effects on the intestine, produce acid fast and in large quantity, capable to produce anti-microbial components other than acid (bacteriocin, diacetyl) that effectively inhibit other undesirable bacteria especially pathogen bacterial (Havenaar and Jos, 1992; Daeschel, 1989).

Control of diseases in humans and cattle by probiotic activity has been done for centuries. Probiotics are foods containing living microorganisms that actively promote health. This is achieved by correcting the balance of intestinal flora if taken alive and in adequate amounts. According to Fuller (1987), in common there are three working models of probiotics are: (1) reduce microbial populations through competition by producing antimicrobial compounds or through competition of nutrients and where sticking to the wall intestinal; (2) alter microbial metabolism by increasing or lower enzyme activity and (3) stimulate immunity by increasing antibody levels or activity of macrophages, while the effectiveness of probiotics is determined by its ability to provide healthful effect, nature is not pathogenic, non toxic and also because of its ability to survive and metabolic activity in the intestine. Lactic acid bacteria can also lower cholesterol by inhibiting the activity of enzyme 3-hydroxy-3-coenzyme A reductase metilglutaril (HMG CoA reductase), cholesterol degradation to de-conjugated compounds of co-prostanol and bile salts.

The objective of this study was to test the potential as probiotic agents that lowering cholesterol levels and lipoprotein profiles of *Lactobacillus plantarum* JR64 strain

by using mice as test animals. *Lactobacillus plantarum* JR64 strain was isolated from microbes in the beverage *badeg pace*. *Badeg pace* is a traditional drink that similar to noni juice (*Morinda citrifolia*). *Badeg pace* is fermented spontaneously and it has health properties. *Badeg pace* has become the beverage of Ponorogo from generation to generation that believed to provide health effects. One strain of lactic acid bacteria isolated from these beverages is *Lactobacillus plantarum* JR64. However, growth in media of noni fruit juice has not been able to achieve an effective concentration as probiotics.

Limited growth problem of *Lactobacillus plantarum* JR64 in origin, media, namely noni juice, so it can not achieve effective concentrations as probiotic agents. This problem can be overcome through the substitution of growth medium containing sufficient nutrients for growth. Media formulations have been conducted using corn milk was able to support the nutritional needs during the formation of biomass and metabolites are expected.

As the benefits of lactic acid bacteria in general as mentioned above and with the discovery of *Lactobacillus plantarum* JR64 isolates that are expected as a candidate probiotic, then need to be tested in-vitro and in-vivo. In-vivo testing will be conducted using test animals such as white mice. Rats are mammals that have a significant role in human life, whether beneficial or harmful. Advantageous properties especially in terms of its use as a laboratory animal, such as rats (Rattus norvegicus albino strain). Rats as omnivorous animals, can consume all types of food consumed by humans, both derived from animals and plants. Therefore, rats are assumed to have similar digestive tract and metabolic processes with humans.

II. MATERIALS AND METHODS

Twenty Rats were grouped into 4, each group consisted of 5 Rats. *First group* was a negative control given standard normal diet of 20 gr/day plus aquadest. *Second grup* was a positive control given cholesterol normal diet 20 gr /day plus Propil Tio Urasil (PTU) 60 mg/kg body weight/day. *Third group* was supplemented with normal diet 20 gr /day plus Propil Tio Urasil (PTU) 60 mg kg body weight /day and 10^{12} Cfu *Lactobacillus plantarum* JR64. *Fourth group* was the same as third group unless the probiotic using commercial probiotic *Lactobacillus bulgariccus at* 10^{12} Cfu (FNCC41, UGM),.

Blood samples were withdrawn for measurement of total cholesterol, triglyceride, High Density Lipoprotein (HDL), and Low Density Lipoprotein (LDL) cholesterol every week and measured by using spectrophotometer with 546 nanometers wavelength. Before blood samples witdrawn, rats must free from food and drink more less 16 hours. Blood samples take with hematokrit and fill in eppendorf tube contained EDTA and serum was obtained by centrifugation at 10.000 rpm for 5 min. Total cholesterol was measured using CHODP-PAP method (Cholesterol Oxydase Phenol Amino Phenazon) and tryglyserida with GPO method (Glycerol-3-phosphate-oxydase).

