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The Potential Goat Milk and Goat Milk Yogurt in Increase Sod Level on Malaria Infections

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Abstract - **Protein in goat milk is the main source of active biopeptides that can produce antioxidants. One of the processed products of fermented goat milk is goat milk yogurt. Yogurt contains bioactive peptides and has antioxidant activity. Yogurt contain lactic acid bacteria. Plasmodium infection will cause an imbalance of antioxidant and make SOD enzyme level decreasing. Goat milk dan yogurt goat milk have potential to increase SOD enzyme level. This research is true experimental study with a post-test only group design. Thirty five female Balb/c mice were divided into: K- (control negative group), K+ (infected malaria), K+DHP (malaria, DHP drug), X1 (malaria, goat milk), X2 (malaria, goat milk, DHP), X3 (malaria, goat milk yogurt), X4 (malaria, goat milk yogurt, DHP). Inoculation of Plasmodium was given as much as 10^7/0.2 ml. The intervention was given 24 days. SOD enzyme level data collection was performed on the seventh day post inoculation. Dose of goat milk and goat milk yogurt is 0.5 ml/20gBw. Data was analyzed using Kruskal-Wallis with Post Hoc Mann-Whitney. The result showed a significant increased on SOD level (p <0.05). The mean SOD level in each group: 20,9 ng/ml (K-); 5,67 ng/ml (K+); 17,85 ng/ml (K+DHP); 9,03 ng/ml (X1); 12,11 ng/ml (X2); 10,84 ng/ml (X3); 18,22 ng/ml (X4). The administration of DHP drugs and Yogurt goat milk (X4) is most effective in increasing SOD levels. Giving DHP drugs and Yogurt goat milk (X4) is more effective in increasing SOD levels than only administering DHP drugs (K+DHP). Giving goat milk yogurt with a dose of 0.5 ml / 20g Bw and DHP drugs can be considered to increase SOD levels in malaria infections.**

Keywords: Goat milk; Goat milk yogurt, Malaria, SOD level

I. INTRODUCTION

Goat's milk is one of the solutions for cow's milk allergy. Cow's milk allergy is common in infants and young children. Goat's milk has been shown to have a positive effect on biological functions and easily digested by the body (Yangilar F, 2013; Aristya AL, Legowo AM, Al-baarri AN, 2013; Banjare K et.al, 2017). Protein in goat milk is the main source of active biopeptides that can produce antioxidants (Young Park, 2010; Alyaqoubi S, Abdullah A, Addai ZR., 2014; Alyaqoubi S,et.al, 2014). Goat milk contains antioxidant-forming substances superoxide dismutase (SOD). The protein in goat milk also has anti-inflammatory properties that function as part of the immune system. Protein in goat milk is an important source of angiotensin-converting enzyme (ACE) which functions as an antihypertensive peptide and can also help control infections from pathogenic microbes (Afiarahma AI, Witjahyo RBB, 2015).

One of the processed products of fermented goat milk is goat milk yogurt. Yogurt contains bioactive peptides and has antioxidant activity (Anggraeni RH, Legowo AM, Al-Baarri AN, 2013; Gahruie et.al, 2015; Nguyen L, Hwang ES, 2016). The antioxidant activity of goat milk yogurt is higher than that of cow's milk yogurt (Muniandy P, Shori AB, Baba AS, 2016; Fardet A, Rock E, 2017). Increased antioxidant activity in goat milk yogurt caused from the activity of the lactic acid bacteria (LAB) contained therein. Several studies have shown the effects of LAB in responding to oxidative stress (Padaga MC,et.al., 2018; Nakagawa H, Miyazaki T, 2017). The potential of LAB for human health is a stimulant system that can balance intestinal flora, reduce cholesterol, have anti-aging and antioxidant activity (Nakagawa H, Miyazaki T, 2017; Grażyna C,et.al; 2017). LAB produces exopolysaccharides (EPS) which specifically have immunostimulatory activity and can increase the colonization of the digestive tract (Polak et.al, 2013). LAB can hydrolyze casein a bioactive peptide that has various biological functions Based on previous research, casein in milk yogurt can reduce serum SOD levels and increase Malondialdehyde (MDA) (Nakagawa H, Miyazaki T, 2017).

Malaria is an infectious disease and causes oxidative changes due to plasmodium infection. Plasmodium infection will cause an imbalance of antioxidant (Khalid M et.al, 2013; Gomes QBA, Da-Silva LFD, Gomes QAR, 2015; Fabbri C, De-Cássia MNR, Lalwani P, 2013). In the blood plasma of individuals who experience malaria there will be a decrease in the number of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). SOD is the main antioxidant that catalyzes superoxide radicals into intracellular hydrogen peroxide (Gomes QBA, Da-Silva LFD, Gomes QAR,2015; Li Y et.al, 2018). In malaria infection there will be a decrease in the SOD enzyme. Under these conditions, the use of antioxidants is expected to help prevent oxidative damage and prevent further development of malaria (Gomes QBA, Da-Silva LFD, Gomes QAR,2015).

