Sys Rev Pharm 2020; 11(6): 380 – 383
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

In Vivo Antimalarial Effect of Yellow Root Stem (*Fibraurea tinctoria* Lour) on *Plasmodium berghei*

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Article History: *Submitted: 11.04.2020* *Revised: 12.05.2020* *Accepted: 18.06.2020*

ABSTRACT

Background: Limitations of antimalarial chemotherapy demonstrate the importance of the discovery of new drugs. Indonesia has a high diversity of plants and some have been demonstrated to have activity as an antimalarial. Fibraurea tinctoria Lour (FT) stem are widely found in East Kalimantan. Secondary metabolites i.e. alkaloids, flavonoids, polyphenols and terpenoids/steroids were found from the phytochemical screening of ethanol extract of yellow root stem FT.
Objective: To determine in vivo antimalarial effect of yellow root stem FT against Plasmodium berghei.
Methods: Yellow root stem FT was extracted with methanol. Mice were inoculated with Plasmodium berghei ANKA which injected intraperitoneally. Parasitemia degree was quantitated for 5 days.
Results: There were significant difference found at therapy group treated with 10 mg/g BW extract combined with artemisinin, 1 mg/b BW extract combined with artemisinin and only artemisinin against the reduction degree of parasitaemia at $p < 0.05$.
Conclusion: Therapy group under treatment of methanol extract of yellow root stem FT combined with artemisinin was proven to have effect on in vivo antimalarial activity.
Keywords: Antimalarial, fibraurea tinctoria Lour, plasmodium berghei - in vivo
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DOI: 10.31838/srp.2020.6.60

INTRODUCTION

The intensity and speed of antimalarial drugs resistance highly depend on the consumption intensity of antimalarial drugs. Higher intensity on consuming antimalarial drugs accelerates the resistance to those drugs (Mustofa, 2009; WHO, 2012). Limitations of antimalarial chemotherapy indicate the importance on the discovery of new drugs which ideally aimed at medicating new target. Some approaches regarding with discoveries and development of antimalarial drugs have been carried out including optimizing therapy through existing drugs, developing the existing analogue drugs, discovering new drugs from natural sources and discovering active compound that works on new target. The most innovative approach for chemotherapy is identification on new target and identification on compound that works on the target (Roshental, 2003; Herlina et al., 2007).

Indonesia has a high diversity of plants and some of those have been proven to have activity as an antimalarial (Elfita et al., 2011; Zein et al., 2013). Stem of *Fibraurea tinctoria* Lour is widely available in East Kalimantan where the local people call it as yellow root (Kulip, 1997; Ilona, 2003; Sangat et al., 2003).

Plasmodium berghei is a unicellular protozoa that is a parasite and one of the many species of malarial parasites that infect mammals. Plasmodium berghei is an ideal model for the study of malaria parasites compared to three species of rodentia parasite in West Africa because it can be done on a large scale, the data on gene structure mapping, methods for genetically modifying parasites, and the presence of distinctive clones and lines mutants that are genetically modified (Muti'ah et al., 2010). This in vivo antimalarial activity experiment used Plasmodium berghei ANKA. Plasmodium berghei will be injected intraperitoneally into mice (Mernvanga and Veronique, 2012).

RESEARCH METHOD

Testing Animals

This research used male albino mice strain Balb/c, aged 8-12 weeks with 28-32 grams of weight. The animals were taken care based on ethical conduct standard approved by Medical Faculty, Brawijaya University. Prior starting the experiment, testing animals were acclimated for one week to avoid stress. This research protocol used testing animals which had been approved by Research Ethical Commission of Medical Faculty, Brawijaya University.

Equipment

Some equipment used in this research were: vessel for powder maceration, Mettler Analytical Balance H31 AR, rotary evaporator, porcelain plate, vertical laminar air flow, centrifuge, culture flask 50ml, tweezers, micropipette, microscopes, syringe 1 ml, laboratory bottle 100ml, glass object, nitrogen liquid tank, disposable pipettes 2 ml, flakon tube 5 ml, Eppendorf tube, measuring cup tube, pipette glass, Erlenmeyer tube, stomach probe, sterile scissors, glass objects and measuring glass tube.

