INFLUENCE OF THE COMBINATION OF PASAK BUMI ROOT AND PROPOLIS EXTRACTS ON
PARASITEMIA LEVELS IN MICE INFECTED WITH PLASMODIUM BERGHETI

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ABSTRACT

Objective: Malaria infection remains a global concern due to increasing resistance to artemisinin-based combination therapy. This study examined the antimalarial effects of propolis extract alone and in combination with pasak bumi root extract.

Methods: In the study, 30 mice were divided into six groups including two control groups, two groups of mice treated with propolis alone at concentrations of 90 and 180 mg/kg body weight (BW), and two combination groups of mice treated with 90 or 180 mg/kg BW propolis in combination with 60 or 75 mg/kg BW pasak bumi, respectively. Plasmodium berghei 2% was injected into each mouse, and blood smears were prepared after 8 days to assess parasitemia.

Results: The results revealed no significant difference in parasitemia levels between the positive control and the two combination groups (p=0.136 and 0.289, respectively). However, superior growth inhibition (GI) results were observed in the combination groups (97.97% and 97.83%, respectively) than in the propolis monotherapy groups, whereas better outcomes were observed in the positive control group (98.63% GI) than in the propolis monotherapy groups (23.88% and 51.66%, respectively).

Conclusion: These results illustrate that combination therapy is superior to propolis monotherapy in inhibiting parasitemia.

Keywords: Pasak bumi, Propolis, Mice, Plasmodium berghei, Parasitemia, Oral.

INTRODUCTION

Malaria is an infectious disease caused by Plasmodium parasites and transmitted by Anopheles mosquitoes [1]. According to the World Health Organization (WHO) data, malaria was linked to 438,000 deaths globally in 2015 [2]. In Indonesia, the incidence of malaria has reached 2.9% up from 1.9% in 2013. The incidence and prevalence of malaria are highest in Papua, East Nusa Tenggara, West Papua, Central Sulawesi, and Maluku [3].

Malaria remains prevalent due to resistance to malaria therapy [2]. In Indonesia, chloroquine is used for antimalarial therapy, but resistance to artemisinin-based combination therapy (ACT) first appeared in 2004 [4,5]. Similarly, the resistance of Plasmodium falciparum to ACT has also been detected in Cambodia, Laos, Myanmar, Vietnam, and Thailand [2]. However, the WHO continues to recommend ACT due to its efficacy [6].

At present, other alternatives for treating malaria have been investigated, such as the use of pasak bumi (Eurycoma longifolia) and propolis. Pasak bumi root has displayed antimalarial effects in both in vivo and in vitro studies [7]. In addition, the antimalarial effects of pasak bumi are stronger than those of chloroquine [8]. Research on the usefulness of propolis and its components has been widely conducted. Chalcone compounds in propolis reduce hemolysis induced by isosorbide by ≤40%, demonstrating its good anti-Plasmodium effects [9]. Recently, propolis has increasingly been studied for its antimalarial effects [10]. As antimalarial therapy, pegs can be combined with ACT and chloroquine. Pre-existing research has examined the combination of chloroquine and propolis as antimalarial therapy, but no research has assessed the combination of pasak bumi and propolis. Therefore, this study analyzed the combination of pasak bumi and propolis in the treatment of malaria [7,11].

METHODS

This in vivo study was conducted at the Try Animal Laboratory of the Ministry of Health of Indonesia, Parasitology Laboratory of FKUI, and RIK Laboratory of Depok between April 2016 and December 2016. The study subjects were healthy (active) Mus musculus (Swiss) mice (3–4 months old, 20–30 g). Mice with defects or those that died during experimentation were excluded from the analysis. The independent variables were the concentration and type of treatment given to the experimental groups. The dependent variable was the parasitemia level in mice.

In the study, mice were divided equally into six groups in cages marked with picric acid as follows:

1. Group 1: Negative control (K−) infected with 0.2 ml of Plasmodium berghei 1% without any therapy.
2. Group 2: Positive control (K+) infected with 0.2 ml of P. berghei 1% and treated with ACT 1.7 mg/kg body weight (BW) orally for the first 3 days after infection.
3. Group 3: Treatment 1 (K1) infected with 0.2 ml of P. berghei 1% and treated with oral propolis 75 mg/kg BW for 4 days.
4. Group 4: Treatment 2 (K2) infected with 0.2 ml of P. berghei 1% and treated with oral propolis 150 mg/kg BW for 4 days.
5. Group 5: Treatment 3 (K3) infected with 0.2 ml of P. berghei 1% and treated with a combination of pasak bumi 60 mg/kg BW and propolis 90 mg/kg BW daily for 4 days.
6. Group 6: Treatment 4 (K4) infected with 0.2 ml of P. berghei 1% and treated with a combination of pasak bumi 75 mg/kg BW and propolis 180 mg/kg BW daily for 4 days.

