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The Effects of *Garcinia parvifolia* (Miq) Miq Extracts Against Liver Damage of *Plasmodium berghei* Infected Mice

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Abstract: The study aimed at determining the effects of the stem bark extracts of *Garcinia parvifolia* administered orally to *P. berghei* infected mice in varied dosages to observe Malondialdehyde (MDA) level, SGOT and SGPT and its relationship with liver cell damage and degree of parasitemia. Thirty mice of Swiss strain used in the experiment were assigned into 5 treatment groups: Group 1 (control group) consisted of *P. bergheii* infected mice reacted with physiological salt solution; group 2 (normal) consisted of uninfected mice; group 3 consisted infected mice treated with stem bark extracts of *G. parvifolia* with a dosage of 200 mg/kg BW/day; group 4 consisted of infected mice treated with stem bark extracts of *G. parvifolia* with a dosage of 400 mg/kg BW/day and group 5 consisted of infected mice treated with stem bark extracts of *G. parvifolia* with a dosage of 800 mg/kg BW/day. The test preparation was administered orally for 4 days. After 4 days of administration, MDA level was measured in the plasma using a spectrophotometer and three mice were killed to obtain the liver for histopathological purpose. The study found 400 mg kg^{-1} BW that during malaria infection, MDA production increased and liver damage tended to be milder. Administration of stem bark extract of *G. parvifolia* in oral way could reduce MDA, SGOT and SGPT level ($p<0.05$), particularly with a dosage of 400 mg and 800 mg/kg BW/day.

Key words: Malondialdehyde, *P. berghei*, mice, liver histopathology, malaria, *G. parvifolia*

INTRODUCTION

Malaria is an infectious disease that remains a leading health problem in the world including in Indonesia. Household health survey in 2002 found that during the period of 1998-2001. Indonesia had been in several episodes of malaria extraordinary incidence (KLB) which affected 121 villages in 42 districts of 11 provinces. They included North Sumatera, West Sumatera, Lampung, Kepulauan Seribu, West Java, Central Java, East Java Nusa Tenggara Barat and Nusa Tenggara Timur with total cases of 29.345 including 483 deaths.

In malaria infection, phagocytic cells actually release a number of soluble factors as an antimalarial response. They are ROS and cytokine (Clark *et al*., 1987). However, a number of recent studies had proved that ROS played a role in tissue damage in various forms of malaria pathology (Hunt *et al*., 1992). The description explicitly proves that whether ROS (Reactive Oxygen Species) played a role as antimalaria or as a trigger of pathologic disorder was still under through investigations by the experts. The aim was to explore the potential of antioxidant therapy as antimalaria against complication. Intensive efforts had been made by some researchers to find new antimalarial agent from medicinal plants including Garcinia genus during the last decade. Some research had identified antimalarial activities of the plants of this genus including garciniaxanthone and cowaxanthone from *G. dulcis* and *G. cowa* both of which had been known to have powerful antimalarial properties with respective IC_{50} values of 0.96 and 1.5 μg mL^{-1} (Likhitwitayawuid *et al*., 1998a, b).

Some preliminary studies identified powerful antimalarial activities in the stem bark of *G. parvifolia* both *in vitro* and *in vivo* (Soesanto *et al*., 2007) and identified antioxidant effects of some parts of *G. parvifolia* (Syamsudin *et al*., 2007). The recent study utilized animal model of malaria to determine Malondialdehyde (MDA) production and its relationship with the damage of liver cells in *P. berghei* infection as well as to determine the effects of stem bark extracts of *G. parvifolia* administered orally in various dosage levels.

MATERIALS AND METHODS

Preparation of extracts: The stem bark extracts of *G. parvifolia* were prepared with Percolation Method. Stem bark extracts of *G. parvifolia* were drenched with ethanol in a beaker glass for 3 h. The extracts were then

carefully moved into percolator under constant press. Then, ethanol-extracting solution was added and let out for 24 h. The percolates were concentrated to form thick extracts by using a vacuum evaporator.

Equipments: Percolator, glass, microscope, spectrometer UV-Shimadzu, animal stall, syringe, rotavapor.

Inoculation of mice: Inoculation was conducted to three *P. berghei* infected donor mice. Blood samples were taken from the three donor mice. The blood was taken from the sinus orbitalis by using hematocrit capillaries that had been treated with heparin. The blood samples were put into tubes. The three blood samples were examined for parasitemia in this case, a parasitemia with a degree of 30% could be transmitted to other mice.

***In vivo* antimalarial assay:** Experimental animals used for the study were 30 male mice of Swiss strain, ±2 months of age and 20-35 g of bodyweight. The *P. berghei* infected mice were randomly assigned into 5 groups:

- Group 1: Control group (treated only with physiological solution)
- Group 2: Treatment group, treated with stem bark extract of *G. parvifolia*, 200 mg/kg/day for 4 days
- Group 3: Treatment group, treated with stem bark extract of *G. parvifolia*, 400 mg/kg/day for 4 days
- Group 4: Treatment group, treated with stem bark extract of *G. parvifolia*, 800 mg/kg/day for 4 days
- Group 5: Treatment group, treated with chloroquine, 5 mg/kg/day for 4 days

The treatment groups received the treatment dosage orally for 4 days (Peters, 1987). In each day (from day 1-4), peripheral blood smears were prepared for parasitemia examination.

