Antimalarial Activity of Ethanolic Leaf Extract of *Bauhinia strychnifolia* in Mice Infected with *Plasmodium berghei*

**Voravuth Somsak**, Jariya Noioid, Sukanya Chachiyo, and Sawannee Kraithep

**Department of Clinical Chemistry, Faculty of Medical Technology, Western University, Kanchanaburi 71170, Thailand**

**Abstract**

The emerging resistance of *Plasmodium* species to currently available antimalarial drugs remains a public health problem, hence the need for new effective, safe and affordable drugs. Plant extracts remain a reliable source of drugs. *Bauhinia strychnifolia* (*Yha-nang dang*) is widely distributed and has been traditionally used in Thailand to treat fever, alcoholic toxicity and allergy. The leaf extract of *B. strychnifolia* had potent antioxidant, anti-cancer and anti-microbial activities. The aim of the study was to investigate antimalarial activities of *B. strychnifolia* in malaria infected rodent models. Acute toxicity of ethanolic crude extract of *B. strychnifolia* leaves was assessed in mice up to a dose of 6,000 mg/kg. ICR mice were inoculated with *Plasmodium berghei* ANKA and treated with the extracts (500, 1500 and 3000 mg/kg) orally by gavage. Four day suppressive and curative effect against established infection and prophylactic models of antimalarial studies were carried out. The extract did not show any toxic effect because doses up to 3,000 mg/kg caused no death or alter the behavior of the tested normal mice. For antimalarial studies, the extract (3,000 mg/kg) exerted significant ($P < 0.01$) effects of prophylactic, suppressive, and curative with percent inhibition of 59%, 84%, and 68%, respectively. However, antimalarial effect of chloroquine at 5 mg/kg was higher than the extract in all test models. These results indicate that ethanolic crude extract of *B. strychnifolia* leaves has excellent *in vivo* antimalarial activities against *P. berghei* ANKA. Hence, this plant extract represents a promising source of new antimalarial agents.

**Keywords:** Antimalarial; *Bauhinia strychnifolia; Plasmodium berghei*

**Introduction**

Malaria is an endemic infectious disease that is wide spread in tropical and sub-tropical areas of the world and one of the six most important parasitic disease of human [1]. It is a major public health problem in sub-Saharan Africa, where over 85-90% of all global burden of malaria exists with up to 50% of all outpatient visit in areas with high malaria transmission and 30-50% of all hospital admission are attributed to malaria [2]. Each year 1.1 million people die with this disease. Approximately 1 million deaths are in Africa and an estimate 700,000 of them are children. Although an effective vaccine is the best long term control for malaria, current research on vaccine development is still at pre-clinical stage. Therefore, the strategy for malaria mainly focuses on antimalarial drugs capable of reducing or eliminating parasites [3]. However, antimalarial drug resistant *Plasmodium* species and the emergence of insecticide resistant Anopheles mosquitoes cause not only the spread of malaria to new areas but also its re-emergence in areas where it had previously been eradicated [4,5]. Hence, this has prompted research towards the discovery and development of new, safe and affordable antimalarial chemotherapies. In this respect, plant extracts are potential targets for research and development of alternative antimalarial drugs [6].

*Bauhinia strychnifolia* Craib (Leguminosea-caesalpinioideae species) is well known in Thai as *Yha-nang dang*. It is widely distributed and has been traditionally used in Thailand to treat fever, alcoholic toxicity, allergy and cancer [7]. Several reports showed that leaf extract of *Bauhinia strychnifolia* (*B. strychnifolia*) had potent antioxidant and free radical scavenging activities. Moreover, anti-cancer and anti-microbial properties of this plant extract have also been described [7,8]. However, antimalarial activity of *B. strychnifolia* leaf extract has not yet been reported. Hence, the aim of the study was to evaluate the antimalarial activity of ethanolic leaf extract of *B. strychnifolia* against *Plasmodium berghei* (*P. berghei*) infection in mice.

**Materials and Methods**

**Plant material and preparation of aqueous crude extract**

*B. strychnifolia* leaves were collected from Kanchanaburi province, Thailand, and authenticated by Dr. Sakaewan Ounjaijean, Faculty of Pharmacy, Payap University, and Chiang Mai, Thailand. The leaves were dried in hot air oven at 50°C, and ground to coarse powder. The leaves were then powdered, and the fine powder was stored at -20°C. Before use, powdered *B. strychnifolia* leaves were dried in hot air oven at 50°C, and ground to coarse powder in a mortar. Extraction was carried out by dispensing 100 g of dried powdered plant material in 1 L of 95% ethanol with continuous shaking for 72 h. This was followed with vacuum filtration and powdered crude extract was then obtained using a rotary evaporator. This powdered crude extract was stored at -20°C. Before use, powdered crude extract was freshly dissolved in tween 80 to obtain appropriate doses [7].

**Experimental mice**

Healthy ICR mice (female, 4 weeks old and 30-35 g weight) obtained from National Laboratory Animal Center, Mahidol University were used. The mice were conveniently housed under standard environmental condition at 22-25°C. All mice had *ad libitum* access to commercial feed pellets and clean water throughout the study. All animal experiments were approved and ratified by the Animal Ethic Committee, Faculty of Medicine, Chiang Mai University.

