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The Effect of Rhizome Extract of Curcuma (*Curcuma xanthorriza* Roxb) for Cell Injury in Histopathology of Liver Tissue of Male White Mice (*Mus musculus L.*) Strain BALB/C Infected by *Plasmodium berghei* Anka

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Abstract : Background: Malaria is a dangerous disease that can cause death. Curcuma xanthorriza Roxb. contains curcumin as an antiinflammatory and natural antioxidant which has a hepatoprotector function.
Objective: To know the effect of rhizome extract of curcuma (Curcuma xanthorriza Roxb) on cell injury in histopathology of male mice's (Mus musculus L.) liver tissue which were infected by Plasmodium berghei ANKA.
Methods: The methods of the study are laboratory experimental study with the design arepost-test only randomized group. Using 25 mice divided randomly into 5 groups that are: normal mice group (K1), aquadest group (K2), ginger group 150mg/kgBW (K3), ginger group 100mg/kgBW (K4), ginger group 50mg/kgBW (K5). Onthe fifth day of experiment, liver histopathology observation was performed.
Results: Descriptively the results show a decrease in cell injury in cell histopathology of the liver tissue of mice. The higher the dose given the smaller the results of scoring cell injury. However, the results of the Mann-Whitney U statistical test were of significance between the groups given curcuma xanthorriza Roxb extract. 150 mg/kg body weight, 100 mg/kg body weight, 50 mg/kg body weight more than 0.05 (Sig <0.05).
Conclusion: The administration of 150mg / BB extract of curcuma rhizome (Curcuma xanthorriza Roxb.) Had a descriptive effect but was not analytically significant on cell injury in histopathology of liver tissue of male mice (Plasmodium berghei ANKA).
Keywords : Malaria, ginger rhizome (Curcuma xanthorriza Roxb.), Cell injury.

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Introduction

Malaria is a disease that caused by *plasmodium* parasites, 90% of malaria world's cases mostly caused by group of *plasmodium falciparum* paracites, meanwhile the others are caused by*plasmodium vivax, plasmodium ovale, plasmodium malariae.* Malaria paracites are transmitted by female *anopheles*mosquitoe who bit human[1]. This disease is a death-risk for babies, infants, and pregnant woman. In fact, malaria also cause activity decreasal in workers due its anemia effect.[2]. Previous study stated that malaria could cause symptoms related to liver organ. Cell damage could be seen on histopathology observation, specifically cell injury [3].Cell injury in liver could occur through few mechanisms such as sequestration and imunne respons[4].

Indonesia is an endemic malaria country. Indicators to monitor malaria morbidity in Indonesia using Annual Paracite Incidence. API is the number of positive malaria cases per 1000 population in one year.Based on API, Eastern pary of Indonesia entered in high malaria stratification, medium stratification in some areas such as Kalimantan, Sulawesi and Sumatra while the Java-Bali region in low stratification. The governments's malaria control programs could fail due to a case of resistance to the malaria treatment. Malaria treatment resistance was reported for old drugs (chloroquine, sulfadoxine, pyrimethamine and quinine) in more than 25% Indonesia. This condition make the Indonesia ministry of health decided to change the malaria treatment strategy, using ACT (artemisinin base combination treatment) in 2004. The latest report showed that there is resistance to the use of ACT in Southeast Asia.[5]. Artemisinin resistance is reported for the first time in West Cambodia and spread to several countries in Southeast Asia. In July 2016, has been reported artemisinin resistance in 5 countries along Greater Mekong area (Cambodia, Lao PDR, Myanmar, Thailand and Vietnam).

With the case of malaria drug resistance and the major impact that malaria cause on liver, an innovation and development of malaria drugs can be a solution. Indonesia is a country with many cultures, therefore it has lots of traditional herbal medicines recipes. One of the raw ingredients is Curcuma. Curcuma is recognized as medicinal plant from the past, especially by Javanese people. Curcuma is often used as a single or mixed drug. There are more than 50 traditional recipes using curcuma[6]. Curcuma is processed into traditional medicine and believed to be able to treat stomachache, jaundice, malaria drug, and kidney disease[7]. Many researches on curcuma as a hepatoprotector has been done. Active curcuma component which works as hepatoprotector is curcumin. According to research, curcumin has natural antioxidant effect[6].