III. RESULTS AND DISCUSSION

Feed intake and changes in condition rats

This study used male white Wistar rats with body weights ranging from ± 200-250 gr. White rats fed for six weeks, including one-week adaptation period and 5 weeks of treatment. The observation on the development of rat's body weight during the change took place, it is estimated that a normal body weight of animals would increase in line with age. Increased body weight is influenced by dietary factors. Diets with high fat and cholesterol content causing an increase in body weight faster than diet with low fat content. Besides the existence of abnormalities in metabolism may also affect body weight.

Figure 1 showed that all groups decreased body weight after receiving treatment. This was caused by factors that are influenced by stress hormones cortisol and adrenaline. Adrenaline causes your fat burning while cortisol increased supply of glucose to burning and energy formation. Besides giving propyl tiourasil every day can increase body weight reduction. Propyl tiourasil can affect the adrenal cortex in changing the cholesterol into cortisol (Murray, *et. al.*, 2003).

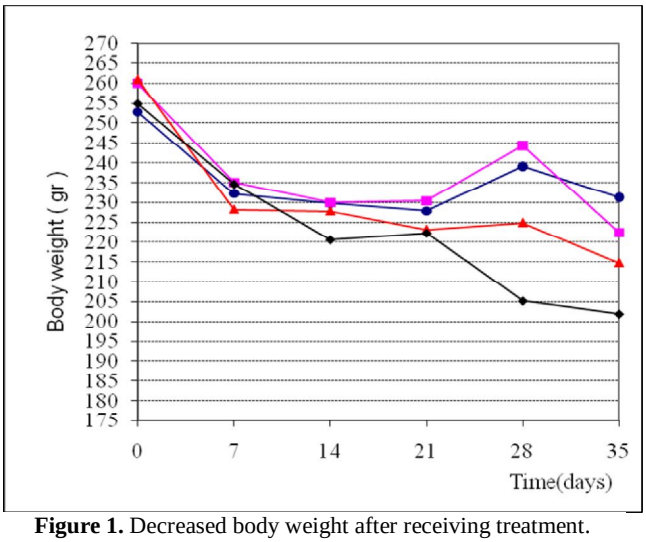

Figure 1. Decreased body weight after receiving treatment. Symbol : ● : Negative control, ■ **:** Positive control, ▲**:** *Lactobacillus plantarum* JR64 ♦**:** *Lactobacillus bulgaricus*

Average total amount of feed for rats treated were presented in Figure 2. Group A which consume more on average 18.5% of the weight of feed given each day as many as 20 gr. While for group B, C and D are respectively 11.9% , 11.3% and 13.4%. In group B, C and D obtain the feed has a relatively high fat content can lead to eating time interval becomes longer, so the amount of feed consumed during the period of 24 hours will be less. The high amount of feed consumed by the group A causes

an increase in body weight in these groups was higher than group B, C and D.

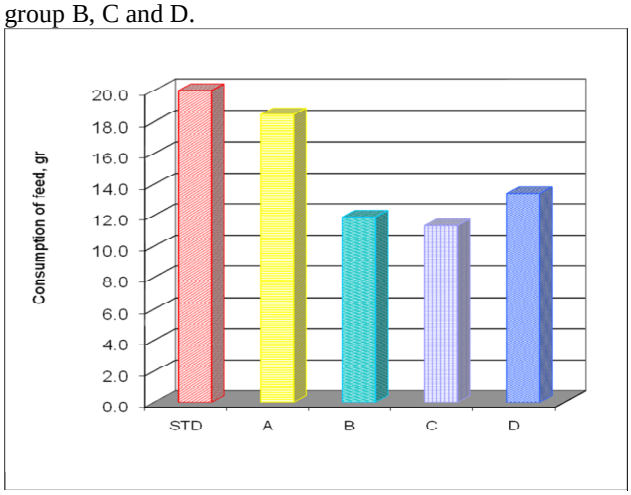

Figure 2. The average of consumption of feed by Wistar rats. Symbol : A : Control Negative, B : Control Positive, C: *Lactobacillus plantarum* JR64 D : *Lactobacillus bulgaricus*

Effect of probiotic agents to decrease LDL cholesterol

Figure 3. showed a relationship between the time of consumption of probiotics with the change in the level of LDL-Cholesterol (mg / dL). Lipid-containing foods undergo a process in such a way before is absorbed by the intestine. That process is the cholesterol ester hydrolysed to cholesterol in food is mixed with the esterified cholesterol and bile cholesterol before absorbed, then this compound will be incorporated into the chylomicron. Chylomicron will react with lipoprotein lipase to form chylomicron remaining, then the remaining chylomicron receptor reacts with Low Density Lipoprotein (LDL) cholesterol and hydrolyzed into cholesterol. Very Low Density Lipoprotein (VLDL are formed in the liver to carry cholesterol into the plasma and converted to Low Density Lipoprotein (LDL) cholesterol receptors will then be taken by the Low Density Lipoprotein (LDL) in the liver and ekstrahepatik network. Approximately 75-80% of Low Density Lipoprotein (LDL) Cholesterol will be converted to High Density Lipoprotein (HDL cholesterol) by the enzyme Lecithin Cholesterol Acyl transferase Kolesteril (HUW) to be transported to the liver and circulated again. (Murray, *et. al.*, 2003).

