The Anti-Malarial Effect of *Thespesia populnea* (L.) Soland ex Correa Extract Using Malaria Mice Model Infected with *P. berghei*

Prawesty Diah Utami*, Herin Setianingsih, Indira Firdha Syafitri, Rico Pratama Wiyono

**ABSTRACT**

Introduction: Malaria is a re-emerging disease that still causes high morbidity and mortality rates. Reports of malaria therapy, encouraging studies to find new therapies based on plants and adjuvant in malaria infection. *Thespesia populnea* or portia tree leaf extract is a plant that has been shown to have anti-inflammatory, antibacterial and antifungal effects. The aim of this study was to analyze the effect of *Thespesia populnea* leaf extract as antimalarial levels, and its effect on hemoglobin levels in BALB/C mice infected with *Plasmodium berghei* ANKA (PbA). Methods: The study was true experimental laboratories using post-test only group design. Using 27 mice were randomly divided into 3 groups: Group with aquades (G1), Group received Chloroquine (G2), and Group with 200 mg/kg bw of *Thespesia populnea* (L) Soland ex Correa extract and Chloroquine (G3). The degree of parasitemia was observed serially from the first day to the fourth day. Observation of the number of leukocytes and hemoglobin on the fourth day after the mice were terminated. Results: The results of statistical analysis showed that the administration of *Thespesia populnea* leaf extract 200 mg/kg bw with chloroquine had the effect of decreasing the degree of parasitemia and increasing hemoglobin significantly than G1 group, but there was no significant difference with G2 group, although descriptively there were differences. Conclusion: Based on the results of this study, it can be concluded that *Thespesia populnea* or portia tree leaf extract has an anti-malarial effect and can also significantly prevent anemia.

Key words: BALB/C, Hemoglobin, Malaria, Parasitemia, *Thespesia populnea* extract.

**INTRODUCTION**

Malaria is an illness that has been a worldwide health concern until now and caused by *Plasmodium* sp. according to World Malaria Report 2019, malaria morbidity increases to 219 million and mortality rates to around four hundred thousand, and no substantial reduction in malaria morbidity and mortality rates was recorded in 2014-2018. In Indonesia in the 2009–2018 period, the malaria morbidity rate has continued to decrease, from 1.8 per 1,000 in 2009 to 0.84 per 1,000 in 2018. There are, many provinces with a high API index, such as Papua with 52.99 per 1,000 population at the highest API index. Other three API provinces are West Papua (8.49), East Nusa Tenggara (3.42) and Maluku (1.16) per 1000 inhabitants. Malaria infection causes major changes in the host’s hematological pattern, including a decrease in hemoglobin and erythrocyte count. The decrease in hemoglobin is due to the mechanism of hemolysis, damage to erythrocytes by parasites, inhibition of erythropoiesis, erythrophagocytosis, and inhibition of reticulocytorelease. Parasitemia level control is expected to prevent anemia which is triggered by lysis of infected erythrocytes due to the development of parasites in them.

*Thespesia populnea* is found in warm tropical and subtropical regions along the coast. *Thespesia populnea*’s phytochemical screening reveals that the leaves are high in proteins, terpenoids and flavonoids. Proteins contribute to the formation and function of live cells, terpenoids play a part in anti-inflammatory and analgesic. Flavonoids have proven to have a wide range of useful substances, including inflammatory substances, estrogenic, inhibitory enzymes, antimicrobial substances. Based on this phenomenon, researchers want to show both the antimalarial effect and the effect on hemoglobin levels in the extracts of the *Thespesia populnea* leaf.

