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The Effect of *Garcinia parvifolia* Miq (Active Fraction) on Phagocytosis by Peritoneal Macrophages During *Plasmodium berghei* Infection in Mice

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Abstract: The aim of this study was to assess the active fraction of the stem bark of *G. parvifolia* Miq to *P. berghei* on total red cells of mice and the number of intraperitoneal macrophage phagocytocide latex after infected by *P. berghei*. Three doses of active fraction of *G. parvifolia* at the dose of 25, 50, 100 and 200 mg kg⁻¹ b.wt., respectively were used in this study and chloroquine was used as the positive control, while distilled water was used as a negative control. Phagocyte activity was measured by the ability of mice peritoneal macrophages to phagocytize latex particles *in vitro*. The result of this research indicated that at the dose of 200 mg/kg/day could reduce the number of parasite in the blood up to 54.90%. From the results indicated that the reduction of the number of parasite which circulated in the blood occurs at the doses of 50 and 100 mg/kg/day, although its activity is much lower than malaria drug of chloroquine. Macrophages activity (87.19%) increased at the dose of 200 mg/kg/day, compared to the control group (52.99%), these indicated that the fraction of *G. parvifolia* induced immune response. The increase of the phagocyte activity of the macrophage was stimulated by the active fractions of *G. parvifolia* (dose dependent) which was given to the *P. berghei*-infected mice.

Key words: Macrophages, phagocyte, *P. berghei*, active fraction

INTRODUCTION

Malaria remains one of the most infamous widespread diseases in the world. It is estimated that there are 300 to 500 million clinical cases of malaria every year and 1.7 to 2.5 million deaths (WHO, 1996). Recently, interest in plants as the potential sources of antiparasitic drugs was encouraged by the isolation of artemisinin from *Artemisia annua*, which was traditionally used in Chinese medicine (Dua *et al.*, 2004).

Macrophages as the trigger in the cellular immune system play a role in the eradication of plasmodium, mainly in the erythrocyte stage. After the macrophages are activated by gamma interferon (IFN γ) produced by native T, they change into phagocyte to do the phagocytosis, to eliminate the parasites (Abbas *et al.*, 1994; Yaneto *et al.*, 2003). The macrophage phagocytosis activities could be activated with the administration of immunostimulant drugs, not only in the form of vaccine but also certain natural chemicals (Subramaniam *et al.*, 2000). A pharmacological screening of n-hexane, ethylacetate and n-butanol extracts of the stem bark of *G. parvifolia* Miq (Clusiaceae) exhibited significant activity against the drug-resistant clone of *P. falciparum* (FCR-3) and the sensitive clone of *P. falciparum* (3D7) (Soesanto *et al.*, 2007). In this study, the effect of the *Garcinia parvifolia* (active fraction) on the evaluation of experimental *P. berghei* infection and on the activation of infected macrophage *in vitro* was assessed.

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MATERIALS AND METHODS

Preparation of Extracts

The stem barks of *G. parvifolia* Miq were collected in Nang Kalis Village, Borneo, Indonesia, June 2005 and were identified by comparison with authentic specimens at the Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Science and specimen samples were preserved. The stem bark was dried, powdered and macerated with dengan n-hexane (1, 5 L×3), using rotavapor to dry and evaporate to solvent, to obtain 9 g dry extract. After that, The n-hexane dry extract was fractionated with methanol and then separated with a centrifuge (5°C, around 10 min), to obtain the extract in the methanol as the solvent (7.2 g) (fraction A). The active fraction in 2% gum acacia was pulverized into a fine paste and prepared into a suspension of the desired concentration with 2% gum acacia for oral administration.

Animals

Male Swiss albino mice, 4-6 weeks' old, from the Litbangkes were used. The animals were divided into 5 groups of 50 mice each. The mice were infected with 0.1 mL of the *P. berghei* suspension at a concentration of 1.0×10^7 parasite per mouse on day 0. The drug was administered intraperitoneally for 4 days from day 0 to 3 at doses 25, 50, 100 and 200 mg kg⁻¹ in the experimental group. The control group was given the solvent in equal volume for the same duration (Peters, 1987). During the experimental period, the animals were housed under standard laboratory conditions with *ad libitum* water and balanced food.

