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Antimalarial Activity and Survival Time of *Andrographis paniculata* Fraction (AS202-01) on *Plasmodium berghei* Infected Mice.

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ABSTRACT

The rapid spread of resistance encourages the search for new active compounds. *Andrographis paniculata* commonly known as sambiloto was empirically used as traditional medicine in Indonesia to cure malaria. This study aims to determine antimalarial activity and survival time of *A. paniculata* fraction from 70% ethanolic extract (namely AS202-01) on *Plasmodium berghei* infected mice. Antimalarial in vivo study was conducted based on Peter's 4-days suppressive test. Thirty healthy Balb/c male mice, divided into 6 groups (n = 5), were infected intravenously with 1×10^6 parasitized erythrocytes of *P. berghei*. Infected mice were treated at a dose of 6.25, 12.5, 25 and 50 mg/kg BW, twice a day for four days, the untreated group of mice received CMC-Na 0.5% and the control group treated with chloroquine (10 mg/kg BW). Thin blood smears giemsa staining were made every day for seven days and observed under microscope to count parasitemia and calculate inhibition of parasite's growth. Probit analysis was conducted to determine effective dose (ED₅₀) value. Observation of antimalarial effect of AS202-01 was continued until 14 days to evaluate survival time of infected mice. The results showed that AS202-01 was inhibited *P. berghei* growth with ED₅₀ value of 6.75 mg/kg BW. It was classified as a highly active antimalarial substance. The AS202-01 was also significantly able to increase survival time of infected mice compared to untreated group.

Keywords: *A. paniculata* fraction (AS202-01), antimalarial, survival time, *Plasmodium berghei*

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INTRODUCTION

Malaria is one of the most important infectious diseases in the world. This disease is caused by Plasmodium species and transmitted by the bite of female Anopheles mosquito [1]. Estimated 214 million people infected malaria in the world. Despite the decrease of malaria infection about 18% from 2000 to 2015, but a new problem is antimalarial drug resistant Plasmodium parasites and the emergence of insecticide resistant Anopheles [2]. Resistant not only the spread of malaria to new areas but also its reemergence in areas where it had previously been eradicated [3]. Therefore, the search for new antimalarial, either synthetic or natural, is important for killing of malaria parasites [4].

The use of medicinal plant extract for treatment of malaria has a long and successful tradition [5]. For example, quinine was isolated from *Chinchona* species (Rubiaceae) and artemisinin from *Artemisia annua* [6]. Diterpenoids, flavonoids, saponin, polyphenols, alkaloids, kaempferol, and acetogenin from plants were known to have antimalarial activity [7, 8]. This explains why a lot of current researches focus on natural and plant-derived products as they can be sourced easily, can be locally available, and can be selected on the basis of their pharmacological use [9, 10].

Andrographis paniculata commonly known as sambiloto was empirically used as traditional medicine in Indonesia to treat people suffering from malaria. It also possesses antimicrobial effect, antiinflammatory, antioxidant, antidiabetic, antiHIV, antifungal, hepatoprotector, antifertility and anti cancer [11, 12]. The major constituents of *A. paniculata* is andrographolide, a diterpene lactone compound, which has antimalarial activity both in vitro and in vivo [13]. Andrographolide inhibited *P. berghei* growth with ED₅₀ value of 3.82 mg/kgBW [14]. The ethyl acetate fractions of *A. paniculata* from 70% ethanolic extract (AS202-01) is an andrographolide rich fraction. Previous study reported that andrographolide content of AS202-01 was higher among other extracts and fraction [15]. This study aims to determine antimalarial activity and survival time of AS202-01 on *Plasmodium berghei* infected mice.

MATERIALS AND METHODS

Plant materials:

A. paniculata dried powder with batch number PP01J1125027 was obtained from PT. Kimia Farma (Persero) Tbk, Bandung, Indonesia on February 2015

Experimental animals:

Male mice BALB/C strain were obtained from LPPT-Universitas Gajah Mada, Yogyakarta. They were weighting between 20-30 g and maintained on standard animal pellets and water ad libitum at Animal Laboratory of Institute of Tropical Disease, Universitas Airlangga. Permission and approval for animal studies were obtained from Faculty of Veterinary Medicine, Universitas Airlangga No:489-KE/2015.