Curcumin mechanism as antioxidant occurs because curcumin was able to catch and breakdown the bond of ROS (Reactive oxygen species) thus preventing damage on liver. This effect proved by researchers who wanted to test the rhizome extract of curcuma. (*Curcuma xanthorriza Roxb*) against liver cell damage repair caused by malaria parasites. This research is an experimental research using BALB/C mice strain as the experimental animal induced by Plasmodium berghei ANKA. Plasmodium berghei ANKA is a parasite that attacks the few mammals other than humans (mice). The results of this research are expected to know the histopathology description of mice liver that have been induced by Plasmodium berghei ANKA and the effect of curcuma extract application that could depress cell injury in liver.

Experimental

This research is an experimental study using the post-test only control group design. In this design model both the experimental group and the control group were randomly obtained, and no pre-test was carried out because observations of cell injury with histopathology of white mice liver tissue (*Mus musculus L*) were carried out after termination. The experimental group was intervened (treatment). After the post-test was done, the difference between the experimental group and the control group will be seen.The study was conducted at the Institute of Tropical Disease, Universitas Airlangga, Surabaya, starting from the preparation stage of the animal, the pre-treatment, until the treatment and dissection stage of experimental animals was carried out at the Institute of Tropical Disease Universitas Airlangga and histopathological observation was carried out at the Pathology lab. RSUD Dr. Soetomo, Surabaya.

The sample used was male BALB/c strain (Mus musculus L.) 7-9 weeks old. The reason for using male white mice is because it does not experience an estrus cycle that can affect the physiology of experimental animals. In addition, these mice chosen in this study are easy to maintain and can adapt well to the new environment. Another reason miceare used as medical testing models is their genetics, biological characteristics and behavior are very similar to humans, and many symptoms of the human condition can be replicated in mice.

Mice used in this research have inclusion criteria in the form of mice weight an average of 25 grams, about 7 to 9 weeks of age, for 1 week in a healthy state, in mice can be seen; soft and slippery fur, and normal mobility. While being excluded if it has a characteristic weight less than 19 grams or greater than 29 grams, pain within 1 week at the time of adaptation, which can be seen from coarse and slightly erect hairs, and reduced mobility, and if the level of parasitemia (-) . And if during the experiment it was found that mice died and suffered from other diseases, which were not a result of treatment, the rats would be excluded from the study subjects (drop out).

The independent variables of this study were Curcuma xanthorriza Roxb extract obtained from Temulawak (Curcuma xanhorriza Roxb) which contained flavonoids and curcumin. Extract was obtained through extraction with 96% ethanol (whole extract). Given to male mice BALB/c strain with a dose of 150 mg / KgBW (K3), 100 mg / KgBW (K4), 50 mg / KgBW (K5) every day for 4 days. While the dependent variable of this study is cell injury. Cell Injury is a condition in cells that are exposed to physiological or external stress which results in reversible abnormalities in cells[8]. Specific histopathological observation of the liver tissue of mice affected by malaria will be damaged[3]. Measurements using a ratio scale.

In this study 5 groups of male mice (Mus musculus L.) BALB/c were used in each group consisting of 5 mice. So that the total mice used were 25 mice. The detail 5 groups of mice are:

1. The first group (negative control) consisted of 5 normal male mice (Mus musculus L.) BALB/c strains.
2. The second group (negative control) consisted of 5 male mice (Mus musculus L.) BALB/c infected with Plasmodium berghei ANKA and given CMC-Na for 4 days (H0 - H3).
3. The third group consisted of 5 BALB/c male mice (Mus musculus L.) infected with Plasmodium berghei ANKA and obtained ginger rhizome extract (Curcuma xanthorriza Roxb) at a dose of 150 mg / kgBW / day orally every day for 4 consecutive days -according to (H0 - H3).
4. The fourth group consisted of 5 male mice (Mus musculus L.) BALB/c strain infected with Plasmodium berghei ANKA and obtained ginger rhizome extract (Curcuma xanthorriza Roxb) at a dose of 100 mg / kgBW / day orally every day for 4 consecutive days -according to (H0 - H3).
5. The fifth group consisted of 5 male mice (Mus musculus L.) BALB/c infected with Plasmodium berghei ANKA and obtained ginger rhizome extract (Curcuma xanthorriza Roxb) at a dose of 50 mg / kgBW / day orally every day for 4 consecutive days -according to (H0 - H3).

During the 4 days of treatment, each mouse in the four groups of mice was examined for blood to calculate the number of parasites. There were two types of blood that were examined, namely thick and thin blood smear preparations. Thick blood smear to detect malaria parasites in the blood when parasitemia is low. Thick preparations are always used to look for malaria parasites. This preparation consists of many layers of red blood cells and white blood cells. When coloring, hemoglobin in soluble red blood cells (dehemoglobinization), so that large amounts of blood can be examined quickly and easily[9]. Thin preparations are used to confirm the species of malaria parasites, when thick preparations are difficult. This is only used to look for parasites under certain conditions.

