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Antimalarial activity of Virgin Coconut Oil against *Plasmodium berghei* ANKA in mice

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ABSTRACT

Malaria remains a health problem in various parts of the world. A decrease in antioxidant levels during malaria infection cause oxidative stress, therefore exogenous antioxidant is needed. Virgin Coconut Oil (VCO) can be a source of exogenous antioxidants due to the antioxidant activity of VCO, which was contributed majority by phenolic acids content. Antioxidants play a role in countering the effects of free radicals by inhibiting fat peroxidation that cause erythrocyte cell wall stronger and not easily ruptured. Pharmacological properties of VCO including anti-inflammatory, anti-oxidant, antiviral, and antiprotozoal properties have been reported. Therefore, this study aimed to find out the antimalarial activity of VCO by evaluating the parasitemia and percentage of inhibition to the growth of parasite. Thirty male BALB/c mice were infected intraperitoneally with 1×10^6 *Plasmodium berghei* ANKA-infected erythrocytes. The mice were then randomly divided into five groups: positive control (PC) group was given 187.2 mg/kg BW of dihydroartemisinin phosphate, negative control (NC) group was only given sterile water; G1, G2, and G3 groups were given 1 ml, 5 ml, and 10 ml/kg BW of VCO, respectively. VCO treatments were given for 4 consecutive days started from 24 hours post infection. Parasitemia was determined daily on Giemsa-stained tail blood smear, further the percentage inhibition was calculated. The results showed that parasitemia in VCO-treated mice were lower than that of NC group, but higher than that of PC group, indicated the antimalarial activity of VCO. The inhibition of VCO to the growth of parasite showed that G2 was higher (48.70%) than that of G1 (13.04%) and G3 (33.9%). The use of antioxidant therapy as a supportive therapy is one of alternatives in supporting the healing process of malaria patients and may reduce various risks that could potentially occur in patients.

Keywords: virgin coconut oil, parasitemia, *plasmodium berghei* ANKA

INTRODUCTION

Malaria is an infectious disease caused by *Plasmodium* sp which is transmitted to humans through the *Anopheles* sp mosquito [1]. *Plasmodium* species that infect human are including *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi* [2]. Malaria remains a health problem in various regions of the world, resulting in 405,000 deaths, with 228 million cases occurring in Africa and Southeast Asia [3]. Indonesia is a country with the highest incidence of malaria in Southeast Asia, especially in the provinces of Papua, West Papua, and East Nusa Tenggara (NTT) which are endemic areas [4]. Changes in biochemical parameters and hematological complications are often experienced by malaria patients [5].

Anemia, thrombocytopenia, leukocytosis, decreased antioxidants, increased lipid peroxidation, lactic acidosis, coma and death are some of the symptoms/events of malaria [6]. Almost all deaths from malaria are caused by *Plasmodium falciparum* [7]. Complications of hematological disorders as well as spleen and liver are the cause of death.

The existence of parasite resistance to several antimalarial drugs, including chloroquine, artemisinin, and the combination of artesunate with mefloquine makes it difficult to treat malaria which results in death and malaria cases [8], thus encouraging the need of new antimalarial agents.

Malaria infection cause the increase production of Reactive Oxygen Species (ROS) caused by activated neutrophils and degradation of hemoglobin by the parasite. The increased of ROS increases the vascular permeability due to endothelial damage [19]. A decrease in antioxidant levels at the time of infection causing oxidative stress, therefore antioxidants from outside the body are needed (exogenous antioxidants). Exogenous antioxidants can be obtained from natural ingredients [10], such as virgin coconut oil (VCO). Pharmacological properties of VCO including anti-inflammatory, analgesic, antipyretic, anti-oxidant, anti-stress, antimicrobial, antiviral, and antiprotozoal properties have been reported [11] [12]. Therefore, this study aimed to find out the antimalarial activity of VCO by evaluating the parasitemia and percentage of inhibition to the growth of parasite in mice infected with *Plasmodium berghei* ANKA.

