Effect of After-Treatment of *Strychnos ligustrina* Extract on The Percentage of Parasitemia in Mice Infected with *Plasmodium berghei*

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Abstract. Antimalarial treatment is usually given up to 4 days which reduces the number of parasitemia, but malaria can still occur. Therefore, a study to determine the percentage of parasitemia after drug administration is important. *Strychnos ligustrina* has been investigated in vitro to inhibit the growth of *Plasmodium berghei* and reduced the number of parasitemia during 4 days of treatment. The purpose of this study was to determine the percentage of parasitemia in mice infected with *P. berghei* after 4 day treatments with *S. ligustrina* extract. *S. ligustrina* was extracted by maceration method using Ethanol 25%, 50%, and 75% (E25, E50, E75, respectively), and Aquades (EA). This study used 91 male mice divided into 5 groups: E25, E50, E70, EA, each extract consisted of 3 doses (200, 400 and 800 mg/kg BW) and Drug Control (DC). For Drug Control (DC) was using a combination of Dihy-droartemisin dose 25 mg/kg BW and piperaquine phosphate dose 197 mg/kg BW. Mice infected with 1x 10^6 *P. berghei* intraperitoneally. Blood samples were taken on day 5 after treatment with *S. ligustrina* extract for 8 days (days 1-8 after treatment). Preparation of blood smear was stained with Giemsa to calculate the percentage of parasitemia by counting the number of infected erythrocytes divided by 500 erythrocytes and multiplied by 100%. The percentages of parasitemia day 7 with 3 kinds of doses of 200, 400, 800 mg/kg body weight in E25 (11,54%, 2.60% and 11.54%, respectively), in E50 (3.44%, 0%, 3.81%, respectively), in E75 (19.25%, 0.73 %, 9.75 %, respectively), in EA (0.77%, 4.48%, 8.67%, respectively) and in DC 2.10%. At the stage of schizogony, which is one of the life cycles of malaria found in the liver, this parasite is not visible in the blood circulation. The results showed that *P. berghei* was still found in the blood of mice after administration of *S. ligustrina* extracts up to 4 days in all treatments with different percentages of parasitemia. Based on these results it is recommended that the administration of drugs with *S. ligustrina* extract as antimalarial drugs for more than 4 days.

Keywords: *Strychnos ligustrina, Plasmodium berghei.*

1 Introduction

Malaria is a disease caused by protozoa, the genus *Plasmodium* and to treat malaria using anti-malarial drugs [1]. Chloroquine is widely used as an antimalarial drug but there is a problem of *Plasmodium* resistance to the drug, so in 2004 the Republic of Ministry of Health determined the change of drugs from chloroquine to Artemisinin-based Combination Therapy (ACT) [2]. Some research shows there is a failure of artemisinin therapy at the Thai-Cambodja border. It is possible that *Plasmodium* begins to develop resistance to the drug [3]. Because it is necessary to look for other plants as antimalaria drugs. This study uses *S. ligustrina* as an antimalarial drug.

The use of *S. ligustrina* wood as an antimalarial in the form of aqueous extract in vitro can inhibit the growth of parasites by 100% [4]. In addition, in vitro studies of *S. ligustrina* ethanol extract also showed inhibition of *Plasmodium* growth [5].

In this study using, *P. berghei* is a species of *Plasmodium* that infects the blood cells of mice because it is used as a model to study the pathogenesis of malaria [6].

The use of antimalarial drug ACT artesunate-amodiakuin was carried out for three days [7] as well as the administration of dihydroartemisinin-piperaquine (DHP) taken once a day for three days [8]. During the treatment, a period can reduce the percentage of parasitemia but malaria recurrence can still occur. Because it is necessary to do research to determine the percentage of parasitemia after treatment.

The purpose of this study was to determine the percentage of parasitemia after treatment with *S. ligustrina* extract in mice infected with *P. berghei*.

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2 Materials and Methods

2.1 Preparation of extract

Strychnos ligustrina wood is dried, ground and filtered with a size of 40-60 mesh so that it becomes powder. Then the wood powder was extracted by the maceration method using 25% Ethanol (E25), 50% Ethanol (E 50), 75% Ethanol (E75) and Aquades (EA). The extraction process is carried out for 24 hours. The extracted filtrate was concentrated with a rotary evaporator and then used to test for *P. berghei* in mice.

2.2 Treatment of mice

The mice used were DDY strain male mice with a bodyweight of 20-25 g aged 1.5 - 2.5 months. Before the mice were infected, they were acclimatized for seven days and given worm medicine, antibiotics, and anti protozoa. This study used four kinds of extracts (E 25, E50, E70, EA), each extract consisting of three doses (200, 400 and 800 mg/kg BW) and Drug Control (KO), each treatment consisted of seven mice. For Drug Control (DC) using a combination of Dihydroartemisin dose of 25 mg/kg BW and piperaquine phosphate dose of 197 mg/kg BW. Mice infected with 1x106 *P.berghei* intraperitoneally**.**

The extract and drug are given orally once a day, on the third day after *P. berghei* infection because mice have shown positive for *P. berghei* infection as a result of blood examination. The duration of administration of the extract and drug is four days (on the third to sixth day after infection).

2.3 Blood collection and blood smear

Blood collection is carried out for eight days, since the last administration of extracts and drugs (the first day to the eighth day after the last administration of extracts and drugs).