After the 4th day the mice will be terminated to see the anatomic pathology of cell injury. The scoring method used in this study is the mordue method modified by the author, where the lowest value is 0 and the highest is 4. Observation is done by observing one field of view which is divided into 4 parts, if one part has one cell that experiences lesions cellor necrosis, then the observed part is given a score of 1, if cell injuryor necrosis occurs in two parts of one field of view, then the section is given a score of 2, if cell injury (cell injury) or necrosis occurs in all three parts of one field of view, then the section is given a score of 3, if in all four parts there is cell injuryor necrosis, then one field of view is given a score of 4[10].

The research data will be analyzed statistically. Statistical analysis of this study uses the Statistical Product and Service Solution® (SPSS) program. If one of the normality and homogeneity tests shows normal and homogeneous data, the parametric ANOVA test will be used. If one or both of the conditions are not met, Kruskal-Walis non-parametric test will be carried out.

Results

Tabel 1. Sample Characteristic

Characteristic	**K1**	**K2**	**K3**	**K4**	**K5**
N	5	5	5	5	5
Cell Injury Score	0	1.4	1.4	1.6	2.4
Parasitemia Mean Number	0	6.1	2.172	2.804	3.984

K1: *Normal group of mice;* ***K2:*** *Group of mice inoculated with Plasmodium berghei and in aquades therapy;* ***K3:*** *Group of mice inoculated with Plasmodium berghei and treated with temulawak extract (Curcuma xanthorriza Roxb) at a dose of 150mg / KgBW;* ***K4:*** *Group of mice inoculated with Plasmodium berghei and treated with temulawak extract (Curcuma xanthorriza Roxb) at a dose of 100 mg / KgBW;* ***K5:*** *Mice inoculated with Plasmodium berghei and treated with temulawak extract (Curcuma xanthorriza Roxb) at a dose of 50 mg / KgBW.*

After 4 days treatment, each groups of mice were terminated to make histopathology slides to observe the mice's liver cell injury.The examination results of cell injury can be seen in Table 1. It was found that the highest mean of cell injury value is group 5 (K5) with a mean value of 2.4, whereas the group with the lowest mean value is group 1 (K1) with a value of 0. Group 2 and 3 (K2 and K3) have the same mean value of 1.4. Group 4 (K4) has an mean value of 1.6. While the levels of inoculated parasitemia can be seen that the highest growth percentage is at K2 while the lowest percentage is K3.

Table 2. Normality Test and Comparison Between Group

Group	N	**Histopathology Mean Score**	***Normality Test****	***Kruskal-Wallis P-Value*** **
K1	5	0	-	0.005
K2	5	2	0.814	
K3	5	1.4	0.006	
K4	5	1.8	0.006	
K5	5	2.6	0.006	

****: Data distributed normally if p-value >0.05; **:Significant if p-value<0.05***

Before conducting a hypothesis test, it is necessary to do normality test first to determine the type of hypothesis test used. If data is normally distributed, then the bivariate analysis test used is a parametric type. But if the data is not normally distributed, then nonparametric bivariate analysis will be used. The test used is Saphiro Wilk test because the number of samples used is less than 50.

Based on the normality test in Table 2. It can be seen that the result of significance (p) in the variable number of liver cell injury K1 is 0 K2 is 0.325, K3 is 0.006, K4 is 0,000, K5 is 0.006. Because the significance value is $<\alpha$ ($\alpha = 0.05$), it means that the data is not normally distributed. Then the next step is to do a non-parametric test, namely the Kruskal-Wallis test

In Table 2. With the significance value (p) of 0.005 means that there is a difference in the curcuma extract application to cell injury in liver tissue of the inoculated Plasmodium berghei male white mice (*Mus musculus L.*) in the BALB/c strain between treatment groups.

Post-Hoc Test.

Table 3. Post-Hoc Comparison Between Group

Comparison Between Group	N	Histopathology Mean Score	*VS*	N	Histopathology Mean Score	*P-Value*
K1 vs K2	5	0	**VS**	5	2	0.018*
K1 vs K3	5	0	**VS**	5	1.4	0.018*
K1 vs K4	5	0	**VS**	5	1.8	0.005*
K1 vs K5	5	0	**VS**	5	2.6	0.005*
K2 vs K3	5	2	**VS**	5	1.4	0.910
K2 vs K4	5	2	**VS**	5	1.8	0.656
K2 vs K5	5	2	**VS**	5	2.6	0.125
K3 vs K4	5	1.4	**VS**	5	1.8	0.549
K3 vs K5	5	1.4	**VS**	5	2.6	0,031*
K4 vs K5	5	1.8	**VS**	5	2.6	0,058

**: P-Value <0.05 (Significance)*

After the Kruskal-Wallis test, it was found that there were differences in the treatment group. Furthermore, to find out whether there are differences in each treatment group, the Mann-Whitney U post-hoc test was carried out. The Mann-Whitney U test is a test to determine the cell injury level. It can be seen whether there is a significant effect between 5 treatment groups.

