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Histomorphological Studies of the Organs of Malaria Mice Model After Administration Combination of Leaf and Stem Bark Extract of *Syzygium cuminii* with Chloroquine

Lilik Maslachah¹, Lucia Tri Suwanti², Hardany Primarizky³, Thomas V Widyatno⁴, Dyah Fitria Ratna Kusuma⁵, Ridhoafuriyati Winanda⁵, Ryanika Wahyuprasetyo⁵

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**Abstract**

This study aims to assess the histomorphological finding of the in spleen, brain, kidneys and lungs organ in malaria mice model after administration combination of Leaf and Stem bark extract of *Syzygium cuminii* with chloroquine. Thirty-five male albino mice were randomly assigned into 7 treatment groups. K0: not infected, K1: infected, K2: infected+chloroquine, P1: infected+*Syzygium cuminii* leaf extract, P2: infected+chloroquine+*Syzygium cuminii* leaf extract, P3: infected+*Syzygium cuminii* stem bark extract, P4: infected+chloroquine+*Syzygium cuminii* stem bark extract. Therapy was given once a day for 4 days after 24 hours post infection. The 21⁴ day, mice were euthanasia. The organs were taken in order to make histopathology preparations, stained with hematoxylin and eosin. The combination of *Syzygium cuminii* leaf extract and chloroquine in mice infected with *P. berghei* can reduce cerebrum and cerebellum microglia cell counts, white pulp diameter size, hemorrhages damage, alveolar congestion, edema and thickened alveolar septa of lung, decreased tubular necrosis, and interstitial nephritis of renal.

**Keywords**: chloroquine, histomorphological, mice, P. berghei, Syzygium cuminii

**Introduction**

In 2018, an estimated 228 million cases of malaria occurred worldwide (95% confidence interval [CI]): 206–258 million, there were an estimated 405 000 deaths from malaria globally[1]. Malaria infection in erythrocytes causes clinical symptoms of fever and anemia. Then, complications in severe malaria can cause multisystem disorders that can affect different organs (brain, lung, liver, kidney and spleen). In patients with childhood malaria, clinical complications are showed by cerebral malaria, severe anemia and metabolic acidosis. While in adults sufferers are shown to have cerebral malaria, metabolic acidosis, acute kidney failure, jaundice and acute respiratory disorders[2].

The pathogenesis mechanism of severe malaria is associated with immune cell activation and upregulation of pro-inflammatory cytokine, endothelial dysfunction, dysregulation of coagulation pathways and microvascular obstruction mechanisms by the sequestration of infected erythrocytes. In erythrocytic phase, hemoglobin degradation by parasites causes free heme (Fe³⁺). This molecule plays a role in inducing oxidative stress. Oxidative stress has implications for lipoprotein oxidation and serious damage to different organs through the formation of reactive oxygen.
intermediates and nitrogen intermediates (ROI and NO) by host cells. LDL oxidation will upregulate the expression of adhesion molecules, and also sequestration and adhesion of infected red blood cells in endothelial cells which endanger the permeability of blood vessels in vital organs[3].

Therapy for malaria is not enough to only provide antimalarial primary drugs, but also the addition of therapeutic adjuvants such as antioxidants is needed to increase efficacy and reduce complications that directly action specific biological pathways[4]. Leaf and stem bark extract of *Syzygium cumini* contains flavonoids, terpenoids, tannins and polyphenols which have scavenging antioxidant activity to reduce the effects of free radicals[5], so it is expected from this study that the combination of *Syzygium cumini* leaf and stem bark extract with primary antimalarial drugs as an adjuvant therapy can reduce the damage to mice organs in the malaria model.

**Materials and Methods**

**Ethical approval**

This study has obtained approval by certificate no 722-KE from Animal Care and Use Committee on Veterinary Medicine Universitas Airlangga Surabaya Indonesia.

