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The Effect of Artemisinin-Based Combined Therapy with Salacca Edullis Reinw Seed Extract on Malondialdehyde (MDA) Level in Mice Malaria Model

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ABSTRACT

Background: Malaria is an infectious disease caused by parasite *Plasmodium sp.*, while infection with malaria can cause an excessive increase in free radicals and damage several organs. This study evaluated the effect of artemisinin-based combined therapy with *Salacca edullis reinw* (Zalacca) seed extract on malondialdehyde (MDA) level in mice malaria model.

Methods: Using a post-test design randomized method, 48 male white mice were divided into 6 groups, including 2 control and 4 treatment groups, while they were inoculated with *Plasmodium berghei* ANKA. Follow-up was undertaken for 14 days when comparing the groups. Artemisinin and zalacca seed extract were used in treatment groups of malaria infection.

Results: The treatment with artemisinin and zalacca seed extract revealed a significant decrease in MDA level in mice infected to malaria.

Conclusion: Zallaca seed extract was demonstrated to reduce MDA level in mice infected to malaria. Even administration of artemisinin alone could reduce the MDA level, but was not significant. Treatment with a combination of artemisinin and zallaca seed extract was shown to significantly lower MDA level in malaria infection.

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Introduction

Malaria is an infectious disease caused by the parasite *Plasmodium sp.* Which is transmitted through the bite of the female Anopheles mosquito (1, 2). Malaria infections are one of the infectious diseases that attack the entire population of the world (3). *Plasmodium sp.* which often causes malaria infection is divided to *Plasmodium vivax* and *P. falciparum*. Some studies mentioned that *P. falciparum* is a malaria plasmodium that causes severe malaria infection, whereas *P. vivax* is a Plasmodium that causes milder infections. Apart from mild infections, several studies have reported that *P. vivax* can also cause clinical complications such as anemia, acute kidney failure, shock, and coma (4).

Research on induction of *P. berghei* ANKA in mice shows the emergence of oxidative stress which

is characterized by increased free radical activity through fat peroxidation and is associated with pathological abnormalities. This can be known from the increased levels of malondialdehyde (MDA) and indirectly through a decrease in the levels of superoxide dismutase (SOD) which functions as a scavenger (5). Other studies have also shown that malaria infection in pregnant women caused pregnancy conditions to become worse, this is associated with the emergence of excessive oxidative stress that is characterized by increased lipid peroxidation of cell membranes detected as MDA (6).

Antimalarial treatment methods have experienced resistance, so WHO recommended treatment measures using Artemisinin Combination Therapy (ACT) as a new malaria drug to be more effective (7). Artemisinin is an endoperoxide (sesquiterpene lactone endoperoxide) which has the ability as an antimalarial agent to be stable and to work faster (8, 9). This medication works by inhibiting the enzyme ATPase and produces free radicals to eliminate the parasite that causes malaria, so that the use of the drug can lead to a buildup of free radicals in the body (5). Free radicals can increase lipid peroxidation which will then decompose into MDA in the blood, while MDA is considered as a marker of cellular damage due to presence of free radicals (10). An excessive increase in free radicals will cause damage and cell apoptosis. Some studies have suggested that antioxidants may help in the treatment of malaria (11).

Salacca edullis reinw (Zalacca) is one of the fruits known to contain antioxidant compounds and is a major commodity in the Special Region of Yogyakarta, especially in Sleman Regency (12) with the potential to be be used in several populations, in both forms of fruits and seeds (13). Tracing benefits of zalacca seeds illustrated that the content of zalacca seeds has antioxidant activity (14, 15). In West Java, the ethanol extract of zalacca seeds was demonstrated to contain tannin, quinone, monoterpene, sesquiterpenes, alkaloids, and polyphenols (16), acting as antioxidant (17). So this study aimed to determine the effect of artemisinin-based combined therapy with *Salacca edullis reinw* (Zalacca) seed extract on MDA level in mice malaria model.

Materials and Methods

In an experimental study using a post-test randomized design from December 2019 to January 2020, 48 male white mice (8-10 weeks old, 25-35 g) were divided into 6 groups, while treatment groups were inoculated with *Plasmodium berghei* ANKA (PBA). Zallaca seed extract and dihydroartemisinin-piperaquine phosphate (DHP) were used for treatment and the follow-up was considered for 14 days. Collection of mice and specimen was carried out in the Laboratory of Experimental Animal of UB's Faculty of Medicine, and Parasitology Section, Faculty of Medicine, Universitas Brawijaya in Malang, Indonesia. Assessment of MDA level was conducted at the Laboratory of the Physiology of the Faculty of Medicine, Universitas Brawijaya in Malang, Indonesia.