MATERIALS AND METHODS

Ethical Approval

The ethical approval is explained on the certificate no 749/HRECC.FODM/XI/2019 from the Health Research Ethical Clearance Commission, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

Parasite, Host and Drugs

The parasite used to infect mice is the *P. berghei* ANKA strain. The mice used were BALB/c strain mice obtained from the Laboratory of Experimental Animals, Department of Biochemistry, Faculty of Medicine, Airlangga University. Male mice, weighing 25-30 grams, and \pm 7 weeks old were used in this study. Antimalarial drug of dihydroartemisinin-piperazine phosphate (DHP) at 187.2 mg/kg BW (Mersipharma, Sukabumi, Indonesia) was used as a therapeutic dose as a control [13]. The VCO used was Javara brand VCO with BPOM RI certificate MD 207928001388.

P. berghei ANKA Infection in Mice

Ten donor mice were infected with 0.2 ml of *P. berghei* ANKA-infected frozen blood per mouse. When parasitemia reached \pm 25%, mice were sacrificed and the blood were infected to the test mice.

Experimental Design

Experimental animals were treated according to international guidelines for the care and use of laboratory animals [14]. A total of 30 mice were infected intraperitoneally with 1×10^6 of *P. berghei*-infected erythrocytes. The mice were then randomly divided into five groups, and each group consisted of 6 mice. The details of each group were as follows: PC group: positive control group

were given DHP 187.2mg/kg BW, NC: negative control group, mice were only given sterile water; G1 group: mice were given 1 ml/kg BW of VCO; G2 group: were given 5 ml/kg BW of VCO, and G3 group: mice were given 10 ml/kg BW of VCO. Treatments were given for 4 consecutive days started from day three post infection. Parasitemia was determined daily on Giemsa-stained tail blood smear [14]

Determination of Parasitemia and Inhibition

Parasitemia was calculated based on the number of infected erythrocytes in counted erythrocytes observed. The formula for parasitemia is as follows:

$$\text{Parasitemia (\%)} = \frac{\text{Number of infected erythrocytes} \times 100}{\text{Counted erythrocytes}}$$

The percentage of inhibition was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{NC} - \text{TG} \times 100}{\text{NC}}$$

NC: mean of parasitemia in negative control

TG: mean of parasitemia in treated-group (G1, G2, G3)

Statistical Analysis

Data were entered into a Microsoft Excel spreadsheet, exported, and analyzed using SPSS. Comparison between treatment, negative and positive control mice was assessed using one-way analysis of variance (ANOVA) for normally distributed data. When the ANOVA showed significant differences, Bonferroni's post hoc multiple comparison test, or Howell's Games was used to assess differences between groups. The Mann-Whitney U test was used to assess differences when the data were not normally distributed. The difference was considered statistically significant at the 95% confidence level ($p < 0.05$).

RESULTS

VCO-treated mice resulted in parasitemia lower than that of NC group, but higher than that of PC group. The DHP antimalarial drug in PC was 100% inhibited the growth of parasite and reached 0% parasitemia on day 4 post treatment. On the other hand, the NC group showed the highest parasitemia which was 23%. The G1 group showed little lower parasitemia than that of NC, followed by G2. However, mice in G3 group showed parasitemia lower than that of G2 group (Figure 1). Based on the mean parasitemia on day 4 of NC, the inhibition of VCO to the growth of parasite was calculated and presented in Table 2