Biochemical studies: The blood was obtained from all animals by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters namely SGOT and SGPT.

MDA level: MDA level was measured from the blood plasma according to the Method of Wills. About 2 mL of sample solution (blood plasma) was added into 1 mL of 20% Trichloroacetic Acid (TCA) and 2 mL of 0.067% Thiobarbituric Acid (TBA). The solution was homogenously mixed through boiling for 10 min. The cooled solution was then centrifuged at 3000 rpm for 10 min. Pink filtrates were measured for absorption at λ 530 nm using UV-Vis spectrometer (Will, 1987). MDA level was measured using MDA standard curve with respective concentrations of 0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6. Liver tissues were put into 10% formalin for histopathology examination.

Serum hepatospecific markers: Activities of Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) were estimated by the Method of Reitman and Frankel (1957). About 0.05 mL of serum with 0.25 mL of substrate (aspartate and α-ketoglutarate for SGOT, alanine and α-ketoglutarate for SGPT in phosphate buffer pH 7.4) was incubated for an hour in case of SGOT and 30 min for SGPT 0.25 mL of DNPH solution was added to arrest the reaction and kept for 20 min in room temperature. After incubation, 1 mL of 0.4 N, NaOH was added and absorbance was read at 505 nm in uv-vis spectrophotometer activities were expressed as 1 UL.

RESULTS AND DISCUSSION

Examination of the blood smears provided parasitemia degree for each treatment and control group as shown in Fig. 1.

The study revealed that the degree of parasitemia increased with the observation time (day 1 through day 4). In the control group (malaria), a declining trend was observed on day 4 compared to that of treatment groups. Measurement of MDA level was determined by observing staining reaction with Thiobarbituric Acid (TBA). The underlying principle of the method is that acid and heat lead to decomposition of lipid peroxide and eventually to MDA formation. MDA that had been formed react to TBA that lead to stain changes as measured with a spectrophotometer. Data on the measurement of MDA level in each treatment group are shown in Table 1. The

Table 1: Effect of extract *G. parvifolia* (Miq) Miq on serum GOT, GPT and MDA in infected mice

	Serum		
Treatments	SGOT (U L^{-1})	SGPT (U L^{-1})	Liver (MDA (nmol mg^{-1} protein))
Control	48±4.35	58±1.27	3.0±0.004
Infected	97±3.75	97±2.35	5.2±0.006
Extract dose 200 mg kg^{-1}+infected	87±2.35	84±1.41	4.7±0.002
Extract dose 400 mg kg^{-1}+infected	79±1.35	79±1.73	3.9±0.007
Extract dose 800 mg kg^{-1}+infected	67±1.41	61±1.31	3.2±0.003
Chloroquine	57±1.81	62±1.30	3.3±0.002

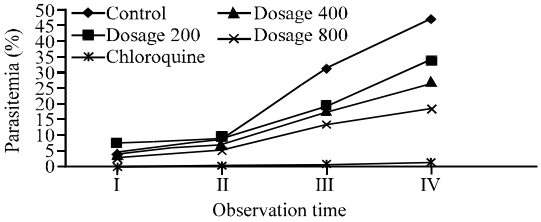

Fig. 1: Development of parasitemia in each group with oral administration

result findings shown in Fig. 1 was comparable to those shown in Table 1. In Table 1, MDA level in malaria group (negative control) was found to be higher that that of treatment groups. This means that lipid peroxidation increased during the infection episode. Lipid peroxidation referred to oxidative deterioration of long-chain unsaturated fatty acid (Polyunsaturated Fatty Acid or PUFA). PUFA in the plasma membrane was prone to oxidation by superoxide radical ($O_2^{-}\bullet$) through free radical reaction chain called lipid peroxidation. End product of lipid peroxidation was hydrocarbon and carbonyl strains (C = O), particularly aldehyde such as Malondialdehyde (MDA). MDA could be used as a standard in determining the lipid peroxidation level in the tissues or in the organs (Halliwel and Gutteridge, 1998).

During the malaria infection episode, phagocytic activities tended to increase and eventually increase the release of ROS (Reactive Oxygen Species) such as superoxide ($O_2^{\bullet}$) and Hydrogen peroxide (H_2O_2) of monocytes and neutrophils. Therefore, infected erythrocytes would be sequestered between the hepatic macrophages and the process would stimulate oxidative reaction and free radical production. In biochemical parameter investigation, the group of *P. berghei* infected mice indicated increasing activity of GOT and GPT enzymes. GOT and GOT enzymes are markers or damaged liver functions. MDA level in blood plasma of mice was found to be significantly correlated to the degree of parasitemia but no severe damage was observed in the liver tissues. Liver damage was characterized with hypertrophy and mild but extending sinusoid as well as mild necrosis on day 4th (Fig. 2 and 3).