Materials

Materials used in this research were: Methanol, distilled water, yellow root stem powder, *Plasmodium berghei* isolates: NaCl 12%, NaCl 1.6%, NaCl 1.9%, glucose 0.2%, Complete media 15%, medium RPMI, Gentamicin, Nabic, HEPES solution, O blood serum, PBS 10% and aquadest. Artesunate (*Wanhui Double Crane Pharmaceutical*, China), 0.5% cmc Na solution, blood taken from mice infected with malaria, buffer Giemsa, Giemsa, PA methanol and oil emersion.

Method

Making the Yellow Root Stem (*Fibraurea tinctoria* Lour) Methanol Extract

The stem simplisia of yellow root plant (*Fibraurea tinctoria* Lour) was put into vessel, poured with methanol 80%, covered and allowed at its condition for 5 days. After 5 days, the mixture was filtered and the supernatant was washed with filtering liquid at adequate amount. Macerat was replaced into a closed-vessel at a cool place for 2 days and then sieved. Macerat was then vapored at low pressure at temperature not more than 50^{o}C until reached expected consistency. Extract

was then placed into oven at temperature 50°C until reached thick extract.

Thawing Isolate *Plasmodium berghei*

Parasite isolates were obtained from *liquid Nitrogen Tank* added with NaCl 12%, 1/5 volume (3 gr) which dropped using spuit and waited for 5 minutes. NaCl 1.6% 9x volume (0.4 gr) was added which dropped using syringe, waited for 5 minutes and centrifuge for 5 minutes with 2000 rpm velocity. Supernatant was discharged and 0.9% NaCl (0.225gr), 0.2% glucose 9x volume (0.04gr) were added which dropped using syringe and waited for 5 minutes. Centrifugation was conducted again for 5 minutes at 2000 rpm. Supernatant was discharged again and added with CM (*Complete media*) 15% 6 ml and placed into flask culture. All procedures related with isolate *P.berghei* was conducted in aseptic laminar air flow vertical.

Inoculation of *Plasmodium berghei*

Inoculation of *Plasmodium berghei* was conducted intraperitoneally (i.p) as 10^7 parasites in 0.2 ml of blood for each mice. The number of erythrocytes per ml blood and parasitemia of the donor mice that would be transferred were counted first. As many as 10 µL of blood infected by *Plasmodium berghei* was taken and then diluted by 10^4 x using PBS solution. The number of erythrocytes was counted in *eri Naubauer* counting chamber to determine the number of erythrocytes/ml blood by using (N x 5 x 10^4 x dilution) formula where N was the number of erythrocytes. The following step was to count the parasites number of the donor mice by multiplying the number of erythrocytes/ml blood with the percentage of parasitemia.

Measuring the Parasitaemia Degree

Blood smear of mice was created. The smear was then fixed using methanol until dry. Giemsa staining was carried out by mixing fluka Giemsa and buffer Giemsa (1: 9 comparison). Staining was done for 20 minutes and then rinsed with flowing water until no remaining paint left and then dried. Parasitemia was checked using microscope with 1000x magnification by counting number of erythrocytes infected by malaria from total 1000 erythrocytes. Parasitemia (%) was number of erythrocytes infected by *Plasmodium berghei* from total 1000 erythrocytes.

Data Analysis

In order to determine the difference of parasitaemia degree between control and treatments groups, One Way Anova (Analysis of Variance) was conducted. This research was significant different if $p < 0.05$. If significant difference occurred, Post Hoc analysis with Tukey test was conducted.