The sample size for each treatment group was determined using the provisions of the Institutional Animal Care and Use Committees (IACUC) (2002), namely: a minimum of 6 mice in one study group. To anticipate a drop out of 30%, each group was added by 2 animals so that the number of samples used in each group to be 8. This study used 6 treatment groups, so that the total sample size in the study group to be 48 mice. This study was approved by the Ethics Committee of Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia (No. 472/UN27.06/KEPK/2019). Guidelines of the Declaration of Helsinki were followed in the research.

The 6 groups were deined as follows: Group of mice that were not infected by *P. Berghei* and were not given treatment (C-); Group of mice that were infected by *P. Berghei* and not given treatment (C+); Group of mice infected with *P. berghei* and given DHP dose of 0.0117 tablets/30 g orally for 3 days (P1); Group of mice infected with *P. berghei* and given a DHP dose of 0.0117 tablets/30 g orally for 3 days+ZSE dose 0.52 g for 14 days (P2); The group of mice infected with *P. berghei* and given ½ DHP dose 0.0058 tablets/30 g orally for 3 days+ZSE dose 0.52 g for 14 days (P3); Group of mice infected with *P. berghei* and ZSE dose of 0.52 g for 14 days (P4).

The independent variables of this study were zalacca seed extract, and DHP. The dependent variable of this study was MDA level. All cages were placed in the same room with the same level of lighting, temperature, and humidity. Feeding was ad libitum in the form of a standard mice pellet. After an adaptation period of 7 days, the animals were devoted to 6 groups, two control groups, and four treatment groups. All animals were inoculated with *P. berghei* ANKA originating from donor mice containing 107/mL of erythrocyte parasite, which was inoculated as much as 0.1 mL intraperitoneally, using a 1 mL syringe.

In the treatment group, zalacca seed extract was administered for 14 days after mice were infected to malaria with a percentage of parasitemia of more than 5%. Antimalarial administration of DHP was given on the 5th day post-inoculation for 3 days. On the 15th day after inoculation, blood samples were taken from the mice to check the levels of MDA. Data analysis was performed using SPSS software

Table 1: Comparative analysis of median malondialdehyde (MDA) in different groups.

Group	MDA (median)		*P* value
C-:C+	15.785±8.849	33.218±11.288	0.02
C-:P1	15.785±8.849	9.338±1.880	0.005
C-:P2	15.785±8.849	7.300±4.789	0.008
C-:P3	15.785±8.849	7.724±5.672	0.02
C-:P4	15.785±8.849	8.997±2.123	0.01
C+:P1	33.218±11.288	9.338±1.880	0.003
C+:P2	33.218±11.288	7.300±4.789	0.004
C+:P3	33.218±11.288	7.724±5.672	0.003
C+:P4	33.218±11.288	8.997±2.123	0.004
P1 P2	9.338±1.880	7.300±4.789	0.08
P1:P3	9.338±1.880	7.724±5.672	0.556
P1:P4	9.338±1.880	8.997±2.123	0.51
P2 : P3	7.300±4.789	7.724±5.672	0.29
P2:P4	7.300±4.789	8.997±2.123	0.29
P3:P4	7.724±5.672	8.997±2.123	0.94

*Mann Whitney U Test. Source: Primary data (2020). Note: Group of mice that were not infected by *P. Berghei* and were not given treatment (C-); Group of mice that were infected by *P. Berghei* and not given treatment (C+); Group of mice infected with *P. berghei* and given DHP dose of 0.0117 tablets/30 g orally for 3 days (P1); Group of mice infected with *P. berghei* and given a DHP dose of 0.0117 tablets/30 g orally for 3 days+ZSE dose 0.52 g for 14 days (P2); The group of mice infected with *P. berghei* and given ½ DHP dose 0.0058 tablets/30 g orally for 3 days+ZSE dose 0.52 g for 14 days (P3); Group of mice infected with *P. berghei* and ZSE dose of 0.52 g for 14 days (P4) *were significantly different (P<0.05)

(Version 20, Chicago, IL, USA) applying Kruskal Wallis and Mann Whitney U tests. A p value less than 0.05 was considered statistically significant.

Results

The median measurement of MDA obtained the values of K- (15.785±8.849), K+ (33.218±11.288), P1 (9.338±1.880), P2 (7.300±4.789), P3 (7.724±5.672), and P4 (8.997±2.123). The treatment had a significant effect on MDA level (P=0.0001). Table 1 shows a comparison of the median values of the negative control group with the positive control revealing a significant difference (P=0.020). Based on the comparison of the median value of all treatment groups with the negative and positive control groups, there was a significant difference too (P<0.05), so the therapy had a positive effect.