Administration of 800 mg/kg BW/day stem bark extract of *G. parvifolia* could reduce the degree of parasitemia and MDA level to a greater extent that those of *P. berghei*-infected mice. Histopathologic profile revealed that mice treated with 800 mg/kg BW/day stem bark extract of *G. parvifolia* had milder necrosis and less striking increase of Kupffer cells compared to mice not treated with the same extracts. Likewise, the activities of GOT and GOPT enzymes decreased with increasing doses of the extract administered. The decline of parasitemia degree and MDA level as well as improvement in liver histopathology as observed in the group of mice treated

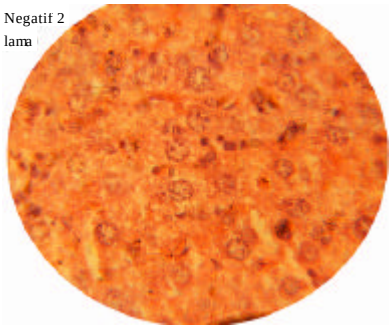

Fig. 2: Liver tissues of *P. berghei* infected mice

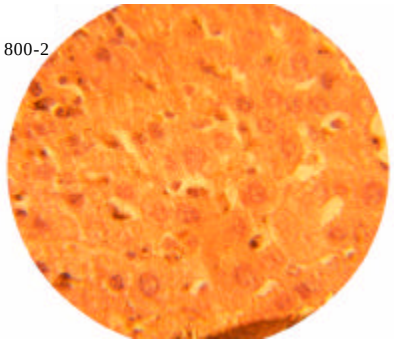

Fig. 3: Liver tissues of *P. berghei* infected mice after the treatment with 800 mg kg^{-1} of stem bark extract of *G. parvifolia*

with stem bark extract of *G. parvifolia* might be attributable to the active compounds that provided antimalarial effects in the stem bark extract of *G. parvifolia*. Extract filering from all parts of *G. parvifolia* showed that the extract of stem bark had an antioxidant activity with IC_{50} <100 μg mL^{-1} as found by using DPPH Method (Syamsudin *et al*., 2007). Other studies found that stem bark extract of *G. parvifolia* in a dosage of 1000 mg kg^{-1} administered by Intra-peritoneal (IP) injection had % growth inhibition of 80.09% compared to 94.5% chloroquine in *P. yoelii* infected mice (Soesanto *et al*., 2007).

CONCLUSION

Stem bark extract of *G. parvifolia* had an anti-plasmodial activity and improved liver damage in *P. berghei* infected mice.

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REFERENCES

Clark, L.A., N.H. Hunt, G.A. Butcher and W.B. Cowden, 1987. Inhibition of murin malaria (*P. chaubaudii*) *in vivo* by recombinant interferon or tumor necrosis factor and its enhancement by butylated hydroxyamide. J. Immunol., 139: 3483-3499.

Halliwel, B. and J.M.C. Gutteridge, 1998. Free radica in biology and medicine. 3rd Edn., Oxford University Press, New York.

Hunt, N.H., M. Koop and R. Stockler, 1992. Free Radicals and Antioxidants in Malaria In: Biochemstry and Clinical Application, Ong, A.S.H. and L. Packer (Eds.). Birkhauser verlag, Basel, pp: 337-353.

Likhitwitayawuid, K., T. Phadungcharoen and J. Krungkai, 1998a. Antimalarial xanthones from Garcinia cowa. Planta Med., 64: 70-72.

Likhitwitayawuid, K., W. Chanmahasathien, N. Ruangrungsi and J. Krungkai, 1998b. Xanthones with antimalarial activity from *Garcinia dulcis*. Planta Med., 64: 281-282.

Peters, W., 1987. Chemotherapy and Drugs Resistance in Malaria. Vol. I, Academic Press Inc., New York, ISBN: 978012552, pp: 145-273.

Reitman, S. and S. Frankel, 1957. Colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. Am. J. Clin. Pathol., 28: 56-63.

Soesanto, S., T. Subagus W. Darmono and Mustofa, 2007. *In vitro* and *in vivo* antiplasmodial activities of stem bark extracts of *Garcinia parvifolia* Miq. Int. J. Trop. Med., 2: 41-44.

Syamsudin, S. Kumala and B. Sutaryo, 2007. Screening of some extracts from *Garcinia parvifolia* Miq. (Guttiferae) for antiplasmodial, antioxidant, cytotoxic and antibacterial activities. Asian J. Plant Sci., 6: 972-976.

Will, E.D., 1987. Evaluation of Lipid Peroxidation on Lipid and Biological Membranes. In: Biochemical Toxicology, Snell, K. and B. Mullock, (Eds.). 5th Edn., Press Oxford, Washington DC, pp: 139.