**Materials**

*Plasmodium berghei* parasite strain ANKA from Institute of Tropical Diseases Universitas Airlangga, male albino swiss mice with 20g-30g and aged 2.5 months from the Veterinaria Farma Center in Surabaya. Chloroquine Pro Analysis (PA) from Sigma Chemical Co dose 25 mg /kgBW. The leaf and stem bark of *Syzygium cumini* from Kediri, East Java and then identified in the laboratory of Purwadadi botanical garden Pasuruan dose of 600mg / kgBB.

**Dose of Plasmodium berghei infection in mice**

The dose of *Plasmodium berghei* which is infected in mice were 1×10⁵ in 0.2 ml, and given intraperitoneally. Infection that occurred in mice was examined with thin blood smears taken from veins tail and stained with 25% Giemsa for 30 minutes then examined with a microscope using 400 x magnifications.

**Preparation of leaf and stem bark of *Syzygium cumini***

The leaf and stem bark of *Syzygium cumini* are cleaned, aerated until dry and pounded in order to make fine simplicia. 500 grams of fine simplicia from the leaves and stem bark of *Syzygium cumini*, each soaked with 2 L 96% ethanol solvent, then ultrasonic for 6 minutes. After that, it was filtered using a Buchner funnel, the filtrate was evaporated until a thick extract was formed and then freeze dried into a dry extract.

**Treatment of the experimental animals**

Mice were adapted for two weeks, given feed and distilled water in ad libitum. Mice were randomly divided into 7 treatment groups. The details of the treatment group are as follows, control group: K0: mice were not infected. K1: mice were infected. K2: mice were infected and treated chloroquine25 mg/kgBW. Treatment groups P1: mice were infected and treated *Syzygium cumini* leaf extract 600mg/kgBW. P2: mice were infected treated chloroquine 25 mg/kgBW and *Syzygium cumini* leaf extract 600mg/kgBW. P3: mice were infected treated *Syzygium cumini* stem bark extract 600mg/kgBW. P4: mice were infected treated chloroquine 25 mg/kgBW and *Syzygium cumini* stem bark extract 600mg/kgBW. Therapy is given once a day for 4 days after 24 hours post infection. The 21st day, mice are euthanasia in order to take the organ.

**Organ collection to make histopathology preparation**

Mice were anesthetized with ketamine 40 mg/kgBW intramuscular, the four legs were fixed in the supine position, thoracotomy was performed until the viscera organs were seen. Perfusion of the heart at the end of left ventricular using PBS buffer by cutting the right auricle until clean blood fluid is replaced with PBS buffer. The taken organ was put into the specimen pot fixed with 10% formalin, then alcohol dehydration followed by xylo1 clearing, after that the paraffin organ infiltration stage was soaked in paraffin liquid, then cut with a thickness of 3-4 μm, deparaffinization in xylol then stained with hematoxylin and eosin. Assessment of the histopathology examination results is using microscope with scoring system.
Data Analysis

Data of histopathology changes of brain, lung, spleen and kidney organs were analyzed using Kruskal Wallis Test then proceed with Mann-Whitney Test. Statistical analysis in this study using SPSS (Statistical Program of Social Science) program.

Results

The results of scoring the number of microglia cells in the cerebrum and cerebellum can be seen in table 1, white pulp diameter spleen in table 2, the lungs histopathological changes in table 3 and renal

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cerebrum (Mean ± SD)</th>
<th>Cerebellum (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K0</td>
<td>6.20a ± 0.84</td>
<td>6.80a ± 0.84</td>
</tr>
<tr>
<td>K1</td>
<td>11.80c ± 0.84</td>
<td>9.60b ± 2.30</td>
</tr>
<tr>
<td>K2</td>
<td>6.60a ± 0.55</td>
<td>6.80a ± 0.84</td>
</tr>
<tr>
<td>P1</td>
<td>6.60a ± 0.89</td>
<td>6.60a ± 0.89</td>
</tr>
<tr>
<td>P2</td>
<td>6.40a ± 0.55</td>
<td>6.40a ± 0.55</td>
</tr>
<tr>
<td>P3</td>
<td>8.20b ± 0.45</td>
<td>7.60ab ± 1.52</td>
</tr>
<tr>
<td>P4</td>
<td>7.40ab ± 0.89</td>
<td>7.40ab ± 0.55</td>
</tr>
</tbody>
</table>