No. of Parasitaemia Analysis

The method of Giemsa Blood Smear was used to count the number of the parasites. The blood in the periphery was taken from the tails of mice and prepared to a sample of thin-and-thick-smear-blood method with Giemsa coloring. The number of parasitemia was calculated by determining the percentage of red-blood cells infected by *P. berghei* in 1000 red-blood cell (Markell *et al.*, 1987).

Macrophage of Phagocytocyte Analysis

The test of non-specific phagocytosis activity was conducted in vitro, in reference to Leijh *et al.* (1986), using latex particles with the diameter of 3 μm. The latex particles were resuspended in PBS to obtain the concentration of 2.5×10^7 mL⁻¹. Peritoneum macrophages, cultured a day before, were washed twice in the RPMI medium and then added with latex suspension of 200 μL/well and incubated in a CO₂ 5% incubator, 37°C, for about 60 min. After that, the cells were washed with PBS 3X to remove the unphagocytosed particles, dried in the room temperature and fixated with absolute methanol. Once dried, the cells attached to the cover slip were colored with Giemsa 20%. The percentage of cells phagocytizing latex particles and the number of phagocytized latex particles were counted from 100 cells, using a light microscope with the zoom of 400x (Leijh *et al.*, 1986).

Acute Toxicity Assay

The LD₅₀ of *G. parvifolia* (active fraction) was determined in mice using the Lorke method (Lorke, 1983). Fifty Swiss mice of both sexes weighing 20-25 g were divided randomly into 5 groups of 10 animal each (5 males and 5 females). The tested Extracts were prepared to suitable dose levels in water solution. The test groups received a single dose intraperitoneally of the extracts at different concentrations. The control group was treated with distilled water, used in all extracts testing. The LD₅₀ was expressed as the 50% lethal dose, which corresponds to the dose leading 50% deaths, 14 days after extract administration.

Statistical Analysis

The results are expressed as mean±SEM. The group differences were analysed in each week using non-parametric analysis of variance (one-way ANOVA), followed by the Tukey test or Kruskal-Wallis test and then by the Dunns test, for multiple comparisons. The level of significance used was 0.01.

RESULTS

The *in vivo* Effect of *G. parvifolia* (Active Fraction) on Phagocytosis

The drug showed a dose dependent stimulation of phagocytosis of opsonized *Plasmodium*. At an optimum dose (200 mg kg⁻¹) the phagocytosis was 87.19%, compared to 52.99% in the control mice. The treatment also increased the activity and capacity of macrophages in the peritoneal cavity (Table 1).

The *in vivo* Antimalarial Activity of *G. parvifolia* (Active Fraction)

The procedure followed is the classical 4 day suppressive test of Peters (1987) where the tested compound is administered during four days to the malarial mice. With a dose of 200 mg/kg/day, parasitaemia is reduced by 54.90% (Fig. 1). Three mice out of five survived more than 20 days, while the other four mice (treated with 0.9% saline solution) died after 4 days (Lorke, 1983).

Acute Toxicity Assay

Daily feeding for 15 days with *G. parvifolia* (active fraction) 2,000, 4,000 and 8,000 mg kg⁻¹ did not result in any change in general behaviour of the animals. Body temperature and state of the excretion were also not influenced by the drug treatment. The weights of some organs (liver, kidneys and spleen) as well as food and water intake were not significantly altered by the drug administration.

Table 1: Effect of *G. parvifolia* Miq stem bark (active fraction) treatment to mice on phagocytosis by peritoneal macrophages

<i>G. parvifolia</i> , active fraction (mg kg ⁻¹)	No. of macrophages/ mouse ($\times 10^6$)	Phagocytosis (%)
0 (control)	17.0±1.2	52.99±4.7
25	19.2±0.8*	61.48±2.8*
50	27.2±1.3**	69.16±3.5*
100	46.8±4.1**	78.19±3.5**
200	53.6±1.5**	87.19±4.5**

