Research Journal of Pharmacology 1 (1): CCCC, 2008
ISSN: 1815-9362

In vivo Antiplasmodial Activity and Acute Toxicity of the Fraction of the *Garcinia parvifolia* Miq. Stem Bark

[1]Syamsudin, [2]S. Wahyuono, [3]S. Tjokrosonto and [4]Mustofa
[1]Department of Pharmacology, [2]Department of Biology Pharmacy,
Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia
[3]Department of Parasitology, [4]Department of Pharmacology and Toxicology,
Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia

Abstract: The study of *in vivo* antiplasmodial activity and acute toxicity of the active fraction of *Garcinia parvifolia* Miq has been conducted. The fraction was obtained by maceration of n-hexane extract with methanol. A standard 4-day test on P. berghei infected Swiss mice was used to evaluate the *in vivo* antiplasmodial activity after an oral administration of the fraction in series dose of 25-200 mg/kg BW once daily for 4 consecutive days. The *in vivo* antiplasmodial activity was expressed by the dose inhibiting 50% of parasite growth (ED_{50}). Acute toxicity was evaluated in Swiss mice after oral administration of the active fraction in series dose of 2000-8000 mg/kg BW. The acute toxicity was expressed by the dose leading 50% deaths (LD_{50}). The results showed that the active fraction of *G. parvifolia* Miq was active against P. berghei in mice with an ED_{50} of 74.45 mg/kg BW/d. In addition, the active fraction was also relatively safe as expressed by the LD_{50} of 8.000 mg/kg BW.

Key words: Antiplasmodial, acute toxicity, active fraction, *Garcinia parvifolia* Miq

INTRODUCTION

Malaria is one of the most important parasitic diseases of subtropical and tropical countries. Malaria is responsible globally for 300-500 million cases of clinical diseases and it presents a public health problem for 2.4 billion people, representing over 40% of the world's population in over 90 countries. Estimates of malaria mortality vary from 1.5-2.7 million deaths worldwide per year with fatality rate being extremely high among young children below 5 years old and pregnant women (Hoffman *et al*., 2002; Phillips, 2001). In addition, since resistance of *Plasmodium falciparum* to currently used antimalarial such as chloroquine is spreading rapidly, the need to develop new and effective antimalarial agents is urgent. Plant-derived natural products are expected to play a seminal role in this regard, especially in view of the success with two important antimalarial agent quinine and artemisinin, both of which are derived from plants and are used in clinic.

In the endemic area in Indonesia where malaria prevails, medicinal plants are often used for malaria therapy such as *Brucea javanica* (L.) Merr. *Carica papaya* Linn, *Phyllanthus niruri* L., *Eurycoma longifolia* Jack., *Swietenia mahagoni* Jacq., *Azadirachta indica* Juss, *Alstonea scolaris* (L.) R. Br.. and *Garcinia* sp. (Mustofa *et al*., 2007; Murtiningsih *et al*., 2005; Leaman *et al*., 1995). Although some of *Garcinia* sp. viz. *Garcina cowa*, *G. dulcis* and *G. viellardi* have been reported to possess antiplasmodial activity (Likhitwitayawuid *et al*., 1998a; 1998b; Hay *et al*., 2004), none of these studies involved *G. parvifolia* Miq.

In our continuing search for natural compounds with antimalarial activities that could provide alternative to choloquine, we have evaluated the *G. parvifolia*, Miq. (Clusiaceae) for its *in vitro* antiplasmodial activity. In the preliminary study, the activity of three extracts of *G. parvifolia*, Miq. stem bark viz. n-hexane, ethylacetate and n-buthanol extracts have been evaluated. Among these three tested extracts, the n-hexane extract exhibited the highest antiplasmodial activity against both P. Falciparum Chloroquine-Resistant (FCR-3) and -sensitive (D10) strains with the IC_{50} ranging from 4.83-6.41 μg mL^{-1} (Syamsudin *et al*., 2007). In this study, we reported *in vivo* antiplasmodial activity and acute toxicity of an active fraction isolated from n-hexane extract of the *G. parvifolia* Miq. stem bark.