Note: Different superscripts on the same column show a significant difference at significant level of 0.05%

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>K0</td>
<td>271.82a±0.26</td>
</tr>
<tr>
<td>K1</td>
<td>458.21b±0.37</td>
</tr>
<tr>
<td>K2</td>
<td>339.75c±0.09</td>
</tr>
<tr>
<td>P1</td>
<td>446.18b± 0.35</td>
</tr>
<tr>
<td>P2</td>
<td>387.22c± 0.11</td>
</tr>
<tr>
<td>P3</td>
<td>405.27b± 0.26</td>
</tr>
<tr>
<td>P4</td>
<td>393.34c± 0.18</td>
</tr>
</tbody>
</table>

Note: Different superscripts on the same column show a significant difference at significant level of 0.05%
Table 3. Lungs histopathological changes of mice (Mus musculus) of control and treatment group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hemorrhage</th>
<th>Alveolar congestion</th>
<th>Edema</th>
<th>Thickened Alveolar septa</th>
</tr>
</thead>
<tbody>
<tr>
<td>K0</td>
<td>0.40d ± 0.14</td>
<td>0.44d ± 0.09</td>
<td>0.52f ± 0.11</td>
<td>0.28e ± 0.18</td>
</tr>
<tr>
<td>K1</td>
<td>1.40a ± 0.24</td>
<td>1.44a ± 1.67</td>
<td>1.52a ± 0.39</td>
<td>1.36a ± 0.17</td>
</tr>
<tr>
<td>K2</td>
<td>1.24a ± 0.38</td>
<td>1.32b ± 1.18</td>
<td>1.24abc ± 0.36</td>
<td>1.24a ± 0.41</td>
</tr>
<tr>
<td>P1</td>
<td>1.28a ± 0.27</td>
<td>1.36a ± 0.26</td>
<td>1.32ab ± 0.27</td>
<td>1.32a ± 0.23</td>
</tr>
<tr>
<td>P2</td>
<td>0.68c ± 0.11</td>
<td>0.72c ± 0.18</td>
<td>0.80de ± 0.20</td>
<td>0.56cd ± 0.09</td>
</tr>
<tr>
<td>P3</td>
<td>1.16a ± 0.33</td>
<td>1.40a ± 0.51</td>
<td>0.96cd ± 0.17</td>
<td>1.16ab ± 0.17</td>
</tr>
<tr>
<td>P4</td>
<td>1.28a ± 0.27</td>
<td>1.48a ± 0.3</td>
<td>1.12bc ± 0.18</td>
<td>0.92b ± 0.41</td>
</tr>
</tbody>
</table>

Note: Different superscripts on the same column show a significant difference at significant level of 0.05%

Table 4. Kidney histopathological changes of mice (Mus musculus) of control and treatment group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tubular Necrosis</th>
<th>Glomerulonephritis</th>
<th>Interstitial Nephritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>K0</td>
<td>0.12c ± 0.11</td>
<td>0.52b ± 0.23</td>
<td>0.00c ± 0.00</td>
</tr>
<tr>
<td>K1</td>
<td>1.80a ± 0.45</td>
<td>1.08a ± 0.30</td>
<td>1.32a ± 0.33</td>
</tr>
<tr>
<td>K2</td>
<td>1.00b ± 0.24</td>
<td>0.64a ± 0.22</td>
<td>0.64b ± 0.33</td>
</tr>
<tr>
<td>P1</td>
<td>1.08b ± 0.30</td>
<td>0.60a ± 0.14</td>
<td>0.76b ± 0.26</td>
</tr>
<tr>
<td>P2</td>
<td>0.96b ± 0.09</td>
<td>0.67a ± 0.29</td>
<td>0.68b ± 0.11</td>
</tr>
<tr>
<td>P3</td>
<td>1.00b ± 0.50</td>
<td>0.96a ± 0.26</td>
<td>0.88b ± 0.30</td>
</tr>
<tr>
<td>P4</td>
<td>1.16b ± 0.48</td>
<td>1.04a ± 0.44</td>
<td>1.08b ± 0.44</td>
</tr>
</tbody>
</table>

Note: Different superscripts on the same column show a significant difference at significant level of 0.05%

Heritability of Ambulatory Blood Pressure in Population of Western Region of Iraq histopathology changes in table 4.