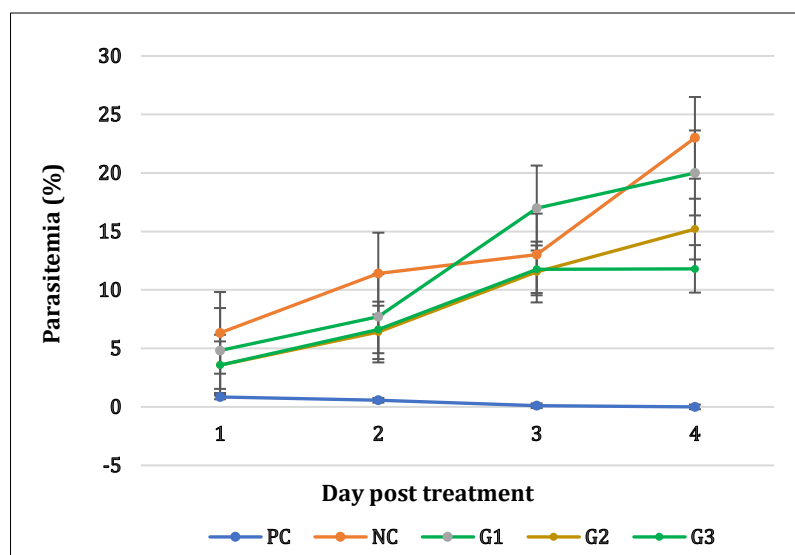


FIGURE 1: The growth of parasites post VCO administration

TABLE 2: Inhibition of VCO on the growth of parasite

Groups	Parasitemia on Day 4 (%) \pm SD	Inhibition (%)
PC	0	100
NC	23 \pm 2.40	
G1	20 \pm 2.70	13.04
G2	11.8 \pm 2.60	48.70
G3	15.2 \pm 5.10	33.91

DISCUSSION

This study has proved antimalarial activity of VCO in mice infected with *P. berghei* ANKA. Parasitemia in mice treated with VCO were lower than that of negative control (NC) group, indicated that VCO was able to inhibit the growth of parasites. Further, the inhibition of VCO was seen in G2 group was higher than that in G3, while in G1 was lower. The data, indicated that VCO at the dose of 5 ml/kg BW was more effective to inhibit parasite growth than lower or higher doses. But it did not mean that the higher dose of VCO is toxic, because there was no mouse died due to VCO administration. The effect of VCO on reducing parasitemia was due to the phenol compounds as anti-oxidants that have anti-plasmodium activity which are able to inhibit the growth of parasite. Some phenolic compounds have been tested against *P. berghei* in mice [15] as well as *P. falciparum* in vitro [16][17].

During malaria infection, the high production of ROS causes the decrease in antioxidant levels in the body, causing oxidative stress, which can cause high levels of free radicals. *Plasmodium* is sensitive to oxidative stress; however, the parasite is susceptible to free radicals and antioxidants [10]. When free radicals in the body is excessive, additional antioxidants are needed [18]. Exogenous antioxidants can be obtained from natural ingredients [11]. VCO can be a source of exogenous antioxidants due to the antioxidant activity of VCO, which was contributed majority by phenolic acids content [19].

Besides, exogenous antioxidants can be obtained from food intake, such as vitamin C, vitamin E, flavonoids, and carotenes [18]. Antioxidants have redox properties that are able to absorb and neutralize free radicals, reactive oxygen species and reduce peroxidation [20]. Antioxidants play a role in countering the effects of free radicals by inhibiting fat peroxidation that cause erythrocyte cell wall stronger and not easily ruptured [21]. Antioxidant content causes a decrease of ROS led reduction in the lipid peroxidation process and a decrease in malonaldehyde (MDA), and maintains the amount of endogenous antioxidant enzymes (superoxide dismutase) [22].

VCO also contains flavonoids that are able to inhibit oxidation reactions through a radical scavenging mechanism by donating one electron to an unpaired electron in a free radical [23]. In an in vitro study, flavonoids are strong inhibitors of lipid peroxidation, as scavengers of reactive oxygen or nitrogen species, and are also capable of inhibiting the activity of lipoxygenase and cyclooxygenase enzymes [21].

Alkaloid content in VCO is high [24]. The antioxidant effect of alkaloid seemed to be higher than that of phenols [25] depend on its source. Alkaloid can be isolated from various parts of medicinal plants and many of them have been reported to have antimalarial activity [26][27][28].

VCO has been well known as a dietary supplement with aim of reducing the risk of certain non-communicable diseases (NCDs) [29]. The VCO has also been proved to have hepatoprotective effect in mice infected with *P. berghei* ANKA [30].

Furthermore, oxidative damage by free radicals is one of the causes of pathological abnormalities in malaria patients. Therefore, the use of antioxidant therapy as a supportive therapy is one of alternatives in supporting the healing process of malaria patients and may reduce various risks that could potentially occur in patients [31].

The production of VCO can be different in the origin of raw materials [24] as well as their preparation that is resulting in different quality and quantity of contents [29], therefore the effect of different VCO found in the market may give different results.

CONCLUSION

VCO can reduce parasitemia in mice infected with *P. berghei* ANKA due to the antioxidant content, and therefore can be an antioxidant source for supporting antimalarial therapy consideration.

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