Corresponding author: Syamsudin, Department of Pharmacology, Faculty of Pharmacy, Pancasila University, Jl. Srengseng Sawah, Jagakarsa, Jakarta 12640, Indonesia

MATERIALS AND METHODS

Plant material: 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. A voucher specimen is kept in Department of Pharmacology, Faculty of Pharmacy, Pancasila University, Jakarta, Indonesia.

Fraction preparation: Five hundred g of the stem bark was air dried for 10 days at 33°C and powdered. The extract was then prepared by maceration with 1500 mL n-hexane for 24 h at room temperature. This maceration was repeated three times and then the three aliquots were pooled and concentrated by a rotary evaporator to obtain 8 g of n-hexane dry extract. The n-hexane extract was then fractionated to obtain 7.2 g of methanol soluble fraction. This active fraction was then refreshed at 4°C until it was analyzed. At the moment of use, the fraction was weighed and reconstituted in gum acacia solution (2%) as vehicle at the final concentration shown in the results.

***In vivo* antiplasmodial activity tests:** The *in vivo* antiplasmodial activity of the fraction was determined by the classic 4-day suppressive test, as described by Peters *et al.* (1975) against *P. berghei*, NK 65 strain infections in mice. The ANKA 65 strain of *P. berghei* was obtained from Research and Development Center for Pharmacy and Biomedicine in Jakarta. Six groups of 5 Swiss female mice with 25±2 g Body Weight (BW) were used in this study. Each mouse was inoculated with 1×10^6 *P. berghei* infected red blood cells intraperitoneally on day 0. At 2 h after inoculation, the mice on group 1-4 were administered orally with 0.2 mL of fraction tested in water solution at different concentration (25-200 mg kg^{-1} of BW per day). The mice on group 5 as positive control and on group 6 as negative control were administered orally with 0.2 mL of chloroquine (5 mg kg^{-1} of BW per day) and vehicle, respectively. The administration of the fraction and control solutions was repeated for 3 consecutive days from day 0 of parasite inoculation. At the 1-5th day after parasite inoculation, blood films were taken from the tail blood and the level of parasitemia was determined on Giemsa stained smears by counting 2000 erythrocytes. Parasite growth inhibition was compared to the level of parasitemia of control group. For the 4th day suppressive method, the ED_{50}, the dose leading to 50% parasite growth inhibition compared to the negative control, was determined from percent of growth inhibition versus the log dose.

Acute toxicity tests: The acute toxicity of the fraction was evaluated according to General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine (WHO, 2000) after single intraperitoneal injection of the fraction. Fifty Swiss mice of both sexes, weighing 20-25 g, were divided randomly into 5 groups of 10 animals each (5 males and females). The tested fraction was prepared for suitable dose levels in gum acacia solution (2%). The test groups received a single dose of fraction in various doses (25-200 mg kg^{-1} of BW per day). The control group was administered orally with gum acacia solution (2%). The animals were observed continuously for 1 h intermittently for 6 h and 12 h and then after 1, 2, 3, 7 and 14 days. The dying animal during the period of observation and those that survived up to the end of the period (14 days) were autopsied and subjected to histopatological analysis. Histopatological studies were made on four organ viz. intestines, heart, lungs and kidneys. The number of mice that died within the period of observation was noted for each group. Subsequently, the LD_{50}, which corresponded to the dose leading to 50% deaths, was calculated by probit analysis.

RESULTS

Table 1 shows the percent parasitemia in P. berghei infected mice after 4 days of treatment with fraction isolated from n-hexane extract of *G. parvifolia* Miq. The mean percent parasitemia of the negative control mice increased drastically within 4 days after treatment, from 27.7% on the day 1-47.9% on the day 4 after treatment. A single oral administration of test fraction (50-200 mg kg^{-1} of BW per day) reduced the parsitemia in mice by more than 59% compared to that in control mice on day 4 after treatment. However, such suppression of the fraction is still inferior to that of chloroquine as positive control producing more than 97% inhibiton parasitemia on day 4 after treatment.

Table 2 shows the parasite growth inhibition after 4 days of treatment with fraction isolated from n-hexane extract of *G. parvifolia* Miq. on *P. berghei* infected mice. The fraction produced a dose dependent chemosuppressive effect although the chemosuppression was never complete. The average percentages of suppression of parasitaemia were 43.27, 49.10, 55.83 and 59.75% at the doses of 25, 50, 100 and 200 mg kg^{-1} of BW per day, respectively. Chloroquine at 5 mg kg^{-1} of BW per day produced 97.10% chemosuppression. The ED_{50} value obtained of the fraction was 74.45 mg kg^{-1} of BW per day.