Increased cholesterol in rats with propyl tiourasil was one way to accelerate the increase in endogenous cholesterol by suppressing the formation of receptor, Low Density Lipoprotein (LDL) in the liver and can increase the activity of enzyme 3-hydroxy-3-metilglutaril coenzyme A (HMG Co A). Besides the increase of cholesterol can also occur due to absorption of fat in the diet (exogenous) and the occurrence of lipolysis in the body (endogenous) (Murray et. al., 2003).

The results showed that probiotic *Lactobacillus plantarum* JR64 could prevent the increase in total cholesterol significantly ($P < 0.05$). It seemed that cholesterol reduction has to do with high levels of fat in feces. This was according to research conducted Usman Pato (1999) which stated that lactic acid bacteria capable of lowering blood cholesterol by sticking in the intestinal wall, then multiply and perform the role of health benefit by producing the enzyme bile salt hidrolase (BSH), which resulted in increased acid conjugated bile that is not easily absorbed from the small intestine compared with bile acid conjugation. Conjugated bile acids by high hence decreasing intestinal absorption of fat and fatty deposits to be reduced because of fats used for synthesized into bile.

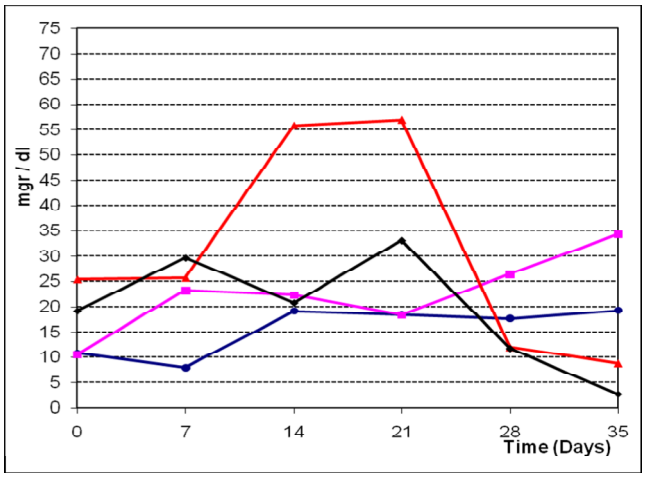

Figure 3. Effect of probiotic candidate to ability lowering Cholesterol (LDL). Symbol : ● : Negative control, ■ : Positive control, ▲: *Lactobacillus plantarum* JR64 ♦ : *Lactobacillus bulgaricus*

In addition *Lactobacillus plantarum* JR64 that is a possibility of lactic acid bacteria strains able to degrade cholesterol to co-prostanol namely a sterol that can not be absorbed by the intestines, then koprostanol and residual cholesterol excreted feces together. Another mechanism in lowering cholesterol levels is by the formation of propionic acid unsaturated fatty acid compound which inhibits the synthesis of cholesterol through the inhibition of enzyme activity of 3-hydroxy-3-metilglutaril coenzyme A (HMG Co A) thus formed no mevalonat (Harianto, 1996 and Napitipulu, 2003).

However, the effect of probiotic *Lactobacillus plantarum* JR64 to decrease triglyceride levels, levels of Low Density Lipoprotein (LDL) and increased levels of High Density Lipoprotein (HDL) cholesterol were not significant ($P > 0.05$). This occurs because of Lactobacillus plantarum are not able to prevent the endogenous synthesis of cholesterol in the body for five weeks, so that cholesterol carried by lipoproteins in the blood are not influenced by probiotics, but directly absorb exogenous cholesterol in the intestine and excress it through faces.

Effect of probiotic agents on the decrease in total cholesterol

Mayes (1997) suggested that the release of cholesterol from the body through several streets of liver cholesterol to form bile, released into the intestine and subsequent cholesterol along with fecal bile acids is lost, lost in the intestinal mucosa and skin, joined the steroid hormones and issued joint urine. The measurement results are presented in total cholesterol.