Blood collection through the vein coccygeal at the tip of the tail of the mice, then blood staining is done by Giemsa staining to calculate the percentage of parasitemia by counting the number of parasites in erythrocytes divided by 500 erythrocytes and then multiplying 100%.

2.4 Data analysis

Data on the average percentage of parasitemia was processed by ANOVA (Analysis of Variants) test. The difference in extract treatment was tested with Duncan at a 5% level.

3 Results and Discussion

In general, there was an increase in the percentage of parasitemia from day one to eight after administration of extracts and drugs for four days except for the treatment of E25D400, E50D400, E75D400 and EAD200, the percentage of parasitemia was lower than Drug Control. On the eighth day after treatment, the percentage of parasitemia with three types of doses of 200, 400, 800 mg / kg body weight at E25 (11.54%, 2.60% and 11.54%, respectively), at E50 (respectively 3.44%, 0%, 3.81%), in E75 (19.25%, 0.73%, 9.75%, respectively), in EA (0.77%, 4.48%, 11.55%, respectively) and in DC 2.10% (Table 1).

Table. 1 The average percentage of mice parasitemia infected with *P. berghei* after administration of *S. ligustrina* extract and drugs.

Treatment	Day observation (after treatment)							
	1	2	3	4	5	6	7	8
E25D200	6,40±6,01^{bc}	6,28±6.03^{bc}	4,40±4,10^{ab}	4,21±3,87^{abcd}	8,47±11,43^{a}	8,54±9,99^{ab}	9,98±7,39^{ab}	11,54±8,52^{ab}
E25D400	0,06±0,09^{a}	1,61±3,14^{ab}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	1,24±1,18^{a}	2,60±1,19^{a}
E25D800	5,61±1,06^{bc}	6,21±1,39^{bc}	4,47±1,70^{ab}	7,29±4,49^{bcd}	10,32±6,86^{a}	9,31±4,93^{ab}	17,59±10,84^{bc}	11,54±8,52^{ab}
E50D200	2,82±4,44^{abc}	1,50±3,34^{ab}	1,22±2,72^{a}	2,68±5,99^{abcd}	3,56±7,95^{a}	3,39±7,58^{ab}	1,99±4,44^{a}	3,44±7,69^{a}
E50D400	3,08±3,75^{abc}	3,54±6,05^{abc}	0,00±0^{a}	0,00±0^{a}	0,00±0^{a}	0,00±0^{a}	0,00±0^{a}	0,00±0^{a}
E50D800	2,33±5,21^{ab}	2,21±4,94^{abc}	5,10±7,21^{ab}	1,19±2,65^{ab}	1,49±3,34^{a}	2,53±4,67^{ab}	5,18±8,37^{a}	3,81±6,05^{a}
E75D200	4,90±2,58^{abc}	4,14±2,79^{abc}	3,56±3,76^{ab}	8,86±5,70^{cd}	9,94±10,33^{a}	9,63±11,32^{ab}	23,29±29,32^{c}	19,25±24,17^{ab}
E75D400	2,59±3,65^{abc}	2,07±1,01^{abc}	2,24±1,16^{ab}	1,03±0,38^{ab}	1,45±0,50^{a}	0,72±0,51^{a}	0,75±0,36^{a}	0,73±0,65^{a}
E75D800	14,35±4,99^{abc}	0±0^{a}	6,29±6,89^{ab}	1,73±3,46^{ab}	2,60±4,23^{a}	1,11±1,93^{a}	6,48±11,22^{ab}	9,75±13,78^{ab}
EAD200	0,74±1,54^{abc}	1,48±3,11^{ab}	0±0^{a}	0,09±0,17^{a}	0,11±0,15^{a}	0,35±0,23^{a}	0,62±0,03^{a}	0,77±0,75^{a}
EAD400	0,04±0,05^{a}	1,27±2,10^{ab}	3,93±4,52^{ab}	6,36±8,36^{abcd}	6,63±9,35^{a}	5,03±7,79^{ab}	3,63^{a}	4,48±5,77^{a}
EAD800	3,05±3,92^{abc}	4,23±5,01^{abc}	3,95±6,05^{ab}	6,06±8,51^{ab}	9,67±11,29^{a}	12,78±17,82^{ab}	7,72±9,18^{ab}	11,55±20,01^{ab}
DC	6,43±2,19^{bc}	4,51±1,02^{abc}	5,12±0,86^{ab}	1,95±1,22^{abc}	1,92±0,97^{a}	1,10±0,96^{a}	2,11±1,36^{a}	2,10±1,39^{a}

Different superscript letters in the same column represent significant differences at the level of P <0.05.

[b] Extract Ethanol 25%, 50%, and 75% (E25, E50, E75, respectively), Extract Aquades (EA), Drug Control (DC).

[c] Extract consisted of three doses: 200,400 and 800 mg/kg BW

The presence of parasites after treatment can be caused by the presence of a schizont stage from *Plasmodium* which is still found in the tissues, for example in the liver [9]. The stage is not visible in the blood circulation so the percentage of parasitemia cannot be calculated. To prove that there are still *Plasmodium* parasites in the liver histopathological examination will be done. In addition, there may be other factors that can cause parasites after treatment, namely the activity of cytochrome P 450 isoenzymes, CYP2D6 [10,11,12].

4 Conclusion

The results showed that the percentage of parasitemia was still found in all treatments due to infection with *P. berghei* and after 4 days of treatment with *S. ligustrina* extract and drugs. Based on these results it is recommended for further research that administration of *S. ligustrina* extract and DHP drugs as antimalarial drugs for more than four days.

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