



Antimalarial activity of parenteral administration of eurycomanone – artesunate combination in *Plasmodium berghei* infected mice

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ABSTRACT

Malarial multiple-drugs resistant parasite is the main problem in treatment of malaria and need efforts in finding and development of new effective antimalarial drugs. Combination of antimalaria with synergistic activity is one of recommendation of World Health Organization by using artemisinin based combination therapy. This study is aimed to evaluate the antimalarial activity of eurycomanone alone and in combination with artesunate on *Plasmodium berghei* strain ANKA infected mice. In vivo antimalarial activity were done by using modified 4-days suppressive test. One hundred adult male albino mice were randomly divided into ten groups with 10 animals each. Experimental mice consist of malarial infected mice (group I) that only received aqua pro injection. Malarial infected mice in groups II to VI received 3.75; 7.50; 15; 30 mg/kg BW/day of eurycomanone and 4 mg/kg BW/day of artesunate, respectively. Group VII to X are malarial infected mice which were given combination of eurycomanone-artesunate according to the doses above and all treated drugs used intramuscularly for four days. The result of this study have been shown that eurycomanone possessed ED_{50} value 16.29 mg/kgBW. The percentage of growth inhibition of *P. berghei* by combination of eurycomanone-artesunate with doses 30 mg/kgBW-artesunate 4mg/kgBW is 99.34%. Based on statistic analysis this combination can be used as potential antimalarial drug.

Keywords: antimalarial combination, eurycomanone, artesunate, intramuscular, *Plasmodium berghei*

INTRODUCTION

Increasing of morbidity and mortality rate of malaria infection taken placed in nearly all developing countries and one of them caused of resistant parasite to antimalarial drugs[1]. The mechanism of resistance is due to a genetic mutation that occurs naturally, so that the malaria parasite is able to avoid the attack of drugs and still be alive[2]. One of the efforts which is recommended by World Health Organization (WHO) to prevent the emergence of resistance by using the combination therapy, particularly in combination with artemisinin derivatives as known artemisinin based combination therapies[3]. One of the artemisinin derivatives are most commonly used in combination with other antimalarial is artesunate that currently used as first-line therapy in case of infection by chloroquine-resistant *P. falciparum*[4]. In vivo antimalarial activity of eurycomanone and its combination with artesunate on *Plasmodium berghei* infected mice (*Mus musculus*) were done to evaluate their potency in combat malarial infection. Eurycomanone and its combination with artesunate are used with a few variations of doses eurycomanone. Eurycomanone is a pentacyclic quassinoid from pasak bumi or tongkat Ali roots (*Eurycoma longifolia*, Jack) in the family of Simaroubaceae[5,6,7]. Previous studies have been investigated that eurycomanone possessed a promising *in vivo* and *in vitro* antimalarial activity[6,7,8]. The other study of *in vitro* antimalarial activity

of eurycomanone on chloroquine-resistant *P. falciparum* (strain W-2) has IC₅₀ value 14.912 ng / mL and on chloroquine-sensitive *P. falciparum* (strain D-6) 26.094 ng / mL [9]. The potency of eurycomanone in growth inhibition of chloroquine-resistant *P. falciparum* (strain W-2) also have been shown by Kardono *et al.* (1991) with IC₅₀ value 48.1 ng / mL and 47.7 ng / mL on chloroquine-sensitive *P. falciparum* (strain D-6) [10]. Yusuf *et al.* (2013) has been investigated that the most potential eurycomanone from Kalimantan (Borneo) possessed antiplasmodial activity with IC₅₀ value 0.0047 nM on chloroquine-sensitive *P. falciparum* (3D7 strain) [11]. Based on the things stated above, it is predicted that this important research is done in effort to determine the potential antimalarial activity of eurycomanone-artesunate combination. The results of this study are expected to provide information about the activity of the eurycomanone-artesunate combination and hoped this information useful for development of antimalarial drug.

EXPERIMENTAL SECTION

Reagents and chemicals

Eurycomanone with formula C₂₀H₂₄O₉ (MW 408.20) was isolated from pasak bumi (*Eurycoma longifolia*, Jack) root extract. The chemical structure of eurycomanone was determined by detailed spectroscopic analysis. Its purity is 87.26% (LCMS-ESI Positive Ion). Artesunate was purchased from Sigma Aldrich. All reagents and chemicals that used in this study were analytical grade. Methanol; Giemsa; Aqua pro injection; Dimethyl sulfoxide; Immersion oil, all of them were purchased from Merck, except Saline 0.9% (Otsuka).