RESULT AND DISCUSSION

Stem of *Fibraurea tinctoria* Lour contained secondary metabolite alkaloids, flavonoids, polyphenols and terpenoids/steroids (Fikriah, 2008). Compound from alkaloid has been known to have ability to inhibit the growth of Plasmodium through choline intracellular transport and terpenoids has ability to inhibit the growth of Plasmodium through inhibiting protein synthesis (Hayati *et al.*, 2012). In vivo antimalarial test aims to determine the degree on reducing parasitaemia. This study was carried out using five groups i.e. positive control group, 10 mg/g BW extract group, combination between 1 mg/g BW extract and artemisinin group, combination between 10 mg/g BW extract and artemisinin group and artemisinin 0.04 mg/g BW group. The objective of this research was to determine the reduction on the degree of parasitaemia. Test of in vivo antimalarial activity was carried out using Zein et al. (2013) method which modified.

Based on the test on in vivo antimalarial activity of yellow root *(Fibraurea tinctoria* Lour*)* stem extract by observing 5 treatments from day 0^{th} to 5^{th}, it was found that the reduction of parasitaemia degree were different.

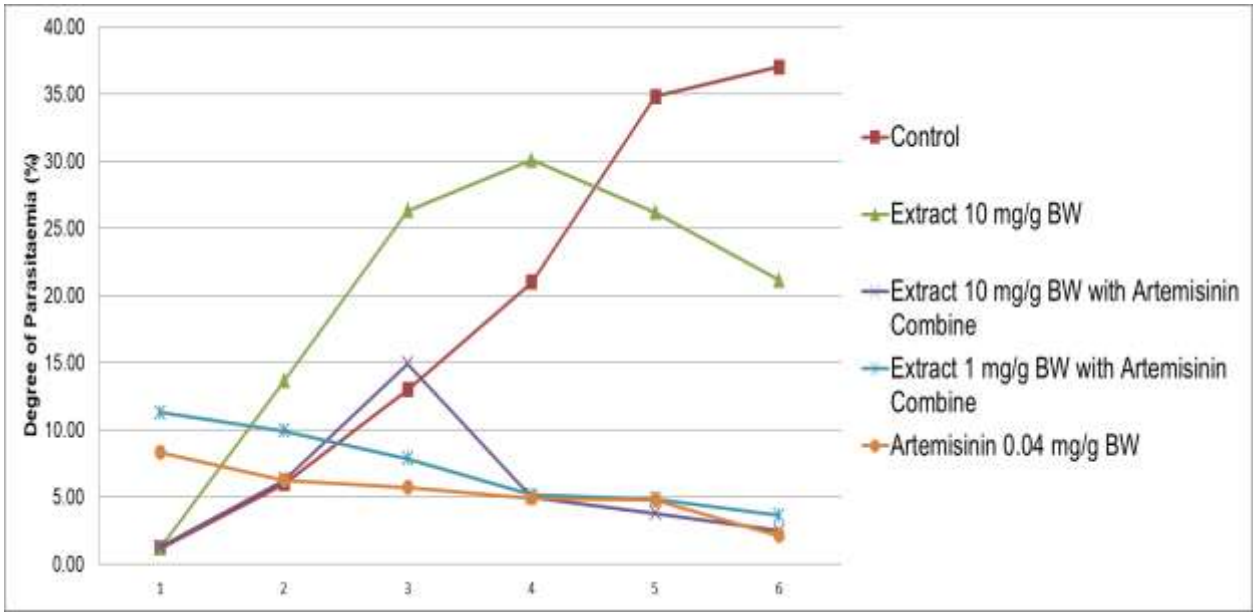

Figure 1: Degree of Parasitaemia Reduction

Figure 1, at positive control group, there was no reduction on the degree of parasitaemia from the 0th to 5th day. The longer the mice were infected, the degree of parasitaemia increased. Group of 10 mg/g BW extract didn't show reduction on the degree of parasitaemia from the 0th to 5th day. Group of 10 mg/g BW combined with artemisinin showed reduction on the degree of parasitaemia. Group of 1 mg/g BW combined with artemisinin showed reduction on the degree of parasitaemia. Group of artemisinin treatment showed reduction on the degree of parasitaemia. This indicated that the growth of *Plasmodium berghei* on mice was inhibited by the therapy of extract combined with artemisinin (Figure).