Figure 4 illustrated changes in total cholesterol level of each group during the treatment. At the end of adaptation (week 0), total cholesterol level for all groups under normal conditions (69-81 mg / dl). Normal cholesterol levels from a variety of research rats varies according to age, species and type of feed given. In the induction phase (weeks 1-4) of total cholesterol in the control group is positive, probiotic agents has increased except in the negative control groups. Rising levels of cholesterol caused by feeding high cholesterol and delivery of PTU (propyl Tiourasil).

From the results of tests, the probiotic group had significantly different values (P <0.01) with negative control group and has a value not significantly different (P> 0.05) with the positive control group. Therefore there was no difference with the positive control groups, probiotics did not have a significant effect on decreasing blood cholesterol levels.

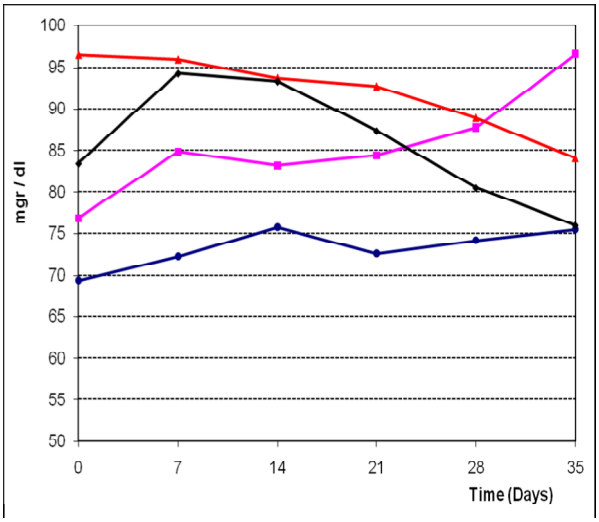

Figure 4. Effect of probiotic candidate to ability lowering Cholesterol Total. Symbol : ● : Negative control, ■ **:** Positive control, ▲**:** *Lactobacillus plantarum* JR64 ♦ **:** *Lactobacillus bulgaricus*

Effect of probiotic agents to decrease triglyceride levels

Figure 5 showed that the changes in triglyceride levels during treatment. Late adaptation (week 0) triglyceride level of all groups under normal conditions (58-86 mg / dl). Induction phase (week 1 to week 4) the average positive control group, triglyceride levels decreased from 74.38 mg / dl to 63.34 mg / dl. From the results of further tests showed that probiotic agents is not significant (P> 0.05) with negative control group but were highly significant (P <0.01) toward a positive control. This indicates that the agents can lower triglyceride levels probiotics at the same level or near-normal cholesterol levels.

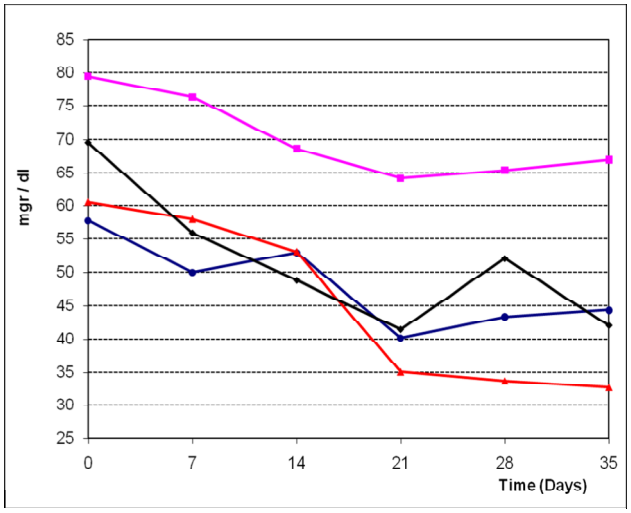

Figure 5. Effect of probiotic candidate to ability lowering Triglycerides. Symbol : ● : Negative control, ■ **:** Positive control, ▲**:** *Lactobacillus plantarum* JR64 ♦ **:** *Lactobacillus bulgaricus*

IV. CONCLUSION

Probiotic *Lactobacillus plantarum* JR64 isolated from noni juice significantly (p < 0,01) reduce Low Density Lipoprotein (LDL) and Triglyceride *in vivo* and tend to reduce High Density Lipoprotein (HDL) and total cholesterol.

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