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**EFFECT OF TONGKA LANGIT BANANA JUICE
TO IMMUNE RESPONSE MICE MODEL OF
MALARIA THROUGH THE LEUKOCYTES
DIFFERENTIAL OBSERVATION**

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ABSTRACT

Leukocytes are the cells of the immune system that consist of two main types, granulocytes and agranulocytes. These types involved in protecting human body against infectious disease and foreign invaders, including *Plasmodium*. The aim of this research is to find out the effect of tongka langit banana (*Musa troglodytarum*) juice on immune response mice (*Mus musculus*) model of malaria through the leukocytes differential observation. The research used Completely Randomized Design (CRD) with three replications. There are a total samples of 20 healthy male laboratory mices strain Balb/C, aged and weigh at ± 2 months and ± 20 gr. The samples consist of 5 donor mices and 15 treatment mices which divided into 5 groups namely K1 or normal control (not infected with *Plasmodium berghei* and not treated with tongka langit banana juice treatment); K2 or negative control (infected with *Plasmodium berghei* and no tongka langit banana juice treatment); and groups that are infected with *Plasmodium berghei* and treated with different dosage of tongka langit banana juice treatment at 0.55 gr/mice/day (K3); 0.65 gr/mice/day (K4); and 0.75 gr/mice/day (K5). The research found that tongka langit banana juice treatment affect leukocytes differential of lymphocytes parallel to the increased tongka langit banana juice dosage given. Whereas for the neutrophils and monocytes,

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the average percentage tend to decrease. Of the three different given dosages, the highest dosage at 0.75 gr/mice/day shown the most effective treatment.

INTRODUCTION

Malaria is one of the infectious diseases which remains a major health problem to combat in many countries of the world including Indonesia. Malaria is caused by malaria parasites (*Plasmodium*) transmitted to people through female Anopheles mosquitos. Malaria parasite infection is responsible for erythrocytes severity and mortality [1-3]. The same thing will be repeated when the parasites invade other erythrocytes [4]. *Plasmodium* infection will change the shape of erythrocytes and trigger erythrophagocytosis in the lymph, then induced leucocytes as the immune response to fagocytosis [5], a protective mechanism done by the fagocyte cells (mononuclear and polymorphonuclear) by devouring microbes and foreign invaders which causes the infection. Fagocyte cells will cooperate after receiving chemical signal from infected tissue by *Plasmodium*. Therefore, infection triggers leukocytosis and leukopenia [6, 7].

One of the immunologic reaction that characterized malaria infection is enlarged lymph which is probably due to the increased number of lymphocytes and or macrophage and other blood cells. The intensity of those cells are influenced by the hemopoietic stimulating factor produced by the immune cell after particular malaria antigen stimulus. Besides, *Plasmodium* infection can also due to complication in its human host which is indicated by specific symptoms such as increased lipid peroxidation, decreased antioxidant, trombositopenia, leukocytosis, enlarged lymph, or even coma and death [8].

Utilizing Malaria vaccine or anti malaria medication to inhibit parasites infection by activating the immune system to reduce clinical manifestation at erythrocytic stadium often resulted in resistance. Thus, there is compelling needs for alternative medication to prevent a more severe complication of the disease [9]. Many attempts have been done to deal with Malaria, from establishing malaria policies to research activities for new malaria cure in both modern and traditional medication using natural substances [10].

Tongka Langit Banana (*Musa troglodytarium*) is a unique and endemic species to Maluku and believed by the native people to cure fever, hepatitis, malaria, boost men stamina and improve bladder performance. Researches [11] has shown that Tongka Langit banana

contains high Karotenoid namely β -karoten. β -karoten is Vitamin A precursor and Vitamin A is essential to improve our immune system to fight infection [12].

Based on the explanation above, this research is conducted to find out the therapeutical effect of tongka langit banana (*Musa troglodytarum*) juice on immune response mice (*Mus musculus*) model of malaria through the leukocytes differential observation.

METHOD

This research employs Completely Randomized Design (CRD) with 3 replications using 5 different treatment groups. Tongka Langit banana is the material for the research. There are two kinds of tongka langit banana which mainly differ in length (short and long). The long tongka langit banana is used and in the form of juice. There are a total samples of 20 healthy male laboratory mices strain Balb/C, aged and weigh at ± 2 months and ± 20 gr. The samples consist of 5 donor mices and 15 treatment mices which divided into 5 groups namely K1 or normal control (not infected with *Plasmodium berghei* and not treated with tongka langit banana juice treatment); K2 or negative control (infected with *Plasmodium berghei* and no tongka langit banana juice treatment); and groups that are infected with *Plasmodium berghei* and treated with different dosage of tongka langit banana juice treatment at 0.55 gr/mice/day (K3); 0.65 gr/mice/day (K4); and 0.75 gr/mice/day (K5). Each group consists of 3 laboratory mices which is acclimatized a week prior to the research and then normally fed throughout the research completion.