Rodent malaria parasite

Plasmodium berghei ANKA strain was originally obtained from Eijkman Institute for Molecular Biology, Jakarta. The parasite has been maintained at Institute of Tropical Disease, Universitas Airlangga by a combination of passage in male BALB/C mice and cryoscopic storage.

Methods:

Procedure 1: Preparation of *A. Paniculata* fraction (AS202-01)

A. paniculata dried powder was extracted by maceration method using ethanol 70% as a solvent. Liquid ethanol extract then evaporated using rotary evaporator. The concentrated extract then further separation by liquid-liquid fractionation using ethyl acetate and water to obtain ethyl acetate fraction namely AS202-01.

Procedure 2: In vivo antimalarial activity test

In vivo antimalarial activity was performed based on Peter's test (The 4-days suppressive test) [16]. AS202-01 was tested using 30 mice which divided to 6 groups (n=5): untreated group (CMC-Na 0.5%), control group (chloroquin), AS202-01 dose of 6.25 mg/kg BW, AS202-01 dose of 12.5 mg/kg BW, AS202-01 dose of 25 mg/kg BW, AS202-01 dose of 50 mg/kg BW.

Each mice was infected intraperitoneally with 0.2 ml *P. berghei* (1×10^6). Infected mice were treated at a dose of 6.25, 12.5, 25 and 50 mg/kg BW, twice a day for four days. The untreated group of mice received CMC-Na 0.5% and control group (chloroquin at dose 10 mg/kg BW). Thin blood smears were made every day for 5 days (day-0 until day-4) and stained using 10% giemsa dye. Thin blood smear were observed under microscope to count the percentage of parasitemia and calculate inhibition of parasite's growth.

Procedure 3: Determine of mean survival time (MST)

Determination of MST was performed based on Somsak et al, 2016 [4]. The survival time of *P. berghei* infected mice was observed during 14 days (day-0 until day-13).

Data Analysis**In vivo antimalarial activity test**

Percentage of parasitemia and percentage of inhibition growth of *P. berghei* were calculated using the formula below.

Percentage of parasitemia:

$$\frac{X_e}{X_k} \times 100\%$$

Percentage of inhibition:

$$100\% - \left(\frac{X_e}{X_k} \times 100\% \right)$$

Xe: % parasitemia growth of experimental group

Xk: % parasitemia growth of untreated group

Inhibition percentage was analyzed using Probit analysis SPSS to determine Effective Dose (ED₅₀) value.

Determine of mean survival time (MST)

The mean survival time (MST) was calculated using the formula below.

$$MST = \frac{\text{Sum of survival time of all mice in a group}}{\text{Total number of mice in that group}}$$

RESULTS AND DISCUSSION**Antimalarial Activity**

Andrographis paniculata belongs to the family Acanthaceae that has been used as traditional medicine in Indonesia to cure malaria. Many reports are also available on the antimalarial activity of *A. paniculata*. Mishra (1992) was reported that crude ethanol extract and fractions of *A. Paniculata* inhibited *P.*

berghei growth. Methanol, chloroform, and petroluem ether extract of *A. paniculata* inhibited *P. falciparum* growth on gametocytes and schizont stage [17,18].

The present study assessed the antimalarial activity of AS202-01, *A. Paniculata* fraction obtained from 70% ethanol extract of *A. Paniculata*. The results showed than AS202-01 inhibited parasite (*P. berghei*) growth. The average of parasitemia percentage of infected mice which treated by AS202-01 on first-day observation until fifth day (D0-D4) tended to increase, but it was still lower than untreated group, this is showed that AS202-01 inhibited the growth of *P. berghei* (ANKA strain) in mice. The results are summarized in table 1.