P. berghei was grown and propagated in donor mice. P. berghei obtained from the Department of Parasitology at FKUI was injected intraepitoneally into donor mice at a volume of as much as 1 ml. The
mice were then incubated until a parasitemia level of 2% was achieved as determined through blood sampling. Blood was then diluted using Roswell Park Memorial Institute at a ratio of 1:9. In total, 0.2 ml of diluted blood was injected intraperitoneally into mice. Treated mice were incubated for 24 h, after which blood was obtained daily through the tail vein without anesthesia to assess parasitemia through Giemsa staining using standard procedures [12].

Parasitemia in 1000 erythrocytes was examined using a microscope with ×1000 magnification and oil emersion. The percent parasitemia was determined using the following formula:

\[
\text{% Parasitemia} = \left( \frac{\text{Number of infected erythrocytes}}{\text{Total number of erythrocytes}} \right) \times 100
\]

The percent growth inhibition (GI) was calculated using Peter’s 4-day suppressive test method through the following formula:

\[
\text{GI} = \left( \frac{\text{Parasitemia K(−)−Experimental group}}{\text{Parasitemia K[−]}} \right) \times 100
\]

Note: K (−) = Negative control.

The dihydroartemisinin-piperaquine (DHP) dose was determined as follows:

\[
\text{Human dose} = \frac{\text{Mouse dose} \times \text{mouse factor}}{\text{human factor}}
\]

\[
4 \text{ mg} = \text{Mouse dose} \times \frac{3}{37}
\]

\[
\text{Mouse dose} = 49.3 \text{ mg/kg BW}
\]

The DHP dose obtained using the formula was 49.3 mg/kg BW. This dose was diluted to 1 mg/ml.

One propolis tablet contains 500 mg of propolis. The crushed tablet was then diluted with distilled water to doses of 90 and 180 mg/kg BW versus the use of doses of 25, 50, and 100 mg/kg BW per day in a prior study [13].

Pasak bumi extract was generated by diluting pasak bumi powder with Aqua Dest. The available extract concentration is 2 g/50 ml water (65% active ingredient). The concentration of the extract was adjusted to the total volume administered daily using the following formula:

\[
V1 \times N1 = V2 \times N2
\]

Formula description:

- \( V1 \) = Administration volume.
- \( N1 \) = Parasitemia concentration of donor mice.
- \( V2 \) = Volume of pasak bumi extract.
- \( N2 \) = Concentration of parasitemia desired.

RESULTS

Figure 1 presents microscopic images of erythrocytes from control and infected mice. On the 4th day, the positive control erythrocytes remain intact and healthy (Fig. 1a). Conversely, the infected parasites appeared pale (Fig. 1b).

As shown in Table 1, the parasitemia level increased each day in the negative control group. Conversely, parasitemia levels remained low in the positive control group, with the lowest level observed on day 3.

Meanwhile, parasitemia levels were lower in the propolis monotherapy groups than in the negative control group, whereas the levels were dramatically elevated versus those in the positive control group. However, a dose-dependent effect of propolis was observed.

In both combination treatment groups, parasitemia levels increased until day 1 before declining on subsequent days, with stronger antimalarial effects observed with the higher doses of pasak bumi and propolis. On day 4, the levels of parasitemia in both combination groups were similar to those of the positive control.

GI

The results for inhibition of parasitemia are summarized in Figs. 2 and 3.

The results illustrated that the greatest GI was observed in the K+ group followed by the K3 and K4 groups. Meanwhile, weaker effects were observed in the K1 and K2 groups, particularly the K1 group. Table 2 outlines the relationship between the level of parasitemia and GI from day 0 to day 4. The data illustrated that the parasitemia level was inversely proportional to GI.

Statistical analysis of parasitemia

Differences in parasitemia between days 0 and 4 were examined through a suppressive test method. These data were tested for normality using the Shapiro–Wilk test, whereas the Kruskal-Wallis test was used to identify significant differences. The results of the analysis are presented in Table 3.

Post hoc analysis of these differences was performed using the Mann–Whitney U-test as presented in Table 4.

