Research Article

The Effect of *Curcuma xanthorriza* Roxb Extract on Hemozoin Pigment Deposition in the Liver Tissue of BALB/C Mice Infected with *Plasmodium berghei* ANKA

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ABSTRACT

Hepatocellular damage is one of the histopathologic features of malaria infection. The presence of large amounts of hemozoin pigment in the liver is associated with hepatocellular damage. *Curcuma xanthorriza* Roxb / curcumin as an anti-inflammatory and natural antioxidant which has a hepatoprotector function. The purpose of these study was to determine the effect of curcuma rhizome (*Curcuma xanthorrhiza* Roxb) extract on hemozoin pigment in the liver tissue of BALB/C mice infected with *Plasmodium berghei* ANKA/ PbA. Method: using 25 groups of BALB/C mice, divided into 5 groups: negative control group; positive control group and 4 treatment groups infected with PbA with extract dose of 150 mg / KgBW, 100 mg / KgBW, and 50 mg / KgBW for four days. On the fifth day the observation of liver tissue to determine deposition of hemozoin pigments by histopathological examination. Results: Statistical analysis showed that descriptively there was a decrease in hemozoin pigment in the liver tissue of mice (150 mg / KgBW extract dose decreased the most hemozoin pigment compared to other doses), but there was no statistically significant difference between the treatment groups with positive controls ($p > 0.05$). Conclusions: The administration of curcuma extract (*Curcuma xanthorriza* Roxb) has the potential to reduce the deposition of hemozoin pigment in liver tissue in BALB/C mice infected with *Plasmodium berghei* ANKA although statistically it is not significant.

Keywords: Malaria, *Curcuma xanthorriza* Roxb, Hemozoin Pigment, BALB/C mice, *P. berghei* ANKA and Live

Introduction

Malaria is an infectious disease caused by parasites from the *Plasmodium* genus which transmitted through the bite of female *Anopheles* mosquitoes [1]. The liver becomes an important organ that is directly involved in the malaria life cycle, which is in the exoerythrocytic phase. The liver becomes the first organ where sporozoites develop into merozoites, then merozoites enter the circulation to infect erythrocytes [2].

Hepatocellular damage is characterized by jaundice and liver dysfunction is associated

with a high degree of parasitemia. The hemozoin (Hz) pigment is released when infected erythrocytes rupture to release new merozoites into the circulation, in the intraerythrocytic cycle. Infected erythrocytes and Hz pigments will be phagocytosed by various phagocytic cells, including liver cells, as evidenced by histopathological examination of liver tissue of malaria patients which shows the greatest deposition of Hz pigment compared to other organs [3]. Hz pigment deposition has also been shown to be associated with the severity of malaria infection in both humans and experimental ani-

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mals. The pathogenesis of hepatocellular damage in malaria infection is poorly understood, in animals showing that the imbalance of pro-inflammatory and anti-inflammatory effects on the immunological response of malaria is the cause of hepatocellular damage [4].

The administration of artemisinin based combination/ ACT therapy against malaria was less effective and the absence of a malaria vaccine [5], were reasons to explore supporting therapies that accelerate and increase the effectiveness of malaria therapy.

Curcuma xanthorriza Roxb / curcumin contains active components flavonoids and curcumin have roles as anti-tumor, natural antioxidant which has a hepatoprotector function, immunomodulator, anti microba and antiparasmodia [6][7]. Previous studies have shown that the flavonoid content in *Curcuma* extract is around 21.31-33.81 mg QE / g [8] and also has 8.7 -11.5 % of curcumin[9]. Based on the information obtained about *Curcuma xanthorriza* as an antioxidant and antiparasite, a bioassay test was performed using experimental study to determine the effect of *curcuma* rhizome (*Curcuma xanthorrhiza* Roxb) extract on hemozoin pigment in the liver tissue of BALB/C mice infected with *Plasmodium berghei* ANKA.

Material and Methods

This research was an experimental study using the post-test only control group design. The study was conducted at the Institute of Tropical Disease, Airlangga University starting from the preparation stage, pre-treatment, treatment and dissection stage of experimental animals. Histopathological observation was carried out at the Pathology Anatomy Department of Faculty Medicine, Airlangga University Surabaya. The study was approved by the ethics commission of the Faculty Medicine, University of Hang Tuah No. 01 / HC / EC / KEP UHT / XI / 2018.