In Table 3. showed a significant difference in liver tissue damage between the negative control group (K1) and the other treatment groups either given aquadest (K2) therapy, or given with curcuma extract (*Curcuma xanthorriza Roxb*) 150 mg / KgBW (K3), 100 mg / KgBW (K4), and 50mg / KgBW (K5). In the treatment group showed a significant decrease in the level of liver tissue damage comparable to the increase in curcuma extract dose (*Curcuma xanthorriza Roxb*), the higher the dose of ginger extract the higher the decrease in the amount of liver tissue damage to mice. In group 2 given aquadest (K2) and group 3 who were given curcuma extract (*Curcuma xanthorriza Roxb*) the dose of 150mg / KgBW (K3) was the lowest rate of liver tissue damage in the treatment group with the same mean value. While group 5 which was given curcuma extract (*Curcuma xanthorriza Roxb*) dose of 50mg / KgBW (K5) had the highest damage rate.

Discussion

Based on the results of histopathological observation of cell injury in liver tissue, different mean values were obtained. In group 1 (K1) the mean value is zero because this group is a negative control that not given any treatment. In group 2 (K2), the treatment group that was given aquadest and group 3 (K3), the treatment group, given an extract dose of 150 mg / kgBW / day had the same mean value of 1.4. This is not in accordance with the measurements results of the parasitemia level where K2 obtained a value of 6.1 while in the K3 group value is 2.172.

A research by Reddy et al (2004)[11], oral administration of curcumin with dose of 100mg/KgBW was proven to inhibit the growth of parasitemia levels. But there are possibility with the same dose has not given significant results on tissue histopathology observation, due to Chika Klarissa (2016)[12] research it was found a significant difference results in mean value of cell necrosis in aquadest group and those given curcuma (*Curcuma xanthorriza Roxb*) extract group with dose of 400 mg/kgBW, 800 mg/kgBW, 1600 mg/kgBW. This condition showed that authors used were not effective enough to show significant differences on tissue histopathology.

In group 4 (K4) and group 5 (K5) have a mean value of 1.6 and 2.4. This is in accordance with the measurements results of the parasitemia levels, where K4 has a mean value of 2.804 and K5 has a mean value of 3.984. If the treatment group is compared, then it can be concluded descriptively that the greater the dose of curcuma extract (*Curcuma xanthorriza Roxb*) can reduce the cell injury mean value.