Table 1: Percent parasitemia in P. berghei infected mice after 4 days of treatment with fraction isolated from n-hexane extract of G. parvifolia Miq

Treatment	Dose (mg kg^{-1} of BW per day)	No. of mice tested	% Parasitemia (mean±SD) on the following day after treatment D 1	D 2	D 3	D 4
Fraction	25	5	25.8±1.41	26.3±3.65	27.3±3.97	27.4±2.34
	50	5	25.7±3.65	24.6±2.56	23.8±4.43	24.4±5.48
	100	5	24.0±2.62	23.2±5.94	22.8±2.61	21.2±5.24
	200	5	19.4±1.67	18.5±2.23	19.1±2.28	20.20±4.3
Chloroquine	5	5	0.18±1.04	1.25±1.07	2.29±0.95	1.39±1.25
Control	-	5	27.7±0.32	31.7±0.38	40.1±0.13	47.9±0.57

Table 2: *In vivo* antiplasmodial activity (ED_{50} in mg kg^{-1} of BW per day) of fraction of G. parvifolia Miq. on P. berghei infected mice

Treatment	Dose tested (mg kg^{-1} of BW per day)	Parasitemia (%±SEM)	Suppression (%±SEM)	Mortality (n/N)[b]
Fraction	25	27.36±2.34	43.27±1.17	0/6
Estimated ED_{50} : 74.45 mg kg^{-1} of BW per day	50	24.41±5.48	49.10±1.15	0/6
	100	21.18±5.24	55.83±1.26	0/6
	200	20.20±4.34	59.75±2.10	0/6
Chloroquine	5	1.39±0.57	90.70±1.08	0/6
Control[a]	-	47.96±0.57	0	0/6

[a)] Vehicle, [b)] Mortality is defined as n/N, where n is the number of dead mice and N is the number of living mice in each group

Table 3: The mortality rate of male and female mice after single intraperitoneal injection of fraction of G. parvifolia Miq. in various doses

Dose of fraction (mg kg^{-1} of BW)	Male mice Dead	Living	Mortality rate	Female mice Dead	Living	Mortality rate
2000	0	5	0/5	0	5	0/5
4000	0	5	0/5	2	3	2/5
8000	0	5	0/5	2	3	2/5
Control	0	5	0/5	0	5	0/5

Table 4: Histopatological description of mice organs after intraperitoneal injection of fraction of G. parvifolia Miq

Organ	Doses of fractions (mg kg^{-1} of BW) Control	2000	4000	8000
Intestines	No change (normal)	No change (normal)	Flaking of vili epithel and debris of the lumen	Flaking of vili epithel and debris of the lumen
Liver	No change (normal)	No change (normal)	Bleeding of the liver cells	Bleeding of the liver cells
Kidneys	No change (normal)	No change (normal)	Bleeding of the tubulus and mild leucocytes infiltration	Bleeding of the tubulus and mild leucocytes infiltration
Heart	No change (normal)	No change (normal)	No change (normal)	No change (normal)
Lungs	No change (normal)	A mild thickening-of alveolus	Thickening of alveolus	Thickening of alveolus

In order to evaluate the possible toxicity of the fraction of the *G. parvifolia* Miq., acute toxicity test has been conducted after oral administration of Swiss mice. Table 3 shows the mortality rate of male and female mice after oral adiministration of the fraction at various doses. The results indicated that male mice were better able to tolerate the lethal of the fraction than the male ones since there was no mortality observed in male mice. In contrast, the mortality was observed in female mice after a single dose ingestion of the fraction at 4000 and 8000 mg kg^{-1} of BW. The oral administration of moderate doses of the fraction (4000 mg kg^{-1} of BW) indicated only mild Central Nervous System (CNS) stimulation. However, at the highest doses (8000 mg kg^{-1} of BW), the oral administration caused rapid respiration, twitchy, writhing, tremor and generalized convulsion. In addition, at these highest doses the mortality rate was less than 50% or 2 of 10 male and tested female mice (Table 3) indicated that the LD_{50} of the fraction was more than 8000 mg kg^{-1} of BW.