In general, One Way ANOVA (Analysis of Variance) showed that there were significant difference ($p < 0.05$) among groups. Tukey Test analysis resulted that there were significant difference ($p < 0.05$) found at therapy group under combination of 10 mg/g BB extract and artemisinin, 1 mg/g BW extract and artemisinin and artemisinin compared with positive control group. The reduction on parasitaemia degree under the therapy of 10 mg/g BW extract and artemisinin was better than group treated with only artemisinin. This could be due to the active compound that contained in *Fibraurea tinctoria* Lour methanol extract. This active compound was assumed to have synergistic effect with the antimalaria compound i.e. artemisinin. No significant difference was found in 10 mg/g BW extract. At this treatment, the extract still contained rough stem extract of *Fibraurea tinctoria* Lour. This condition caused the antimalarial activity was not specific due to the influence of other active compound that existed in thus rough extract.

Intraperitoneal infections have lower infection ability than naturally. Blood circulation (peripheral blood) is the first place P. berghei into the body of the host that is in the form of sporozoit. The cells of the innate immune system are the first body defenses against microorganisms. Parasites that enter the bloodstream will partially be phagocytosed by neutrophil cells (Schmidt, 2011). The number of parasitic cells that have escaped the circulation of blood is less affected by the infectious ability of the parasite and individual reactions to P. berghei infection are not the same (Fujioka and Aikawa, 2005). There are various influencing factors, such as genetic variation, metabolism, and immune system of each host. The successful parasite will enter the liver to continue its life cycle (Miller et al., 2002).

Some escaped sporozoites will go to the liver. Sporozoites enter the sinusoidal lumen in the same direction or against the bloodstream. This sporozoit can attack Kupffer cells. Kupffer cells are the macrophages present in the liver. If it passes through the cell Kupffer sporozoit will pass through the Disse room to infect the hepatocytes. The irradiation given makes the sporozoites weak so it is difficult to fight Kupffer cells (Frevert et al., 2005). In addition to liver, the spleen is used for the identification of Plasmodium infections. The spleen works against the antigens in the blood. Plasmodium has a defense system to avoid the lymphatic immune system. Plasmodium that escapes the lymphatic immune system causes vascular occlusion within the spleen so that the spleen will enlarge (Mohanty et al., 2005).

Fibraurea tinctoria Lour is species from Menispermaceae genes which has antiplasmodial activity with IC_{50} value ranging from 0.4 – 8.6 µg/ml (Pouplin et al., 2007). The main alkaloid contents of *Fibraurea tinctoria* Lour are Palmatine, Jatrorrhizine and Berberine (Onguene et al., 2013). The antioxidant activity represented by the effective concentration-50 (EC_{50}) value of *Jatrorrhizine* is 98.0 µg/ml while the EC_{50} of *Palmatine* is higher than 100 µg/ml (Keawpradub et al., 2005). Alkaloid group compound has been known having ability to inhibit the growth of parasites through inhibiting cholineintracellular transport thus parasites membrane is not formed and parasite die-off (Hayati et al., 2012).

Choline transporter becomes the target of drug and route for drug administration (Biagini et al., 2004). At infected-erythrocyte, choline enters host erythrocyte membrane through the endogenous choline career or through New Permeation Pathway (NPP) parasite. New Permeation Pathway (NPP) is the new membrane transportation pathway with increased permeability compared with normal erythrocyte membrane permeability. At parasites, NPP is useful in providing the needed nutrients, remove metabolic waste, maintain cytoplasm volume and change the ionic composition of cytoplasm of erythrocytes (Kirk, 2001). Similar with antimalarial choline analog, the medicine enters the host erythrocyte membrane through the NPP and accumulated in the intracellular parasite (Biagini et al., 2004).

CONCLUSION

Combination therapy consisted of yellow root *Fibraurea tinctoria* Lour stem methanol extract and artemisinin were proven to have effect on in vivo antimalarial *Plasmodium berghei.*

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