Scientific hypothesis: Goat milk and goat milk yogurt can increase levels of SOD enzymes in mice infected with malaria.

II. RESEARCH METHODOLOGY

Research Design And Experimental-Animals

The research design used in this study is true experimental with a post-test only randomized control group design. This study used female Balb / c mice which were inoculated by P. berghei ANKA (PbA). The study was conducted by dividing 7 groups: 3 control groups and 4 treatment groups. K group (negative control group that is given standard feed); K + (positive control group fed standard, inoculated PbA and not treated; K (+) DHP (positive control group fed standard, inoculated PbA and receiving anti-malaria therapy (DHP); X1 (treatment group 1, mice that were inoculated PbA , and given goat milk X2 (treatment group 2, mice that were inoculated PbA, received anti-malaria therapy (DHP) and were given goat milk; X3 (treatment group 3, mice that were inoculated PbA and given goat milk yogurt); X4 (X4 (mice) treatment group 4, mice that were inoculated with PbA, received anti-malaria therapy (DHP) and were given goat milk yogurt. SOD data collection was performed on the seventh-day post-inoculation. Dose of goat milk and goat milk yogurt is 0.5 ml / 20gBw.

Research location for making yogurt, analysis of protein content and antioxidant activity of goat milk and goat milk yogurt was conducted at the Integrated Laboratory of Diponegoro University, Semarang. The location of mice maintenance, malaria inoculation, and measurement of SOD were carried out at the Integrated Biomedical Laboratory (IBL) Faculty of Medicine, Sultan Agung Islamic University Semarang (FK-UNISSULA). The goat milk used was obtained from the Kuncen Farm Farmers Group located in the Bubakan Village, Mijen District, Semarang City. This study was approved by the Health Research Ethics Commission of the Sultan Agung University School of Medicine with No. 196 / III / 2019 / Bio-commission.

Intervention Goat Milk And Yogurt Goat Milk

Goat milk and yogurt goat milk are given orally using a sonde. The dosage given is based on the maximum volume of gastric mice that is 1 ml / 20 g BW (Jumilawaty E, Hutahaean S , 2012; Ngatidjan, 2006). The ideal solution volume given is 0.25-0.5 ml and the solution volume on this research using 0.5 ml/20g BW/ day. Goat milk is given for 24 days, which is 21 days before inoculation and 3 days after inoculation.

Measurement of SOD Enzymes Level

Measurement of SOD enzyme levels was measured in the liver of mice. Mice will be euthanasia then dissected by making an incision in the abdomen. The liver of mice used for examination of superoxide dismutase (SOD) enzyme levels was washed first using NaCl. The liver sample is wrapped in aluminum foil and then after that, it is put in a plastic clip that has been labeled with paper and stored at -80 ° C until inspection. Then the liver homogenate is made. Examination of SOD enzyme levels using the Enzyme-linked Immunosorbent Assay (ELISA) method (Setianingrum A,2017).

Statistic analysis

Results were expressed as mean ±SD (for normally distributed data) otherwise it expressed as median (min-max). Statistical difference was analyzed by using a one-way analysis of variance (ANOVA) followed by post hoc Bonferroni for normally distributed data, otherwise, Kruskal-Wallis test followed by Mann-Whitney test was used (SPSS 21). Statistical analyses were done by the computer.

III. RESULTS AND DISCUSSION

The normality of SOD enzyme test results using the Saphiro-Wilk test obtained p-value <0.005 (p = 0.00) it shows that the SOD enzyme data levels were not normally distributed. Then the Kruskal-Wallis test is performed. Based on Table 1, there are statistically significant differences in SOD enzyme levels where the value of p = 0.024 (p <0.05). Giving goat's milk and goat's milk yogurt or a combination of both with DHP drugs affects the SOD enzyme. Next, the Post-Hoc Mann-Whitney test is carried out.