The histopathological studies microscopically of intestines, heart, lungs and kidneys (Table 4) revealed that only mild thickening of alveolus was observed in lungs of the mice after they received 2000 mg kg^{-1} of BW doses of the fraction. However, mice that received 4000 and 8000 mg kg^{-1} of BW doses showed flaking of vili epithel and debris of lumen in intestines, bleeding of cells liver in livers, bleeding of tubulus and leucocytes infiltration in kidneys and thickening of alveolus in lungs, although there were no abnormalities observed in heart after receiving doses of the fraction.

DISCUSSION

The classic 4-day suppressive test using the *P. berghei* infected mice model has been widely used as a preliminary test for the *in vivo* antiplasmodial activity of potential antimalarial agents, as it provides a preclinical indication of any *in vivo* potential bioactivity as well as

possible toxicity of the tested sample. Munoz *et al.* (2000) classified the *in vivo* antiplasmodial activity of an extract or a fraction as moderate, good and very good activity if the extract or the fraction displayed the percentage of suppression equal or greater than 50% at the dose 500, 250 and 100 mg kg^{-1} of BW per day. The results from this study strongly indicate that the tested fraction exhibited very good antiplasmodial activity. In addition, this fraction was well tolerated in mice since there is no mortality observed after 4 days of treatment at the maximum dose of ingestion 200 mg kg^{-1} of BW per day. This result supports the traditional use of the *G. parvifolia* Miq. for the treatment of malaria by traditional healers in endemic areas in Indonesia.

The genus Garcinia, which belongs to the family Guttiferae, is known to be rich in active constituents of prenylated xanthones (Linuma *et al.*, 1994; Xu *et al.*, 2001; Chamahasathien *et al.*, 2003). Xanthones have been reported to possess several biological activities such as antibacterial (Grasvenol *et al.*, 1995; Mackeen *et al.*, 2000), antitumour, antioxidant (Mackeen *et al.*, 2000) and antiplasmodial (Likhitwitayawuid *et al.*, 1998a; 1998b). In their study, concerning antiplasmodial activity of the genus Garcinia, Likhitwitayawuid *et al.* (1998a) isolated 5 xanthones from the bark of *G. cowa* and evaluated their antiplasmodial activity. The results showed that cowaxanthone was the most active against *P. falciparum* with an IC_{50} of 1.5 μg mL^{-1}. The other xanthones from *G. dulcis*, were also evaluated for their antiplasmodial activity. Among the 5 tested xanthones, garcinia xanthone exhibited the most active with an IC_{50} of 0.96 μg mL^{-1} (Likhitwitayawuid *et al.*, 1998b). It is suggested that antiplasmodial activity of the fraction of *G. parvifolia* Miq. is due to its xanthones constituents. Purification of the xanthones from the active fraction and evaluation its antiplasmodial activity will be undertaken.

The LD_{50} values obtained more than 8000 mg kg^{-1} of BW indicated that the fraction of *G. parvifolia* Miq. can be grouped as weak toxic substance (Homburger, 1989). This result is supported by the Therapeutic Index ($IT = LD_{50}/ED_{50}$) value of the fraction showing more than 107.5. Although the fraction did not induce the mortality up to the dose level of 2000 mg kg^{-1} of BW, at high doses the fraction appears to exert their toxic effects on the CNS. Increasing doses of the fraction have graded CNS effects such as twitchy, writhing, tremor, convulsion and finally death. In addition, mice that died after intraperitoneal injection of high doses of the fraction showed pathological change in the intestines, livers, kidneys and lungs.

CONCLUSION

In conclusion, these studies demonstrated the very good *in vivo* antiplasmodial activity with weak toxic effect of fraction isolated from n-hexane extract of the *G. parvifolia* Miq. stem bark. Efforts will be undertaken to continue the bioassay guided fractionation in order to isolate and identify the active compounds, as well as to understand the mechanism of action.

AKNOWLEDGEMENT

We thank all colaborators in the field and especially the traditional healers who gave us plant materials for testing. We also thank Dr. Vet. Rita Marleta Dewi from Research and Development Center for Pharmacy and Biomedicine in Jakarta for scientific support in antiplasmodial testing. For excellent technical assistance in acute toxicity testing we are grateful to all technicians of the Balivet, Bogor-West Java.