The infection of *Plasmodium berghei* on the donor mices is done intraperitoneally with 10^7 in 0.2 ml blood for each mice. Two days after the infection, a drop of blood taken from the mice which further observed under a microscope with 1000x zoom to calculate the number of parasitemia. This process is repeated regularly once in two days until the number of parasitemia reached $\geq 20\%$, *Plasmodium berghei* erythrocytes cycle is 48 hours and incubation is >7 days [12]. If the the number of parasitemia on the mice reaches $\geq 20\%$, the mice will be euthanized, incised, and the mice blood taken from its heart by using syringe (0.3 mL) then put inside heparin bottle.

The donor mice blood is infected intraperitoneally to K2, K3, K4, and K5, treatment mices at 0.1 ml. After the infection, the number of parasitemia is calculated until day 8 (when the parasitemia number reached $\geq 20\%$) and on the next day or day 9, laboratory mices in group K3, K4, and K5, are treated with banana

juice with different dosage at 0.55, 0.65, and 0.75 gr/mice/per day respectively. The laboratory mice are fed with tongka langit banana juice orally 1x1 day for 4 days by using feeding tube. Tongka langit banana juice dosage is determined based on the conversion of human dosage to laboratory mice according to [13].

Tongka langit banana juice treatment is conducted for 4 days and the next 2 days the effectiveness of the juice is observed. On the day 7, all samples (laboratory mice in group K1, K2, K3, K4, and K5) are euthanized and the blood in their heart were taken using syringe (0.3 mL) then put in the heparin bottle for further leukocyte analysis which is based on leukocytes differential using Hematology Analyzer. Leukocyte analysis of laboratory mice in group K3, K4, and K5, is compared with mice

in negative control group in order to obtain the effectiveness of tongka langit banana juice. The result will be analyzed statistically with One way ANOVA and to find out the difference of each each treatment Least Significant Difference test (LSD) is administered.

RESULTS

The leukocytes analysis based on leukocytes differential is presented in the Table 1. A standard normal reference in this research refers to laboratory mice leukocytes in group 1 (normal) because all the mice in this group are not infected with Plasmodium berghei and not treated with tongka langit banana juice.

Table 1. The Percentage of Leukocytes Differential on Mice Model of Malaria

Treatment Groups	Percentage of Leukocytes Differential ± SD		
	Neutrophils (%)	Monocytes (%)	Lymphocytes (%)
K1 (Normal)	2.250±0.91 ^a	4.650±0,77 ^a	92.100±3.11 ^a
K2 (Negative Control)	0.750±0.07 ^b	2.350±0,21 ^b	97.350±0.35 ^b
K3 (Dosage 0,55 gr/mice/day)	3.200±0.00 ^{ab}	6.850±2,33 ^c	87.600±0.98 ^a
K4 (Dosage 0,65 gr/mice/day)	4.950±0.49 ^a	3.400±0,21 ^{ab}	92.250±0.77 ^a
K5 (Dosage 0,75 gr/mice/day)	1.000±0.56 ^a	1.650±2,12 ^c	97.800±0.14 ^b

Note: Different superscripts in the same column show real differences (P<0,05)

Based on the Neutrophyl average percentage, the number of neutrophyl in laboratory mice in group K2 (negative control) is 0.750%. This figure is lower than laboratory mice in group K1 (normal) which is 2.250%. There is a rise of average percentage for laboratory mice in group K3 and K4 at 3.200% and 4.950% respectively which is also higher than K1 (normal). The opposite is true for group K5 as there is a decline in the average percentage at 1.000%.

The percentage of monocytes also show quite similar pattern. Laboratory mice in group K2 (negative control) have 2.350% which is lower than group K1 (normal) at 4.650%. Group K3 has the highest percentage at 6.850%. Group K4 and K5 also experience a decline from 3.400% to 1.650%.

As for the average percentage of lymphocyte, Group K2 is at 97.350% slightly higher than the average percentage of lymphocyte in Group K1 (normal) at 92.250%. But, Group K3 has lower percentage at 87.600%. The percentage for group K4 and K5 are better at 92.250 and 97.800% respectively.