Table 1. Statistical Analysis of SOD Enzyme Levels

Kelompok Perlakuan	Kadar Enzim SOD (ng/ml)	p^a	p^b					
			K-	K+	K+DHP	X1	X2	X3	X4
K-	20,90±1,60	0,024*	-	0,117	0,754	0,602	0,675	0,602	0,917
K+	5,67±1,49		-	-	0,009*	0,028*	0,009*	0,009*	0,009*
K+ DHP	17,85±4,38		-	-	-	0,028*	0,117	0,028*	0,754
X1	9,03±2,67		-	-	-	-	0,347	0,251	0,117
X2	10,64(8,13-21,37)		-	-	-	-	-	0,754	0,465
X3	10,84±1,39		-	-	-	-	-	-	0,530
X4	18,22±1,12		-	-	-	-	-	-	-

Information :

a = Kruskal Wallis

b = *Post Hoc* Mann-Whitney

* = p value <0.005 (significantly)

K^- = Control Group Negative, Healthy

K^+ = Control Group Positive, Infected Malaria

K^+ DHP = Control Group, Infected Malaria and DHP Drug Administration

X1 = Treatment Group, Goat milk- Infected Malaria- Goat milk

X2 = Treatment Group, Goat milk - Infected Malaria- Goat milk and DHP

X3 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt

X4 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt and DHP

Based on the results of the Post-Hoc Mann-Whitney test, there were significant differences in SOD enzyme levels between the K + group and the X1, X2, X3, X4 groups. The administration of goat milk intervention and goat milk yogurt provides a protective effect against malaria infection. There were significant differences in SOD enzyme levels between the K + DHP group with group X1 and group X3. This shows that giving goat milk or goat's milk yogurt alone cannot provide the same therapeutic effect with DHP drugs, so that goat milk or goat's milk yogurt alone cannot be the primary therapy for malaria.

There was no significant difference in SOD enzyme levels ($p < 0.05$) in the K + DHP group with X2 and X4 groups. This shows statistically, there were no significant differences in SOD enzyme levels in the groups with the addition of goat's milk or goat's milk yogurt consumed as a companion to the DHP drug. For more details, the difference in average SOD enzyme levels per group can be seen in Figure 1.

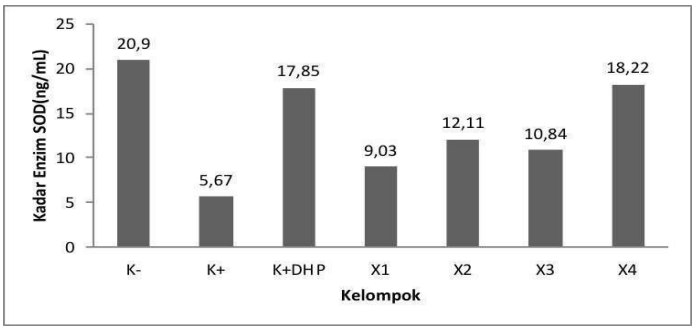

Figure 1. Graph of Mean SOD Enzyme Levels

Figure 1, shows that the highest SOD enzyme was found in the K- group (20.9 ng / ml) which was a healthy control without malaria inoculation and the lowest SOD enzyme was in the K + group (5.67 ng / ml) which was the group who were inoculated with malaria without providing any intervention. The superoxide dismutase (SOD) enzyme which is an endogenous antioxidant will catalyze the reaction of superoxide anion (O2-) free radical dismutase to hydrogen peroxide (H2O2) and oxygen molecules so that superoxide anion (O2-) cannot attack body cells. Malaria infection causes an increase in oxidative stress so that the body experiences a decrease in SOD enzyme levels (Fabbri C, De-Cássia MNR, Lalwani P,2013; Tahro F, Ushio M, 2011). This is consistent with the results of this study in which the K + group had lower SOD enzyme levels than the K- group.

Based on Figure 1, the K group had the highest SOD enzyme level because it was a healthy group, with no malaria infection. The group that had the lowest SOD enzyme levels was the K + group. K + group is a group infected with malaria without any intervention. Low levels of SOD enzymes due to malaria infection without any intervention (Fabbri C, De-Cássia MNR, Lalwani P,2013).

The K + DHP group had higher SOD enzyme levels than the K +, X1, X2, X3 groups. This shows that the administration of DHP drugs is effective in treating malaria. Group X4 had a higher SOD enzyme level than K + DHP. This shows that the administration of goat milk yogurt with DHP drugs is more effective in increasing SOD enzyme levels than with DHP drugs alone. This proves the existence of good interactions between DHP drugs with food ingredients in this case goat milk yogurt. Goat milk yogurt has potential as an adjuvant therapy for malaria sufferers. Yogurt generally contains probiotics which can enhance the immune system by reducing oxidative stress through various mechanisms. Among them is through increased levels of antioxidant enzymes which are the body's natural antioxidants, but also yogurt can reduce oxidative stress that occurs in the intestine, flushing superoxide and hydroxyl radicals, and lower low-density lipoprotein levels and will reduce the redox ratio of glutathione in the blood and intestinal mucosa (Pihlanto, A, 2006; Fodor SI, et.al, 2017).

Groups X1, X2, X3, and X4 had higher SOD enzyme levels than the K + group. This shows that the provision of goat milk and goat milk yogurt can increase SOD enzyme levels. High levels of the SOD enzyme because in milk and yogurt contain casein and whey which can boost the immune system (Fodor SI, et.al, 2017).