Table 1. Activity of AS202-01 on *P. berghei* infected mice

Group	Dose (mg/kgBW)	% Parasitemia					% average Inhibition parasite's growth
		H0	H1	H2	H3	H4	
Negative Control		0.21±0.04	2.16±1.06	5.14±1.07	7.90±1.33	10.46±2.20	-
Chloroquin	10	0.32±0.15	0.31±0.11	0.13±0.05	0.20±0.15	0.23±0.24	99.47±0.79
AS202-01	6.25	0.19±0.06	1.05±0.50	2.09±0.70	3.29±0.23	4.86±0.24	48.33±2.25
	12.5	0.30±0.11	1.26±0.40	2.19±0.61	3.07±0.44	4.24±0.26	58.46±1.27
	25	0.19±0.10	0.91±0.37	1.31±0.47	2.28±0.78	3.33±0.10	64.81±1.27
	50	0.27±0.09	0.73±0.46	1.16±0.31	1.82±0.55	2.86±0.15	70.16±0.78

AS202-01 : *A. paniculata* fraction from

H0-H4 : Day of observation

The data on table 1 showed that inhibition parasite's growth followed a dose dependent manner. The data of inhibition parasite growth was analyzed using log-probit to determine Effective Dose (ED₅₀) value. The result analysis showed that AS202-01 has ED₅₀ value of 6.75 mg/kg BW, it was highly active as an antimalarial substance based on criteria Munoz et al (2000) [19]. The period of activity of andrographolide was found evidently on the ring stage of the parasite. The point of action of this compound in the parasite life cycle corresponds with the protein and nucleic acid synthesis [13]. *A. paniculata* extract and andrographolide includes inhibition of the nuclear transcription factor-kappa B (NF-κB), making it a therapeutic target for the treatment of cancer and autoimmune diseases [20]. The role of NF-κB is also important in malaria as mentioned in a recent study [21]. Plasmodium infected erythrocytes have shown to induce NF-B (kappa) regulated inflammatory pathways in human cerebral endothelium. NF-κB is activated and translocate into nucleus, where it binds to the DNA regulatory site to regulate specific gene expression, especially cell signaling for parasite growth and development. Therefore, the inhibition of transcription factor of *A. paniculata* extract against *P. berghei* ANKA might be a critical process to inhibit blood stage propagation of parasites in vivo [12, 20].

This study results showed that chloroquine had antimalarial activity higher than AS202-01, its inhibition parasite's growth with the value of 99.47%±0.79 at a dose of 10 mg/kg BW. Meanwhile, AS202-01 at the highest dose (50 mg/kg BW) inhibited parasite's growth with the value of 70.16%±0.78. The difference of these activities might be due to the life time of Andrographolide was 2-7 hours, and rapidly elimination within 3-4 hours after administration[22], and the life time of Chloroquine was 150 hour or 6 day on healthy people.

Survival Time

The result showed AS202-01 had higher survival time than negative control. AS202-01 at all doses was capable of significantly increasing the survival time. Survival time was showed dose dependent manner. The higher of AS202-01 dose, then survival time was higher as well. The results are summarized in table 2.

The survival time longer than 12 days by the test compound is regarded as active [23]. AS202-01 at a dose of 12.5 until 50 mg/kg BW was performed survival time more than 12 days. Moreover, survival time which observed was almost as chloroquine for AS202-01 at the highest dose (50 mg/kg BW).

The data on table 2 also showed that the higher of inhibition parasite's growth, then survival time was higher as well. It was possible related to the immune system of infected mice. *A. paniculata* was reported as immunosuppressive agent [24]. Based on the facts, AS202-01 might play a role in immune system which inhibits parasite's growth and then the condition was associated with increased survival time.

Table 2. Survival time of AS202-01

Group	Dose (mg/kgBW)	% average Inhibition parasite's growth	Survival time (Day)
Negative control		-	9.6±2.7
Chloroquin	10	99.47±0.79	14±0
AS202-01	6.25	48.33±2.25	11.8±3.03
	12.5	58.46±1.27	12.6±2.19
	25	64.81±1.27	13.2±1.79
	50	70.16±0.78	13.8±0.45

AS202-01: *A. paniculata* fraction

CONCLUSIONS

AS202-01 was active as an antimalarial on *Plasmodium berghei* infected mice with ED₅₀ value of 6.75 mg/kg BW and classified as a highly active antimalarial substance. The AS202-01 was significantly able to increase survival time compared to negative control.

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