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EFFECT OF BIOACTIVE FROM *Phyllathus niruri* ON BEFORE AND DURING INFECTION OF *Plasmodium berghei* IN MICE

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INTRODUCTION

Malaria is a health problem in many countries, including Indonesia. The problem is difficult to be solved due to resistance on many antimalarial drugs. Therefore, alternative treatment is needed to be developed. *Phyllanthus niruri* before infection can increase the activity of macrophages in mice were infected by *Plasmodium berghei* but there has been no research after infection. This study aims to compare the effect of *P. niruri* between before and after infection with malaria.

MATERIALS AND METHODS

Design of research is posttest only control group. The subject are mice strains Swiss, males, 5 weeks old, weighing 20-25 g, have never been infected by Plasmodium. Independent variable: prophylaxis treatment by *P. niruri* extract (P1); curative treatment by *P. niruri* extract (P2); chloroquine (K+) ; untreated (K-). Dependent variable: parasitemia, spleen and brain histologic.

Twenty mice were divided into four groups so each group consist of five mice. After five days of acclimatization, mice were infected by *P. berghei* in 0.1 ml dose contains 1 x 10^6 P. berghei *i.p.* (Pedroni *et al.*, 2006). Curative treatment was done 5 days after 24 hours infection while prophylaxis was done 5 days before infection. The dosage of Chloroquine is 2 mg/kg BW, 2 mg/kg BW and 1 mg/kg BW respectively in 3 days. The dose of *Phyllanthus niruri* is 1,3 gram/ day (Periyanayagam *et al.*, 2008).

Observation of parasitaemia was done every day for 5 days after infection. It was conducted by examination to the blood smears that colored by Giemsa's. The last day of the study all the mice were sacrificed, taken for spleen and brain histopathological preparation. The difference of parasitemia density among study groups were analyzed by one way anova and histologics appearence were analyzed by nonparametrics analysis *Kruskall-Wallis*.

RESULTS AND DISCUSSION

Result of parasitaemia examination during 1-6 days after infection is presented in Figure 1.

One way ANOVA shows that there is a significant difference on parasitaemia among all study groups ($F_{calculte}$ = 19,634 > F_{table} = 3,34). The analysis continued DMRT (*Duncan's Multiple Range Test*). The result was presented in Table 1. It shows that treatment by *P. niruri* effective to suppress the growth of parasite in blood cell.

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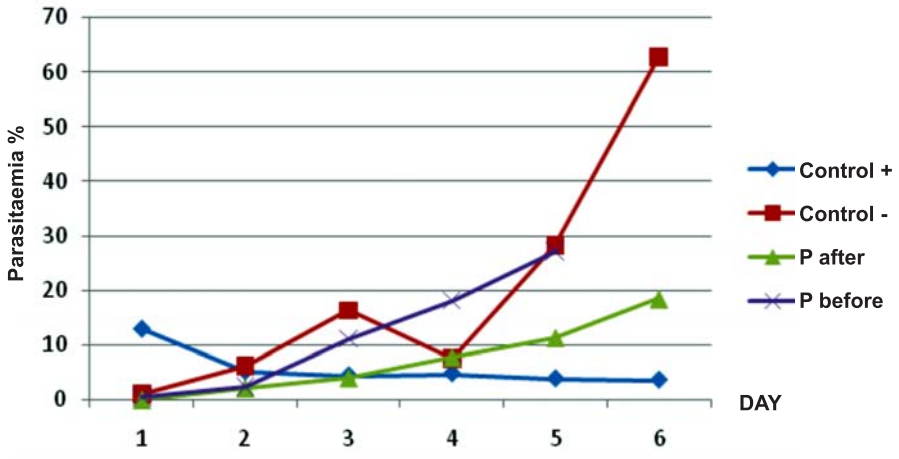

Figure 1. Average of parasitaemia after infection in all study groups

Table 1. Significancy of difference among the study groups

Between Groups	P value
K+ (positive control) – K- (negative control)	0,000*
K+ (positive control) – P2 (after infection)	0,773
K+ (positive control) – P1 (before infection)	0,797
K- (negative control) – P2 (after infection)	0,000*
K- (negative control) – P1 (before infection)	0,000*
P2 (after infection) – P1 (before infection)	0,974

Histology's appearance of spleen and brain examination was conducted to know the influence of *Phyllanthus niruri* towards immune response against malaria. It assumed granting *P. niruri* can promote macrophages activity in spleen.

Table 2. Histological appearance of spleen and brain tissue at 7th day after P. berghei infection in all study groups

Tissue	K- (untreated)	K+ (Chloroquine)	P1 (*P. niruri* before infection)	P2 (*P. niruri* after infection)
Spleen	M<; R	M>	M>	M>
Brain	-	-	-	M

Note: M=macrophage; <=slightly; >=many; R= inflammation cell infiltration

The histological study shows that there were inflammation reaction in spleen tissue in all groups of study proven by increasing the number of macrophage (Table 2). In untreated group there was a tendency in other inflammatory cells in addition to macrophages. While in brain tissue, macrophages only found in P2 (*P. niruri* after infection).

According to Kaur *et al.*, (2005) and Sandhya *et al.*, (2006), *Phyllanthus niruri* contains derivate polyphenol, including tannin and gallic acid. Although the mechanism on the activity of this extract has not been examined, some of these metabolites are believed to have the effect of antiplasmodial. The mechanism is suggested through increasing oxidation of red blood cells and inhibition of the synthesis of protein. Possible natural antiplasmodia in the

extract of *P. emblica*, *T. bellerica* and *T. chebula.* is gallic acid (Rakotondramanana *et al.*, 2007; Ramanandraibe *et al*., 2008) and tannin (Reddy *et al.*, 2007; Dell'Agli *et al*., 2009).

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