Information :

K^- = Control Group Negative, Healthy

K^+ = Control Group Positive, Infected Malaria

K^+ DHP = Control Group, Infected Malaria and DHP Drug Administration

X1 = Treatment Group, Goat milk- Infected Malaria- Goat milk

X2 = Treatment Group, Goat milk - Infected Malaria- Goat milk and DHP

X3 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt

X4 = Treatment Group, Goat milk Yogurt- Infected Malaria- Goat milk Yogurt and DHP

Casein has antioxidant activity including radical scavenger and cation chelator which can inhibit lipid oxidation. Whey contains essential amino acids and a high concentration of branched amino acids such as leucine, isoleucine, and valine. Whey protein has an important role in the regulation of the immune system. Whey protein also contains the amino acids cysteine and methionine, which can enhance immune function through intracellular conversion into glutathione. Glutathione plays an important role in the stability of lysosomal and cell membranes and protects cells from the influence of free radicals (Fodor SI, et.al, 2017; Mahdi et.al, 2018).

Based on Table 1, Group X1 and Group X3 had higher SOD enzyme levels than the K + group. This relates to the content of goat milk and goat milk yogurt which can boost the immune system. Group X3 had higher SOD enzyme levels than group X1. The provision of goat milk yogurt is more effective in increasing the average SOD enzyme level. This is related to lactic acid bacteria contained in goat milk yogurt. Goat milk yogurt contains probiotics that can stimulate the immune system. According to Mahdi's research (2018), goat milk yogurt can reduce MDA and TNF-α levels. Goat milk yogurt contains biopeptides which can induce cellular immune systems and induce anti-inflammatory activity. Goat milk yogurt also contains lactoferrin, lactoferrin can inhibit proinflammatory cytokine production (IL-1, TNF-α, IL-6, iNOS). In Mahdi's study (2018), the administration of goat milk yogurt therapy with a dose of 600 mg/kg BW was able to reduce MDA levels and therapy with a dose of 900 mg/kg BW was able to reduce the expression of TNF-α in animal models of hypercholesterolemia rats (; Mahdi et.al, 2018)

SOD enzyme levels in group X4 were higher than group X2. This is probably due to the content of lactic acid bacteria in goat milk yogurt. Goat milk yogurt in this study used the bacteria S. thermophiles and L. bulgaricus , L. Acidophilus and Bifidobacterium. Lactobacillus bulgaricus bacteria have the advantage of being able to increase macrophages and activate

phagocytes so that they can improve the immune system after being infected with malaria (Denny, Joshua E, 2018).

Antioxidant defense mechanisms can be divided into 4 categories: prevention of active oxidant formation; removal of active oxidants; repair damage and excretion of toxic oxidation products; and adaptive response to ROS. Lactic acid bacteria can show antioxidant activity in different ways.85 Lactic acid bacteria have two antioxidant pathways in boosting immunity. The first pathway is the enzymatic defense system with SOD and GPx enzymes. To prevent excessive oxidative stress a nonenzymatic defense system is needed by having the ability to reduce metal ions and the capacity to "chelating of metal ions". In addition to the ability to increase levels of SOD enzymes, Lactobacillus has the ability to chelating metal ions, the ability to chelating metal ions is higher than its ability to increase levels of SOD enzymes. Research by Zhang et al (2011) shows that Lactobacillus can produce antimicrobial compounds such as organic acids, bacteriocin, and hydrogen peroxide (Zhang et.al, 2011).

Yogurt undergoes a fermentation process, the process will release bioactive peptides from the main protein of milk. The fermentation process will produce antioxidant peptides consisting of 5-11 hydrophobic amino acids. Hydrophobic amino acids include proline, histidine, tyrosine or tryptophan. These amino acids will prevent the formation of free radicals and inhibit the process of lipid peroxidation (Legowo, A. M., Kusrahayu dan S. Mulyani, 2009). During the fermentation process of lactose decomposition, lactose is broken down into glucose and galactose by LAB. Then glucose will be converted into lactic acid, diacetyl and CO2 so that the resulting yogurt with sour aroma, fresh and has a thick viscosity. The yogurt fermentation process involves lactic acid bacteria Gjorgievski N,et.al, 2015; Kumalasari DEK, Legowo MA, Al-Baarri AN, 2013). Lactic acid bacteria have antioxidant activity and can reduce the accumulation of ROS during the digestion process of food and have the ability to degrade superoxide anion and hydrogen peroxide (Legowo, A. M., Kusrahayu dan S. Mulyani, 2009).

IV. CONCLUSION

Giving goat milk and goat milk yogurt can increase the SOD enzyme levels of mice infected with malaria.

V. ACKNOWLEDMENT

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