Preparation of tested drugs

Eurycomanone and artesunate were dissolved in 0.5% dimethyl sulfoxide and diluted with aqua pro injection to obtain the different doses for tested drugs. The tested drugs were filtered aseptically by using sterilized microfilter 0.22 μm. The amount of drug given was calculated based on the body weight. The initial dose of eurycomanone was based on ED₅₀ values of pasak bumi roots extract from previous study (unpublished). The dose of artesunate at 4 mg/kg BW was chosen based on in vivo antimalarial study (unpublished).

Experimental animal ethic

The used of experimental animals in the present study were approved by Committee Ethic of Medical Research, Faculty of Medicine University of Syiah Kuala Banda Aceh, Indonesia with number 305/KE/FK/2015.

Experimental animals

Adult male albino mice 8 to 10 weeks old, weighing between 25 to 30 g were obtained from The Animal House Unit of Medical Faculty, University of Gadjah Mada. One hundred mice were randomly divided into ten groups with 10 animals each. They were acclimatized in cages for 7 days at temperature 23 ± 2°C with 12 hours light and 12 hours dark. The mice were given food and water ad libitum and their sanitation was monitored daily. After acclimatization, then all the mice were transferred to the Laboratory six hours before the experiment started.

Inoculation of malarial parasite and in vivo antimalarial activity test

The malarial parasite *P. berghei* strain ANKA was obtained from Laboratory of Parasitology, Faculty of Medicine, University of Gadjah Mada. The cryopreserved parasites were thawed and inoculated intraperitoneally to infect five donor mice a week before the experiment. Then infected red blood cells (iRBC) were collected into heparinized tubes by cardiac puncture and the parasitemia was determined by using light microscope. The blood with parasitemia of 30%, was diluted with saline 0.9% until contained 5 × 10⁷. On day- 0, experimental mice were injected intraperitoneally with 0.2 mL of infected red blood cells that having 10⁷ *P. berghei*. In vivo antimalarial activity test of various doses of eurycomanone and its combination with artesunate was performed in 4-days suppressive test [12]. The whole groups as below:

- Group I (PbA) mice, received aqua pro injection
- Group II (PbA) mice, received eurycomanone 3.75 mg/kg BW
- Group III (PbA) mice, received eurycomanone 7.50 mg/kg BW
- Group IV (PbA) mice, received eurycomanone 15 mg/kg BW
- Group V (PbA) mice, received eurycomanone 30 mg/kg BW
- Group VI (PbA) mice, received artesunate 4 mg/kg BW
- Group VII (PbA) mice, received eurycomanone 3.75 + artesunate 4 mg/kg BW

Group VIII(PbA) mice, received eurycomanone 7.50 + artesunate 4mg/kg BW

Group IX (PbA)mice, received eurycomanone 15 + artesunate 4 mg/kg BW

Group X (PbA)mice, received eurycomanone 30 + artesunate 4 mg/kg BW

All the groups were given tested drugs intramuscularly after twenty four hours inoculation of malarial parasites. The tested drugs were given as a single daily dose according to body weight on day-1 to day 4. The development of parasitemia in malarial parasite mice were monitored daily until day-5. Blood smears were made by taken a drop of blood through venesection of each mouse tail, onto the edge of a microscope slide. The blood was made a thin blood smear, dried and fixed with methanol. After drying at room temperature then staining with 10% giemsa solution. The number of infected red blood cells (iRBC) after treatment were observed under light microscope with 1000 times magnification. The percentage of parasitemia was calculated by formula:

$$\% \text{ parasitemia} = (\text{number of infected red blood cells} / \text{total red blood cells}) \times 100\%.$$

Then the percentage of malaria parasites growth inhibition was calculated as below:

$$\% \text{ inhibition} = \frac{\text{Parasitemia of negative control} - \text{Parasitemia of tested drug}}{\text{Parasitemia of negative control}} \times 100\%$$

Statistical Analysis

Parasitemia is a parameter which observed and the percentage of parasitemia used for calculation the percentage of malarial parasites growth inhibition. Both data were used for knowing the effective dose 50 (ED₅₀) of eurycomanone by probit regression analysis [13].

RESULTS AND DISCUSSION

The result of antimalarial activity test of eurycomanone and its combination with artesunate by intramuscular administration have shown the Effective Dose (ED₅₀) value of eurycomanone 16.29 mg/kgBW. The percentage of malarial parasite growth inhibition have been shown below (Table 1 and Figure 1)

Table 1. Mean percentage of malarial parasite growth inhibition of eurycomanone and its combination with artesunate in *P. berghei* infected mice, assessed on day five post inoculation

Treatment	The Percentage of malarial parasite growth inhibition
I. Aqua pro injection	-
II. Eurycomanone 3.75 mg/kg BW	11.73
III. Eurycomanone 7.50 mg/kg BW	29.25
IV. Eurycomanone 15 mg/kg BW	58.06
V. Eurycomanone 30 mg/kg BW	78.89
VI. Artesunate 4 mg/kg BW	84.79
VII. (Eurycomanone 3.75 + Artesunate 4) mg/kg BW	54.82
VIII.(Eurycomanone 7.50 + Artesunate 4) mg/kg BW	74.89
IX. (Eurycomanone 15 + artesunate 4) mg/kg BW	96.19
X. (Eurycomanone 30 + artesunate 4) mg/ kg BW	99.34

This study proved the treatment with eurycomanone in various doses to *P. berghei* infected mice showed a dose dependent. The percentage of malarial parasite growth inhibition of eurycomanone and artesunate in groups II to VI are 11.73%; 29.25%; 58.06%; 78.89% and 84.79%, respectively.