The results illustrated significant differences between the K1 group and the K3 and K4 groups. Similarly, significant differences were noted between the K2 group and the K3 and K4 groups. However, the results...
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<table>
<thead>
<tr>
<th>Group</th>
<th>Parasitemia level (%)</th>
<th>Brinkman index</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>Negative control</td>
<td>5</td>
<td>0.52</td>
<td>7.46</td>
</tr>
<tr>
<td>Positive control</td>
<td>5</td>
<td>0.52</td>
<td>1.72</td>
</tr>
<tr>
<td>Propolis 90 mg/kg BW</td>
<td>5</td>
<td>0.74</td>
<td>3.76</td>
</tr>
<tr>
<td>Propolis 180 mg/kg BW</td>
<td>5</td>
<td>0.64</td>
<td>1.34</td>
</tr>
<tr>
<td>Propolis 90 mg/kg BW+PB 60 mg/kg BW</td>
<td>5</td>
<td>0.58</td>
<td>5.5</td>
</tr>
<tr>
<td>Propolis 180 mg/kg BW+PB 75 mg/kg BW</td>
<td>5</td>
<td>1.08</td>
<td>3.46</td>
</tr>
</tbody>
</table>

Parasitemia was assessed starting 24 h after Plasmodium infection. N: Number of mice per group. PB: Pasak bumi. Negative control = Mice infected without treatment. Positive control = Mice infected and treated with artemisinin combination therapy.

### Table 2: Relationship between parasitemia and growth inhibition

<table>
<thead>
<tr>
<th>Group</th>
<th>Parasitemia levels and GI (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 1</td>
</tr>
<tr>
<td>Negative control</td>
<td>5</td>
<td>0.52</td>
</tr>
<tr>
<td>Positive control</td>
<td>5</td>
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</tr>
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<tr>
<td>Propolis 90 mg/kg BW+PB 60 mg/kg BW</td>
<td>5</td>
<td>0.58</td>
</tr>
<tr>
<td>Propolis 180 mg/kg BW+PB 75 mg/kg BW</td>
<td>5</td>
<td>1.08</td>
</tr>
</tbody>
</table>

Parasitemia and growth inhibition were assessed starting 24 h after Plasmodium infection. N: Number of mice per group. P: Parasitemia level, GI: Growth inhibition, Negative control = Mice infected without treatment, Positive control = Mice infected and treated with artemisinin combination therapy, PB = Pasak bumi

### Table 3: Differences of parasitemia levels between days 0 and 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Parasitemia differences</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 1</td>
</tr>
<tr>
<td>Negative control</td>
<td>5</td>
<td>0.52</td>
</tr>
<tr>
<td>Positive control</td>
<td>5</td>
<td>0.52</td>
</tr>
<tr>
<td>Propolis 90 mg/kg BW</td>
<td>5</td>
<td>0.74</td>
</tr>
<tr>
<td>Propolis 180 mg/kg BW</td>
<td>5</td>
<td>0.64</td>
</tr>
<tr>
<td>Propolis 90 mg/kg BW+PB 60 mg/kg BW</td>
<td>5</td>
<td>0.58</td>
</tr>
<tr>
<td>Propolis 180 mg/kg BW+PB 75 mg/kg BW</td>
<td>5</td>
<td>1.08</td>
</tr>
</tbody>
</table>

Kruskal–Wallis test, PB: Pasak bumi, BW: Body weight

### Table 4: Post hoc analysis of differences in parasitemia levels between days 0 and 4

<table>
<thead>
<tr>
<th>Group</th>
<th>Asymp. Sig.</th>
<th>(two-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p value</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td>Negative control</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td>Propolis 90 mg/kg BW</td>
<td>0.136</td>
<td>0.289</td>
</tr>
<tr>
<td>Propolis 180 mg/kg BW</td>
<td>0.251</td>
<td>0.177</td>
</tr>
<tr>
<td>Propolis 90 mg/kg BW+PB 60 mg/kg BW</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td>Propolis 180 mg/kg BW+PB 75 mg/kg BW</td>
<td>0.009</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Post hoc Mann-Whitney U-test, PB: Pasak bumi, BW: Body weight

The results of the study indicated that propolis and pasak bumi had synergistic effects against parasitemia. Prior research similarly demonstrated that pasak bumi was more effective when used in combination with other treatments than as monotherapy [7]. Similar outcomes have also been reported for propolis [15,17].
CONCLUSION
The combination of pasak bumi root extract and propolis had better effects against parasitemia than propolis monotherapy in mice infected with P. berghei.

CONFLICTS OF INTEREST
All authors declare that they have no conflicts of interest.

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