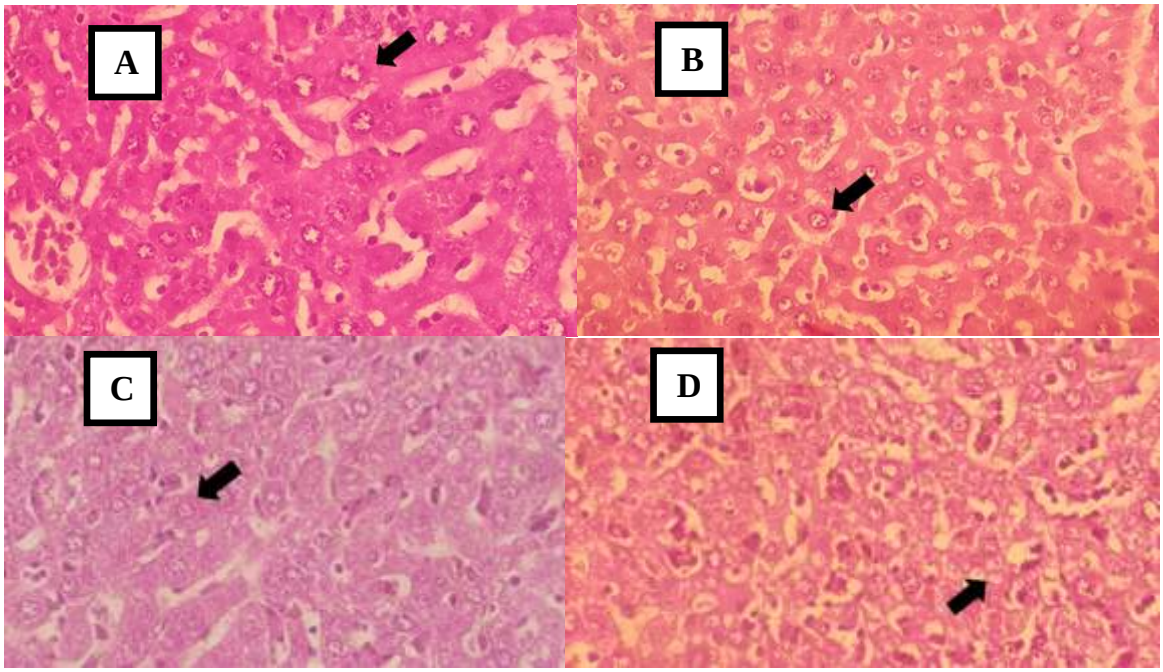

Figure 1. (A)Hepatocytes at K1, no cell injury (cell injury); (B) Hepatocytes in K3, there is mild lesion of light cells; (C) Hepatocytes in K4, there is a moderate degree of degeneration; (D) Hepatocytes in K5, there is a severe degree of degeneration.

Comparison Test

The non-parametric comparison test used is the Kurskal-Wallis test. The value of (p) obtained in this test is lower than 0.005. So there is significant result between groupsin the effect of temulawak extract (Curcuma xanthorriza Roxb) on cell injuryin liver tissue of male BALB/c mice (Mus musculus L.) with Plasmodium berghei ANKA infection. Then Mann-Whitney U test was conducted to find out whether there were differences in each group. Based on table 2, group 1 (K1) compared with groups 2,3,4 and 5 (K2, K3, K4 and K5) have a p-value less than α (α = 0.05) which means that there is significant result in cell injuryof liver tissue between that group.

Group 2 (K2) compared with groups 3,4 and 5 had a p-value more than α (α = 0,05) which meant that there was no significant difference in cell injuryof liver tissue in the group. This might be caused because the dose and period of administration of temulawak extract (Curcuma xanthorriza Roxb) used is too small. In this study, authors used doses of 150, 100, and 50 mg / kg / day for 4 days. The authors used the dose was because it was proven by Reddy et al., (2005)[11] that a dose of 100 mg / kgBW could reduce parasitemia levels> 80%.

In the Klarisa (2016) study[12], there was a significant difference between the mean value of renal tissue necrosis in the aquadest group and those given temulawak extract (Curcuma xanthorriza Roxb) using an extract dose of 400 mg / kgBB, 800 mg / kgBB, 1600 mg / kgBB. In the Klarisa study, the period of administration of extracts was also longer than this study which was 7 days so that the anti-inflammatory effect was significantly proven in kidney tissue. In the study of Reddy et al., (2005)[11] the extract period was carried out for 5 days, so it can be concluded that the dose and period of administration of Curcuma xanthorriza Roxb extract in this study was not enough to show significant results. The author used 4 days because 4 days was the minimum time in the study using extracts to get the benefits of the extract[13].

Group 3 (K3) compared to group 4 (K4) has a P-Value>0.05 which means that there is no significant difference in cell injury of liver tissue in that group. But group 3 (K3) compared to group 5 (K5) has a P-Value<0.05which means that there is a significant difference in cell injury of liver tissue in that group. Group 4 (K4) compared with group 5 (K5) has P-Value>0.05 which means that there is no significant difference in cell injury of liver tissue in that group.

The results of the statistical test in the treatment group giving Curcuma xanthorriza Roxb extract

showed an insignificant. This may occur because the dosage and period of administration of extracts are lacking. In clinical studies, it has been proven that systemic bioavailability of curcumin is low when given orally. This is caused by first-pass metabolism and metabolism in the intestine, especially glucoronidation and sulfation of curcumin[14]. In the study of Reddy et al. (2005) the extract used was only curcumin, whereas in this study, the extract used was the whole extract, so the possibility of antimalarial and antioxidant effects of curcumin in this study was not obtained optimally. Based on this, it can be concluded that the curcuma xanthorriza Roxb rhizome extract of 150 mg / kgBW / day is not statistically significant but can reduce the mean of cell injury valuedescriptively.

Conclusions

In group 5 (K5) has the highest mean of damage value, meanwhile In K2 and K3 have the lowest mean value of liver tissue damage. This is due to the highest dose of curcuma extract in K3 compared to other treatment groups. The anti-inflammatory ability and antioxidants of curcuma are obtained from curcumin and flavonoids. Anti-inflammatory effects are obtained from their ability to inhibit the production of pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS). While the main antioxidant ability of curcuma is obtained from the ability of curcumin and flavonoids as hydrogen atom donors

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