DISCUSSION

Based on the result in findings, the low average percentage of neutrophyl and monocytes and the high average percentage of lymphocytes for laboratory mice in Group K2 (negative control) are due to the fact that they were infected with Plasmodium berghei without having sufficient treatment to help them improve their immune system against the infection. Normally, a laboratory mice or human body is equipped with immune system with very complex mechanism to fight the parasite infection because it involves almost all immune component in the body both internally and externally (through food) [14]. When there is a parasite enters the body, the immune system will respond by releasing cytokines to regulate immune system during the inflammation [5]. However, to recover from Malaria, relying only on natural immune system in the body is not sufficient enough. Hence, external treatment to help strengthening the immune system is necessary to help preventing severe complication of Malaria [9].

Despite being infected with Plasmodium berghei, laboratory mice in group K3, K4, and K5 received tongka langit banana juice treatment. The mice in group K3 and K4 have higher average percentage of neutrophils and monocytes than those in group K2 but in

Group 5 the opposite is true. Furthermore, the average percentage of lymphocyte on laboratory mice in group K3 is the lowest, even lower than K2 but the figure is recovering for both group K4 and K5.

Neutrophils serve as the initial protection base in destroying or eliminating foreign invaders that enters the body, such as bacteria, fungi, virus and parasite. Whereas, monocytes react after neutrophil responses [15]. The high number of neutrophil and monocytes laboratory mice in group K3 and K4 than group K2, show that tongka langit banana juice treatment help to improve laboratory mice immune system to perform fagocytosis by increasing the number of neutrophils and monocytes. When the neutrophil fagocytosis intensify, monocytes activity will also intensify as well due to chemotactic factor released by the neutrophil in triggering monocytes to the infected tissue [15]. This indicates that β -karoten compound in tongka langit banana juice has the potential as a stimulator immunomodulator which enable immunocompetent cell to improve immune system of the body.

The low average percentage of neutrophil and monocytes for laboratory mice in group K5 is believed to be strongly related to the high number of lymphocytes within the group. Another reason for the decline could also be because there is less fagocytosis activity toward foreign invaders in maintaining the damaged tissue. Neutrophil and lymphocytes have almost the same function which is to maintain immune system of the body. Lymphocytes role is to identify "foreign invaders", i.e. bacteria, virus, fungi, parasite then trigger antibody production. On the other hand, neutrophils role is to fight against the infection caused by those invaders. If the number of neutrophils rise, then the lymphocytes will decline or the other way around [16]. It can clearly be seen in the findings that when the average percentage of neutrophil and monocytes in group K3 and K4 are rising compared to group K2 (negative control), the average percentage of lymphocytes in group K3 and K4 tend to rise as well. However, once the dosage of tongka langit banana juice is increased, then the average percentage of neutrophils and monocytes in group K5 is falling, while the average percentage of Lymphocytes is improving.

Besides, the low number of neutrophils in laboratory mice group K5 might be because of higher treatment dosage of banana juice compared to group K3 and K4 which help the mice to have better immune system. On the other hand, below normal level of lymphocytes in group K3 is also due to banana juice treatment. According to [16], the low number of lymphocytes is due to virus infection response and

immunosuppressive medication treatment, lymphocytes respond to immune system quickly.

When compared to negative control group (K2), tongka langit banana juice treatment significantly increase lymphocytes production that helps the immune system to eliminate the number of parasites (Plasmodium) which causing the infection. Lymphocyte is a type of leukocytes that works specifically to fight foreign invaders such as bacteria, virus, fungi, and parasites that enter the body. As a form of cellular and humoral immune, lymphocyte is specific effector in responding to antigen that attached to the macrophages to produce antibody. Any trigger from the antigen will enable T lymphocyte to enter the tissue and destroy any foreign invaders. While B lymphocyte will transform into Plasma cell then produce antibody [17-20].

The immunostimulant effect of karotenoid (β -karoten) compound in Tongka langit banana juice is believed to be the main reason behind the increase number of leukocytes found among laboratory mice after the treatment. β -karoten is Vitamin A pre-cursor which helps the activation of T lymphocyte and cytokines production. It triggers T lymphocyte proliferation to increase and then transform into T helper lymphocyte as well as improving macrophage ability in eliminating Plasmodium in erythrocytes. T helper cell functions during erythrocyte stadium by secreting cytokines to inhibit the growth of merozoit and damage the infected erythrocytes by Plasmodium [21, 22].

CONCLUSION

Based on the analysis and result in the findings, it can be concluded that tongka langit banana juice treatment influence malaria model laboratory mice immune response which can be proved from their lymphocytes differential observation. There is a rise of lymphocytes average percentage parallel to the increased dosage of banana juice treatment, whereas the number of neutrophils and monocytes tend to decline in higher dosage. Of the three different dosages, the highest dosage (0.75 gr/mice/day) is the most effective.

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