REFERENCES

Chamahasathien, W., Y. Li, M. Satake, Y. Oshima, M. Ishibashi, N. Ruangrungsi and Y. Ohizumi, 2003. Prenylated xanthones from Garcinia xanthochymus. Chem. Pharm. Bull., 51: 1332-1334.

Grasvenol, P.W., A. Supriono and D.O. Gray, 1995. Medicinal plants from Riau province, Sumatra, Indonesia. Part 2: Antibacterial and antifungal activity. J. Ethnopharmacol., 45: 97-111.

Hay, A.E., J. Helesbeux, O. Duval, M. Lay, P. Grellier and P. Richomne, 2004. Antimalarial xanthones from Calophyllum caledonicum and Garcinia viellardi. Life Sci., 75: 3077-3085.

Hoffman, S.L., G.M. Subramanian, F.H. Collins and J.C. Venter, 2002. Plasmodium, human and Anopheles genomics and malaria. Nature, 415: 702-709.

Homburger, F., 1989. *In vivo* Testing in the Study of Toxicity and Safety Evaluation. In A Guide to General Toxicity. 2nd Rev. Edn. J.K. Marqius (Ed.). Karger, New York, pp: 198-200.

Leaman, D.J., J.T. Arnason, R. Yusuf, H. Sangat-Roemantyo, H. Soedjito, C.K. Angerhofer and J.M. Pezzuto, 1995. Malaria remedies of the Kenyah of the Apo Kayan, East Kalimantan, Indonesia Borneo: A quantitative assessment of local concensus as an indicator of biological efficacy. J. Ethnopharmacol., 49: 1-16.

Likhitwitayawuid, K., T. Phadungcharoen and J. Krungkrai, 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.

Linuma, M., H. Tosa, T. Tanaka, R. Shimano, F. Asai and Y. Shigetomo, 1994. Two xanthones from root bark of *Garcinia subelliptica*. Phytochemstry, 35: 1355-1360.

Mackeen, M.M., A.M. Ali, N.H. Lajis, K. Kawazu, Z. Hassan, M. Amran, M. Habsah, L.Y. Mooi and S.M. Mohamed, 2000. Antimicrobial, antioxidant, antitumour-promoting and cytotoxic activities of different plant part extracts of Garcinia atroviridis Griff Ex T. Anders. J. Ethnopharmacol., 72: 395-402.

Murningsih, T., Subekti, H. Matsuura, K. Takahashi, M. Yamasaki, O. Yamato, Y. Maede, K. Katakura, M. Suzuki, S. Kobayashi, Chairul and T. Yoshihara, 2005. Evaluation of the inhibitory activities of the extracts of Indonesian traditional medicinal plants against *Plasmodium falciparum* and Babesia gibsoni. J. Vet. Med. Sci., 67: 829-831.

Mustofa, E.N. Sholikhah and S. Wahyuono, 2007. *In vitro* and *in vivo* antiplasmodial activity and cytooxicity of extracts of *Phyllanthus niruri* L. herbs traditionally used to treat malaria in Indonesia. Southeast Asian J. Trop. Med. Public Health. 38 (4): 609-615.

Peters, W.J., J.H. Portus and B.L. Robinson, 1975. The chemotherapy of rodent malaria XXII. The value of drug-reistant strains of *Plasmodium berghei* in screening for blood schizontisidal activity. Ann. Trop. Med. Parasitol., 69: 155-171.

Phillips, R.S., 2001. Current status of malaria and potential for control. Clin. Microbiol. Rev., 14: 208-226.

Syamsudin, S. Tjokrosonto, S. Wahyuono, Darmono and Mustofa, 2007. *In vitro* and *in vivo* antiplasmodial activities of Stem bark extracts from *Garcinia parvifolia* Miq (Guttiferae). Int. J. Trop. Med. 2:41-44.

World Health Organization, 2000. General guidline for methodologies on research and evaluation of traditional medicine. WHO, Geneva.

Xu, J.Y., Y.H. Lai, Z. Imiyabir and S.H. Goh, 2001. Xanthones from *Garcinia parvifolia*. J. Nat. Prod. 64: 1191-1995.