**MATERIALS AND METHODS**

Design experimental study

This analysis was an experimental study focused on post-test control only group design with the sample size determined on the formula of Federer. The inclusion criterion for samples included male strain BALB/C, 7-9 weeks of age; 20-30 g of weight and stable during adaptation time. Male mice were determined based on an estrous cycle not observed in male mice, which resulted in a more stable biological scenario. The study was carried out in the Faculty of Medicine Laboratory of Hang Tuah and had been approved by the Committee for Research Ethics of the Universitas Hang Tuah, Indonesia (No. 187/HC/EC/KPUHT/2015). The 27 of male BALB/C mice were classified into three groups in this study such as: G1 group (*P.berghei* ANKA/PbA-infected mice received standard food and water); G2 group (PbA-infected mice and chloroquine earned two consecutive days in 10 mg/kg bw/day); G3 group (PbA-infected mice received 10 mg/kg bw/
day chloroquine for two consecutive days, and *Thespesia populnea* leaf extract in four consecutive days at 200 mg/kg bw orally). In *vivo* analysis of antimalarial development in *P. berghei*-infected models of malaria mice used a 4-day suppressive research procedure such that *Thespesia populnea* leaf extract was administered in four consecutive days at 200 mg/kg bw orally.10

**Mice models for malaria**

In this research, *Plasmodium berghei* ANKA/PbA was used because of the same biological essential characteristics and susceptibility to pharmaceuticals with *Plasmodium falciparum*. Mice have become infectious with PbA blood of donor mice by administering intraperitoneally the 0.2 mL blood of donor mice (2 × 10³ parasitized erythrocytes).13

*Thespesia populnea* (L.) Soland ex correa leaf extract

The leaves of *Thespesia populnea* derived from the coast of Bumianyar, Madura, East Java, Indonesia. The form of *Thespesia populnea* leaf used in this research is young leaves taken directly from the stem of the plant. It took 8 kg of the leaves of the *Thespesia populnea* plant to make 10 g of whole extract. The leaves of *Thespesia populnea* were washed with distilled water and dried without direct sunlight. After that, the leaves were blended and ethanol was applied, which was then extracted using the maceration system12. The preparation of extract solution every day was achieved by combining 100 mg of whole *Thespesia populnea* extract with 10 mL of purified water (100 mg/10 mL or 10 mg/cc) to be delivered using an intragastric tube. The extract dose of 200 mg/kg bw per day. Thus, the volume of purified water in 1 party (11 mice) per day was 5.5 cc (0.5 cc/mouse/day) or, if rounded to 10 cc, to prevent unintended action in the sample, such as spilled solution. The prerequisite for 10 cc of distilled water was: 10 cc of distilled water × 10 mg/cc of distilled water = 100 mg.

**Preparation of chloroquine solution**

Chloroquine was dissolved in distilled water and delivered orally using an intragastric tube. The dosage was matched to the overall body weight of the mice. The dosage of chloroquine used was chloroquine 10 mg/kg bw mice/day, so that 0.25 mg of mice was required per 25 g of body weight.13 The dosage of chloroquine solution was 0.5 mg of chloroquine/cc of purified water. Requirement for distilled water every day for 22 mice (G2 and G3): 11 cc (0.5 cc/mouse/day) or, if rounded to 10 cc, to prevent unintended action in the sample, such as spilled solution. The prerequisite for 10 cc of distilled water was: 10 cc of distilled water × 10 mg/cc of distilled water = 100 mg.

**Parasitemia level examination**

Parasitemia level was a standard measure to detected *Plasmodium* sp. infection through blood smear and Giemsa staining. Malaria was induced by an intraperitoneal injection of 0.5 mL of donor blood from malaria infected mice. Examination of parasitemia levels on day 0/D0 (after inoculation before treatment) and day 6/D6. The growth of PbA in the blood of mice was observed in this study using a light microscope with 10x magnification of the eye lens and 100x objective lens. Preparations for observation were made, after drying, with 96 per cent alcohol for 3 minutes, then washed with running water and dried at room temperature, then painted with 10 per cent Giemsa (1 drop of Giemsa + 10 drops of distilled water) for 45 minutes, then washed with running water and dried at room temperature. The method used to measure the parasitemia level: An infected erythrocyte/1000 erythrocyte × 100 per cent.14

**Hemoglobin examination**

The hemoglobin assay was conducted on the fourth day/D6 after the mice had finished taking a blood sample from the heart, with the following steps: (1) before the completion of the experiment, a lethal dose of anesthesia was administered to the mice using ketamine and xylazine injected intraperitoneally; (2) After the mice were unconscious, the mice were put on their backs, the abdominal region was opened: A 21 gauge needle and 3 cc syringe were used to take 0.5 ml of blood from the ventricle. The blood was immediately moved to 20 mL capillary pipettes containing appropriate cell count anticoagulants. EDTA anticoagulated blood samples were used to achieve a full blood count using a Mindray Auto Hematology Analyzer.