Discussion

During the infection of Plasmodium sp, the microglia is activated and spreads to the brain of malaria sufferers [6]. Activated microglia cells release proinflammatory cytokines and trigger free radical formation [7]. Active ingredients in Syzygium contains of tannins, alkaloids, steroids, flavonoids, terpenoid, fatty acids and vitamins. The flavonoids contained in Syzygium cumini have pharmacological activities as antioxidants that able to capture free radicals and provide a protective effect on tissues and organs by inhibiting lipid peroxidation [8]. The combination of Syzygium cumini leaf and stem
bark extract with chloroquine can reduce the number of microglia cells. The use of antioxidants as adjuvant therapy combined with antimalarials is important to accelerate the healing process and reduce more severe tissue damage, prevent and inhibit the occurrence of more severe complications by providing slow release which stimulates the immune system, decreases clinical symptoms and decreases disease severity.

Malaria infection is a foreign body if it enters the body and its immediately recognized by the specific immune system so that there is sensitivity of immune cells that causes proliferation of white pulp and causes expansion of the white pulp diameter, because of the increased development of lymphocytes, macrophages and reticular cells in the white pulp. Expansion of the white pulp diameter of spleen causes enlarged spleen size in malaria infections due to increased erythropoiesis and hematopoiesis. Increase of hyperphagocytic activity of macrophages and cytotoxic substances accumulation to fight against the infection. The administration of chloroquine, combination of Syzygium cumini leaf and stem bark extract and chloroquine can reduce the size of the white pulp diameter. This is because chloroquine as an antimalarial and antioxidant content in Syzygium cumini leaf and stem bark extract can improve the host's immune system so that it increases phagocyte cell activity which can reduce the number of plasmodium parasites so that white pulp diameter decreases, can reduce the courses of inflammation that occurred in malaria infected-spleen and also cell swelling reduction, likely that VitC alone does not completely help reducing the damages in both morphology and histology of liver and spleen but should be combination with antimalarial drugs.

Malaria caused by Plasmodium berghei can infect red blood cells causing microvascular obstruction due to parasite sequestration. The increase in free radicals in malaria infection causes cell membrane lipid peroxidation to trigger an inflammatory response and pathological changes in the lung organ. The treatment of Plasmodium berghei infection treated with Syzygium cumini leaf extract and chloroquine showed a decrease in lung organ damage, namely haemorrhages, alveolar congestion, edema, wall thickening of the alveoli septa. This occurs because of barriers to the reactive oxygen species formation. Malaria therapy is not enough just by giving antimalarial drugs, however adjuvant therapy is needed by giving antioxidants from the content of Syzygium cumini leaf extract, such as flavonoids and polyphenols. Flavonoids as antioxidants can inhibit lipid peroxidation. Antioxidants are important in supporting the immune system and reducing oxidative stress. Flavonoids can inhibit cyclooxygenase or lipooxygenase and inhibit leukocyte accumulation which can reduce damage to the lung.

Chloroquine administration in malaria infection can reduce the percentage of parasitemia so that it can prevent cytoadherence parasite which causes a decrease in tubular renal hypoxia so that it can reduce changes in tubular necrosis and interstitial nephritis. Then, chloroquine combination therapy with Syzygium cumini leaf and stem bark extract can protect kidney damage caused by free radicals. Adjuvant therapy can work directly on factors that are being a key to the pathophysiology of malaria such as increased TNF alpha, free radicals and low levels of NO.

Conclusion

The combination of Syzygium cumini leaf extract and chloroquine in mice infected with P. berghei can reduce cerebrum and cerebellum microglia cell counts, white pulp diameter size, haemorrhages damage, alveolar congestion, edema and thickened alveolar septa of lung, decreased tubular necrosis, and interstitial nephritis of renal.

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