In eurycomanone-artesunate combination groups VII to X exhibited the percentage of malarial parasite growth inhibition 54.82%; 74.89%; 96.19%; and 99.34%. The combination of eurycomanone-artesunate showed a higher percentage of malarial parasite growth inhibition compared to control group ($p < 0.05$). The difference is probably related to the synergistic activity between eurycomanone and artesunate. Beside that intramuscular drug administration is a rapid treatment route where drugs absorbed directly into bloodstream. The polarity of tested drugs also play an important role in drug absorption.

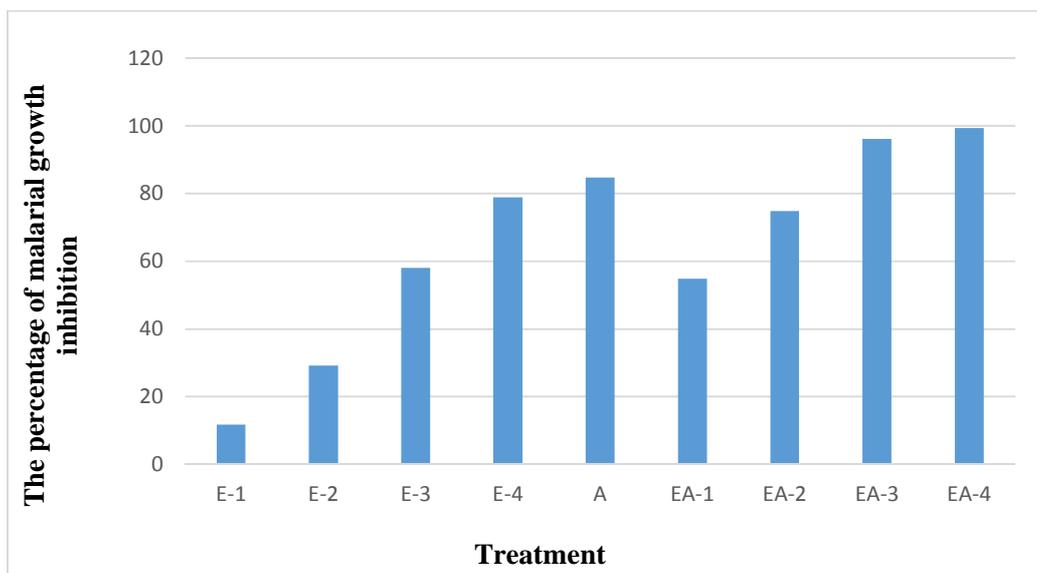


Figure 1. The correlation of various doses of eurycomanone, artesunate to percentage of malarial parasite growth inhibition on day-5, intramuscularly. E-1 = eurycomanone 3.75 mg/kgBW; E-2 = eurycomanone 7.5 mg/kgBW; E-3 = eurycomanone 15 mg/kgBW; E-4 = eurycomanone 30 mg/kgBW; A = artesunate 4 mg/kgBW; EA-1 = eurycomanone 3.75 mg/kgBW-artesunate 4 mg/kgBW; EA-2 = eurycomanone 7.5 mg/kgBW-artesunate 4 mg/kgBW; EA-3 = eurycomanone 15 mg/kgBW-artesunate 4 mg/kgBW; EA-4 = eurycomanone 30 mg/kgBW-artesunate 4 mg/kgBW

Study about antimalarial activity of pasak bumi roots extract (TA164) by Ridzuan *et al.* (2007) through subcutaneous drug administration at dose 10 mg/kg BW significantly inhibited the growth of *P. yoelii* 67% and artemisinin 64% compared to control mice group. The combination of 10 mg/kg/BW of pasak bumi roots extract (TA164) with 1.7 mg/kg BW artemisinin significantly inhibited the growth of *P. yoelii* is 80% compare to control mice, but no significant if compared to artemisinin [14].

CONCLUSION

The Effective Dose (ED₅₀) value of eurycomanone as antimalarial in mice is 16.29 mg/kgBW intramuscularly. The growth inhibition of *P. berghei* by eurycomanone 30 mg/kg BW is 78.89% and its combination with artesunate 4 mg/kg BW 99.34%.

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