**Statistics analysis**

Statistical analysis was used to determine differences in parasitemia and hemoglobin levels between groups was the non-parametric Kruskal-Wallis statistical analysis, since the results were not normally distributed, which was preceded by the Mann Whitney U test. Another objective of this study was to compare the level of parasitemia in each group before (third day) and after receiving treatment (sixth day), so that the statistical analysis used was the related-samples Wilcoxon Signed Rank test because the variables don’t had a normal distribution.

**RESULTS**

The day after the PbA infection process, experiments were made on the stage of parasitemia. All three groups had a positive parasitemia test on the third day, so the research procedure was done on the third to sixth day of infection. Observation of parasitemia levels from day 3 to day 6 could be seen in this figure. Figure 1 revealed that the parasite levels between G1, G2 and G3 showed that there was no substantial variation between the various research experiments (p = 0.749, p>α value). However, there was a significant difference between G1 and G2 and G3 on the fourth day (p = 0.001<α value) and there was no significant difference between G2 and G3 (p = 0.796>α value). The findings of the experiments reveal that the same pattern takes place on the fifth and sixth days of observation. Although the degree of parasitemia in G3 was descriptively lower than in G2, there was statistically no significant difference. The results of descriptive analysis on the level of parasitemia pretest (third day of parasitemia level) and post-test (sixth day of parasitemia level data) could be seen in the following table. The results in Table 1 show that there was a significant difference in the degree of parasitemia pre-test post-test in all groups study. Pre-post analysis for G1 had shown a significant rise in parasite levels (p = 0.008>α value), while G2 and G3 display a significant reduction in parasite levels (p = 0.008 > α value). The Hb level analysis was done once at the end of

**Figure 1:** Observation of Parasitemia Level on 3rd – 6th Day. PbA infected group without any treatment(G1), PbA infected group with Chloroquine (G2), and PbA infected group with *Thespesia populnea* (L) Soland ex Correa extract and Chloroquine (G3). Descriptive and statistically analysis showed that the level of parasitemia on the third day of the 3 groups had not shown a significant difference, but from the fourth to the sixth day it showed that G3 has the lowest level of parasitemia compared to G1 and G2.
the malaria parasite. Inhibiting the production of hemozoin triggers toxic heme depletion pigment that is very important for the survival of parasites. Many literature states that flavonoids have antiplasmodial activity that is poisonous to parasites, contributing to the death of the malaria host erythrocyte cells is the key nutrient for malaria parasite growth

The aggregation of heme (a by-product of Hb breakdown; Hb present in the erythrocytes) is the key nutrient for malaria parasite growth. Therefore, the administration of chloroquine and flavonoids can prevent the aggregation of heme, thereby reducing the parasite load and parasite load was significantly increased in the non-treatment group (G1). The increase in Hb in the G3 group can be attributed to the inhibition of heme aggregation by flavonoids, which prevents the formation of toxic heme derivatives that are toxic to parasites.

DISCUSSION
Observation of parasitemia levels on the third day after infection with P. berghei revealed that there was no substantial variation between G1 and G3 (p = 0.113 > α value). The mean value of parasitemia in G2 was significantly higher than G1 but not substantially different from G3 (p = 0.258 > α value), although the mean value is descriptively distinct.

Table 1: Levels of Parasitemia Pretreatment (3rd Day) and Posttreatment (6th Day).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>Std. deviation</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>3.54</td>
<td>1.16</td>
<td>2.2</td>
<td>4.6</td>
</tr>
<tr>
<td>G2</td>
<td>4.78</td>
<td>0.93</td>
<td>2.9</td>
<td>5.8</td>
</tr>
<tr>
<td>G3</td>
<td>5.21</td>
<td>2.28</td>
<td>2.2</td>
<td>7.8</td>
</tr>
<tr>
<td>Post-Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>8.29</td>
<td>2.78</td>
<td>6.4</td>
<td>15.4</td>
</tr>
<tr>
<td>G2</td>
<td>1.57</td>
<td>0.47</td>
<td>1.0</td>
<td>2.4</td>
</tr>
<tr>
<td>G3</td>
<td>1.46</td>
<td>1.03</td>
<td>0.0</td>
<td>2.7</td>
</tr>
</tbody>
</table>

G1: PbA infected group without any treatment; G2: PbA infected group with Chloroquine; and G3: PbA infected group with Thespesia populnea (L.) Soland ex Correa extract and Chloroquine.