Meanwhile, the comparison of the median value between the treatment groups showed that the intervention provided a change in MDA level that was not much different from the treatment group. The highest change in MDA level was noted in the P2 group (P=0.008) with the lowest median MDA level of 7.30. So administration of DHP with the seed extract was shown to reduce MDA level in mice infected with *P. berghei* when compared to administration of DHP or bark seed extract alone.

Discussion

Our findings indicated that administration of DHP with the seed extract could reduce MDA level in mice infected with *P. berghei* when compared to administration of DHP or bark seed extract alone. The measurement of MDA level itself has been a biomarker for changes in free radicals, while an increase denotes to a rise in free radicals (18) that can cause an oxidative stress because of the imbalance between oxides and antioxidants leading to cell and liver damage (19).

Increased oxidative stress in malaria occurs too due to activation of host neutrophils and hemoglobin degranulation in the parasite. This increase is usually marked by an increase in reactive oxygen species (ROS), nitric oxide (NO), reactive nitrogen species (RNS), and decreased activity of the enzyme superoxide dismutase (SOD) (18). Increased free radicals trigger an increase in permeability of blood vessels and cause damage to the endothelium both in erythrocytes and in the parasite. This not only has an impact on parasitic elimination, but can also be detrimental by causing uncontrolled cell apoptosis damaging several body organs (20).

Free radicals due to malaria infection can be pathogenic, especially in the process of sequestration, causing many complications. Glycosilphosphatidylinositol proteins (GPI) generated by lysis of erythrocytes infected with *P. berghei* can stimulate the activity of macrophages and produce proinflammatory cytokines (8). The excessive inflammatory process in the tissue is mediated by production of cytokines and free radicals (21). The use of antioxidants as supportive therapy

in combination with antimalarial artemisinin has been considered essential to accelerate the process of healing and reduces damages by excessive free radicals (22, 23).

MDA is a dialdehyde compound which is the final product of lipid peroxidation in the body, through an enzymatic or nonenzymatic process (24). The mechanism of the formation of MDA is through lipid peroxidation that begins with the removal of hydrogen atoms (H) from long-chain unsaturated lipid molecules by hydroxyl radical groups (*OH), so lipids are radicals that react with oxygen atoms (O) and form 2 peroxyl radicals (*OO), which subsequently produce MDA (with more than three unsaturated bonds). Free radicals are more dangerous than oxidants which are not considered as free radicals (25, 26).

High MDA concentrations indicated an oxidation process in the cell membrane (27). To protect against ROS attacks, the human body has an organized antioxidant system, both enzymatic antioxidants, and non-enzymatic antioxidants, which work synergistically. Antioxidants protect body cells against oxidative damage and can prevent the production of oxidative products (28). These antioxidant compounds are naturally present in our bodies, such as SOD, catalase, and glutathione peroxidase (29).

Zalacca is a fruit that contains antioxidant compounds, especially zalacca seeds that are widely used (14). The utilization of zalacca seeds has become important tbecause zalacca seeds include 30% of the whole zalacca fruit (30). Previous research on zalacca seeds grown in West Java mentioned that the ethanol extract of zalacca seeds contained tannin, quinone, monoterpene, sesquiterpenes, alkaloids, and polyphenols that act as antioxidants (16). However, these results still need further assessment using several comprehensive methods to determine the antioxidant activity (31).

Research on the use of antioxidants also denoted to an improvement in bleeding conditions in the brain of mice that have been inoculated by *P. berghei* (32). Other researches also showed that antioxidants can reduce the activity of free radicals produced by parasites and the immune system, and can inhibit lipid membrane peroxidation, thus helping to prevent cell membrane damage and to reduce damages to surrounding tissues (21, 33). Antioxidants in zalacca seeds also act as a breaker of free radical reactions in tissues and plasma, especially in tissues with high oxygen partial pressure, such as respiratory tract membranes and the retina. Antioxidants can prevent lipid peroxidation and damage to cell membranes and maintain membrane stabilization, to reduce tissue damages due to presence of free radicals (23, 17). Exogenous antioxidants, such as vitamin A, vitamin C, vitamin E, NAC, and riboflavin can be used as adjunctive/supporting therapies in acute and chronic malaria infections. These antioxidants can accelerate healing, decrease free radical activity, and increase the immunity (22, 34).

Conclusion

Zallaca seed extract was demonstrated to reduce MDA level in mice infected to malaria. Even administration of artemisinin alone could reduce the MDA level, but was not significant. Treatment with a combination of artemisinin and zallaca seed extract was shown to significantly lower MDA level in malaria infection.

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Conflict of Interest

None declared.

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