Preparation Mice Models

The sample used was 25 male BALB /C mice ; 7-9 weeks old, average weight 25 grams. BALB / C mice infected with PbA were laboratory models that are mostly used to ex-

plore malaria pathogenesis and pathophysiology, and to explain liver damage from malaria infections [10]. Total sample was counted using Federer formula [11] based on Ihwah *et al.* research finding that increasing the number of replicates would reduce the error rate, is in line with Montgomery's assertion that replication is used to eliminate errors[11] :

$$t(r-1) \geq 15$$

$$5(r-1) \geq 15$$

$$5r - 5 \geq 15$$

$$r \geq (15+5): 5$$

$$r \geq 5 \text{ (minimum 5 mice/each group).}$$

t: total group research

r; number of replication

Samples were divided into 5 groups as follows:

1. **G1:** consisting of 5 mice without PbA infection and given CMC Na solution with Aquadest as negative control group.
2. **G2:** consisting of 5 mice infected with PbA and given CMC Na solution with Aquadest.
3. **G3:** consisting of 5 mice infected with PbA and treated with *Curcuma* extract (*Curcuma xanthorriza* Roxb) at a dose of 150 mg / KgBW / day orally every day for 4 consecutive days.
4. **G4:** consisting of 5 mice infected with PbA and treated with *Curcuma* extract (*Curcuma xanthorriza* Roxb) at a dose of 100 mg / KgBW / day orally every day for 4 consecutive days.
5. **G5:** consisting of 5 mice infected with PbA and treated with *Curcuma* extract (*Curcuma xanthorriza* Roxb) at a dose of 50 mg / KgBW / day orally every day for 4 consecutive days.

The control group was given CMC Na solution with aquadest, because the treatment group (G3-G5) used CMC Na solvent for *Curcuma* extract to separate and dissolve the oil to prevent clots [12].

Previous studies conducted by Reddy *et al.* (2005) using mice that were inoculated with PbA at a dose of 100mg / KgBB, showed that curcumin could reduce blood parasitemia levels by 80-90%. Therefore, these study used a

dose of 150 mg / KgBB of mice / day, 100 mg / KgBB of mice / day, and 50 mg / KgBB of mice / day [13].

Adaptation to the environment was carried out for 7 days in all groups of mice and maintained their condition, food and drink. Mice are placed in a plastic tub measuring 30 x 20 cm and given husks.

***Plasmodium berghei* ANKA Innoculation Process**

The blood of donor mice that had been calculated as a parasitemia percentage reached $\geq 20\%$ and diluted with PBS, were taken as much as 200 μ l to be intraperitoneally infected to all groups of experimental mice. Each mice were infected with 1×10^6 PbA infected erythrocytes on day 0. Mice that have been infected are marked and put into a cage according to the treatment group. Then the mice were left until the parasite growth in all experimental animals within ± 2 days. If all tested animals have proven to have positive parasitic growth, then all groups of experimental animals are ready to be given treatment. Observation of parasitemia level was made serially from day 1 to the last day of the study (day 5) [14,15].

***Curcuma xanthorriza* Roxb rhizome extract**

Curcuma rhizome (*Curcuma xanthorriza* Roxb) was obtained from the traditional market of Bangkalan of Madura island. The process of identifying rhizome curcuma to determine its species was carried out at the pharmaceutical laboratory of Widya Mandala University.

Preparation of the extraction process begins with the stages of cutting the rhizome into small pieces and dried in the sun. After drying, curcuma rhizome will be ground using a grinding tool with a hole diameter of 0.75 mm.

Extraction process of *Curcuma xanthorriza* Roxb rhizome was done using maceration technique. A total of 5 kg of fresh curcuma rhizome, macerated with 96% ethanol as much as 1 liter to get a 500 mg extract of curcuma rhizome. Soaking was done for 24 hours while stirring occasionally. The resulting extract was filtered with a Buchner funnel to obtain the filtrate. The

residue was remastered 4 times. The extract obtained was concentrated with a rotary evaporator until a thick extract was obtained. The extract was stored at room temperature or heated to a low temperature/ $\pm 30^\circ$ C to evaporate the remaining ethanol until a dry paste was formed [16][17]. The preparation of *Curcuma xanthorriza* Roxb solution was carried out by mixing *Curcuma xanthorriza* Roxb rhizome extract with distilled water and CMC-Na solution as a solvent to be given to the treatment group every day for 4 days continuously using sonde.

Liver Tissue Histopathology Examination

On the 5th day the termination was carried out on mice using ether to take liver tissue. Liver tissue is stored in sterile tubes that have been given formalin 10% with a ratio between tissue and formalin liquid is 1: 10 [18].