In Table 2, the result of the Hb level analysis revealed that G1 showed the lowest Hb level and G3 showed the highest Hb level. Statistical analysis revealed that the difference between G1 and G3 (p = 0.024 < α value) was significant and that there was no significant difference between G2 with G1 (p = 0.113 > α value); and G2 with and G3 (p = 0.258 > α value), although the mean value is descriptively distinct.

Table 2: Observation of Hemoglobin Levels on 6th Day.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>6.00</td>
<td>14.60</td>
<td>9.44</td>
<td>2.77</td>
</tr>
<tr>
<td>G2</td>
<td>8.60</td>
<td>13.80</td>
<td>11.11</td>
<td>1.49</td>
</tr>
<tr>
<td>G3</td>
<td>9.40</td>
<td>13.60</td>
<td>12.40</td>
<td>1.69</td>
</tr>
</tbody>
</table>

G1: PbA infected group without any treatment; G2: PbA infected group with Chloroquine; and G3: PbA infected group with Thespesia populnea (L.) Soland ex Correa extract and Chloroquine.

The administration of 200 mg / KgBB Thespesia populnea extract has an antimalarial effect and can increase Hb levels, although the dosage has not shown a substantial difference when compared to chloroquine alone. Based on these findings, more study is required to determine the optimum dosage of Thespesia populnea extract.

CONCLUSION
The administration of 200 mg / KgBB Thespesia populnea extract has an antimalarial effect and can increase Hb levels, although the dosage has not shown a substantial difference when compared to chloroquine alone. Based on these findings, more study is required to determine the optimum dosage of Thespesia populnea extract.

ACKNOWLEDGEMENTS
We thank EJA for editing manuscript.
Utami, et al.: The Anti-Malarial Effect of Thespesia populnea (L.) Soland ex Correa Extract Using Malaria Mice Model Infected with P. berghei

REFERENCES

**GRAPHICAL ABSTRACT**

Dr. Prawesty Diah Utami, dr., M.Ked., lecturer and researcher at the Faculty of Medicine at Hang Tuah University, Surabaya, East Java, Indonesia since 2006. She has Master’s Degree in Basic Medical Science at the Faculty of Medicine - Airlangga University (2010); She holds a PhD in medical science from the Faculty of Medicine at Airlangga University. (2019). Infectious diseases, immunology, molecular biology, and hyperbaric oxigent are the areas researched.

Dr. Herin Setianingsih, dr., M.Kes., PA working as lectures and researcher of Universitas Hang Tuah Surabaya Indonesia. She holds his Master Degree in Basic medical Program from Universitas Airlangga Surabaya Indonesia. Whereas she had been awarded his Doctoral Program from Universitas Airlangga Surabaya Indonesia. The research Interest: Anti-diabetic, Hyperlipidemia, Cardiovascular, Anti-inflammatory, Hyperbaric Oxygen Therapy and all about molecular biology.

Indira Firdha Safitri, dr., she is a graduate student at the Hang Tuah University Medical Faculty and holds a a medical doctor’s degree in 2018. Now she is practicing her career as a beauty and esthetic consultant at the beauty clinic in Gresik, East Java, Indonesia.

**ABOUT AUTHORS**
Utami, et al.: The Anti-Malarial Effect of *Thespesia populnea* (L.) Soland ex Correa Extract Using Malaria Mice Model Infected with *P. berghei*

Rico Pratama Wiyono, dr.; He is a graduate student in medicine at the Faculty of Medicine, Hang Tuah University, Surabaya, East Java, Indonesia. He began his medical studies in 2012 and graduated with a medical doctor’s degree in 2018. Now he is HDI’s Executive Diamond Leader, medical practitioner, pharmacist, wellness and business consultant, member of SMA Selamat Pagi Indonesia, and BEE University.