Values are mean±SD n = 5, *p<0.01, **p< 0.001

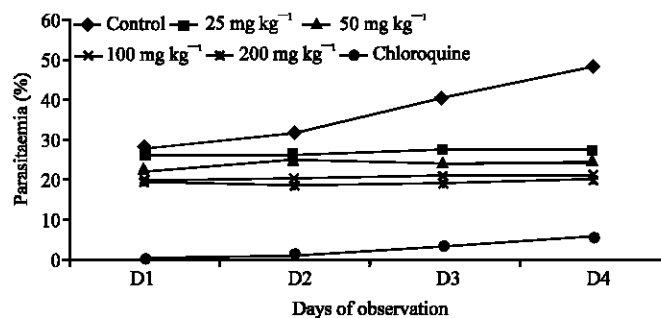


Fig. 1: Effect *Garcinia parvifolia* Miq (active fraction) on established infection

DISCUSSION

This study shows for the first time that the stem bark of *G. parvifolia* Miq extracts have *in vitro* and *in vivo* antimalarial activity (Soesanto *et al.*, 2007). Since macrophages have a major role in stimulating immune function against malarial infection. Other studies are still in progress in this laboratory to identify the effect of the orally-administered active fraction on the activity and the capacity of *P. berghei*-infected macrophages.

The *in vivo* Effect of *G. parvifolia* (Active Fraction) on Phagocytosis

The phagocytosis response includes the phagocytosis activity (the number of active phagocyt in 100 phagocyt cells) and phagocyt capacity (the number of phagocytized plasmodium in 50 active phagocyte cells). The 87.19% increase of activity and phagocytosis capacity of phagocyt cells in the dose group of 200 mg/kg/day might be caused by the chemical components in the active fraction stimulating the lymphocyte cells to mature and self-divide into lymphocyte B and T producing limfokin (cytocin/interleucine) to keep macrophage active. Not only can the activated macrophage produce lisozyme enzyme and the complements, but also increase their capacity to kill through the phagocytosis process on the plasmodium. In the control group, the activity of phagocyt cells was low because the phagocyt cells were not induced.

The *in vivo* Antimalarial Activity of *G. parvifolia* (Active Fraction)

In Fig. 1, the parasitemia deterrent of active fraction of 200 mg/kg/day dose (54.9%) is still lower than chloroquine of 5 mg kg⁻¹ dose (88.35%). The ED₅₀ (effective dose 50) is 74.54±10 mg kg⁻¹ BW. The antimalaria activity of the active fraction could be caused by the chemical components in the stem bark of *G. parvifolia*. Although a number of xanthoness from genus *Garcinia* have been isolated and tested for their antimalaria activity, none of these studies involved *G. parvifolia*. Likhitwitayawuid *et al.* (1998a) isolated five xanthoness from the bark of *G. cowa* and evaluated for their antimalaria activity. The results showed that cowaxanthone was the most active against *P. falciparum* with an IC₅₀ of 1.5 µg mL⁻¹ (Likhitwitayawuid *et al.*, 1998a). The others xanthoness from *G. dulcis*, were also evaluated for their antimalaria activity. Among five xanthoness tested, garcinia xanthone exhibited the most active with an IC₅₀ of 0.96 µg mL⁻¹ (Likhitwitayawuid *et al.*, 1998b). It was suggested that antimalaria activity of stem bark extracts of *G. parvifolia* was due to its xanthoness constituents.

Four mice died in the control group on the second day. The death was influenced by the passage stage or the parasite transfer process from a mouse to another. The greater the passage stage, the greater the virulence. The administration of the active fraction seemed to prolong the life of the mice proportional to the administered dose. Perhaps this is because the active fraction have the effect of antiplasmodium as well as increasing the immune system

Acute Toxicity Assay

The limit dose of 8 g kg⁻¹ did not cause any mortality or any signs of acute toxicity in any of the ten mice tested in the short term (i.e., 24 h) and long term (i.e., 14 days) observatory period. No mortality was also observed when varyng doses 2000, 4000 and 8000 mg kg⁻¹ of *G. parvifolia* (active fraction) were administered orally. The results of the acute toxicity study indicated that the LD₅₀ of *G. parvifolia* (active fraction) is greater than 8000 mg kg⁻¹ oral.

The present results suggest that the control of the infection may be due to the activation of the cellular response in the animals treated with active fraction. When the macrophages are activated, they may have additional effects on *P. berghei*, through the release of pro-inflammatory cytokines. The primary target of most of the immunomodulatory compound is believed to be the macrophages which play a key role in the generation of an immune response (Fenichel and Chiriges, 1984).

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