Liver histopathological examination was done by microscopic examination using a binocular microscope. Observation of hemozoin pigment deposition was done by semi-quantitative scoring: (1) score 0, if there is no deposition of Hz (2) score 1, if there is mild deposition of Hz (3) score 2, if there is moderate deposition of Hz (4) score 3, if there is severe deposition of Hz [19].

Statistical Analysis

Data analysis uses the One Way Anova comparison test if the data is normally distributed and homogeneous, but the results of these study showed that the data were not normally distributed so that the Kruskal Wallis and Mann Whitney tests are used. The significance level of α used is 5% or 0.05 means that in 100 tests there will be 5 incorrect tests.

Results and Discussion

Descriptive and statistical analysis results of hemozoin levels observation can be seen in Table 1. It was found that the highest hemozoin pigment value was based on that in group (G5) which was 2.6, whereas in group (G1/ negative control) the mean value of hemozoin pigment was the lowest.

Table 1. Sample Characteristic & Kruskal Wallis Test

Characteristic	G1	G2	G3	G4	G5
N	5	5	5	5	5
Hemozoin Deposition Mean Score	0	2	1.4	1.8	2.6
Kruskall Wallis test	$p = 0.002^*$				

*: p -value <0.05 (Significance)

Table 2. Post Hoc Comparison Between Group

Comparison Between Group	N	Hemozoin Deposition Mean	Hemozoin Deposition Mean	p -Value
G1 vs G2	5	0	2	0.005*
G1 vs G3	5	0	1.4	0.005*
G1 vs G4	5	0	1.8	0.004*
G1 vs G5	5	0	2.6	0.005*
G2 vs G3	5	2	1.4	0.166
G2 vs G4	5	2	1.8	0.606
G2 vs G5	5	2	2.6	0.166
G3 vs G4	5	1.4	1.8	0.221
G3 vs G5	5	1.4	2.6	0.020*
G4 vs G5	5	1.8	2.6	0.042*

*: p -value <0.05 (Significance)

Based on descriptive and statistical analysis in Table 2. showed a significant difference about Hz levels of liver tissue between the negative control group (G1) and the other treat-

ment groups (G2–G5). The presence of hemozoin pigment deposition indicates the presence of a *Plasmodium* infection. In G1 hemozoin pigment was not found because it had not been inoculated with *PbA* [20].

Figure 1. Hemozoin deposition on liver tissue

Picture of Hz deposition on liver tissue (A) Normal Tissue (B) Mild Deposition Hz (C) Moderate Deposition Hz (D) Severe Deposition Hz.

There was a difference on deposition of Hz between G2 (positive control) with G3 and G4, on G2 group showed that Hz deposition was higher than G3 and G4, but statistical analysis

showed that there was no a significant difference. Reduction on pigment Hz deposition was due to the effect of the administration of curcuma extract, curcuma contains active substances which function to reduce the level of parasitemia, so that it will reduce the production of pigment hz. In the previous study describe that curcumin can inhibit beta hematin

formation, which is the constituent of Hz pigment. Inhibition of the production of Hz pigment causes heme accumulation in cells. Heme was very toxic to cells, so it can cause oxidative stress which induces parasite cell death [21].

On the other hand, flavonoids has a strong antioxidant ability because in their structure, there are more than one phenol group and also has a conjugated double bond, where this structure was needed in binding free radicals. The ability to bind free radicals indicates that in addition to being used as an antioxidant, flavonoids can used as an anti-inflammatory [22].

Hz deposition on G5 is significantly higher than G3 and G4, but there was no a significant difference between G5 and G2, although Hz deposition on G5 was higher than G2. In the results of group 5 (G5) data, higher hemozoin pigment deposition results compared to positive control because it may be influenced by several factors: (1). Lack of dosage as single or main therapy (2). Variation of immune factor differences on mice are still unknown and therapeutic dose conversion with mice body weight is less suitable, (3). Flavonoids have the ability to damage cell walls that cause cell death so as to accelerate the deposition of Hz pigments [22]. So that in group 5 (G5) the level of parasitemia may be decreased by anti-malaria effects, but this effect does not significantly affect mechanism for cleaning Hz pigment deposition in the liver tissue of infected mice.

Conclusion and Recommendation

Based on the study results, it can be concluded that the administration of curcuma extract has potential effect to reduce of hemozoin pigment deposition on liver tissue of BALB/C mice inoculated with *Plasmodium berghei* ANKA, although the decrease was not significant compared to positive control group. In this study only carried out in 3 types of doses, further research needs to be done to determine the right dose of *Curcuma xanthorrhiza* Roxb extract to reduce parasitemia level but on the other hand can prevent deposition of hemozoin in liver tissue significantly.

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