*Corresponding author: Voravuth Somsak, Department of Clinical Chemistry, Faculty of Medical Technology, Western University, Kanchanaburi 71170, Thailand; Tel: +66898809939; E-mail: voravuthsomsak@gmail.com*

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Acute toxicity test

Acute toxicity test of B. strychnifolia extract was carried out as previously described [9]. Groups of mice (5 mice of each) were given 500, 1500, 3000, and 6000 mg/kg body weight of the extract orally by gavage. The mice were then observed for signs of toxicity which include but not limited to paw licking, salivation, stretching of entire body, weakness, sleeping, respiratory distress, coma and death in first 4 h and subsequently daily for 7 days.

Rodent malaria parasite

P. berghei ANKA strain (PbANKA) was used and maintained in our laboratory by weekly serial passage of 1x10⁷ infected red blood cells (IRBC) in naïve mice. Parasite growth was daily measured by Wright stained thin blood smear under light microscope with 100X lens and reported the percentage of IRBC per 100 red blood cells (% parasitemia) as described below.

\[
\text{% parasitemia} = \frac{\text{Number of infected erythrocytes} \times 100}{\text{Total number of erythrocytes}}
\]

Antimalarial drug

Chloroquine (CQ) was used to study in vivo drug susceptibility of PbANKA. The drug was freshly prepared in distilled water and administered orally by gavage. Drug dose, expressed in mg/kg of body weight, was adjusted at the time of administration according to the weight of each mouse. The dose was based on the ED90 (5 mg/kg) of this drug on PbANKA infected mice [10].

Evaluation of prophylactic activity of B. strychnifolia (Repository test)

Evaluation of the prophylactic potential of B. strychnifolia leaf extract was carried out according to the method described previously [11]. The mice were randomly divided into 5 groups (5 mice of each) and treatment with the extract (500, 1500, and 3000 mg/kg) was initiated orally on day 0 and continued until day 4. Two control groups were used, the positive control group was treated daily with 5 mg/kg of CQ while tween 80 was given to the negative control group. On day 5 of the experiment, mice were injected intraperitoneally with 1x10⁷ infected erythrocytes of PbANKA. Seventy two hours later, the mice were divided into 5 groups (5 mice of each). The groups were orally treated with the extract (500, 1500, 3000 mg/kg), CQ (5 mg/kg) was given to the positive control and tween 80 was given to the negative control group. The treatment was carried out once daily for 5 days and parasitemia was then examined by microscopy of Giemsa stained thin blood smear. Moreover, percentage of inhibition was also calculated as previously described.

Statistical analysis

Statistical analysis of the data was performed using GraphPad Prism Software (GraphPad Software, Inc., USA). The one way ANOVA test was used to analyze and compare the results at a 95% confidence level. Values of \( p < 0.05 \) were considered significant. Results were expressed as mean ± standard error of mean (SEM).

Results

Acute toxicity test

Behavioral signs of toxicity observed in mice given 6,000 mg/kg of extract include; paw licking, salivation, stretching and reduce activity. However, the mice survived. Moreover, oral administration of the ethanolic extract of B. strychnifolia at the doses of 500, 1500, and 3000 mg/kg had no toxic effects throughout the 7-day study period. None of the mice showed any signs of toxicity, such as change of skin, eyes and mucus membranes, behavioral patterns, trembling, diarrhea, falling of the fur, sleep or coma. In addition, no significant changes were observed in their body weight. Hence, 500, 1,500, and 3,000 mg/kg of extract were suitable doses for using in this study.

Blood stage propagation of PbANKA infection in mice

For evaluation of blood stage growth of PbANKA, mice were infected intraperitoneally with 1x10⁷ infected erythrocytes of PbANKA, and parasitemia was daily monitored by microscopy of Giemsa stained thin blood smear. As showed in (Figure 1A), parasitemia was first detectable on day 2 post-infection with a parasitemia less than 1%, and maximum parasitemia was then observed with a parasitemia of 70% on day 14 post-infection. Moreover, infected mice died within 14 days (Figure 1B).

Prophylactic activity of ethanolic leaf extract of B. strychnifolia

In order to evaluate protective effect of B. strychnifolia leaf extract, prophylactic activity test was done as previously described. The ethanolic leaf extract of B. strychnifolia exerted significant (\( p < 0.01 \)) reduction in level of parasitemia at 3,000 mg/kg with an inhibition rate of 59% (Figure 2). However, the extract at doses of 500 and 1,500 mg/kg had no activity to reduce in level of parasitemia. Moreover, the standard antimalarial drug, CQ caused reduction of parasitemia significantly (\( p < 0.01 \)) with 90% inhibition, which was higher than those of the extract treated groups (Figure 2).

Suppressive test of B. strychnifolia (4-day suppressive test)

The standard 4-day suppressive test against PbANKA was employed [12]. ICR mice were inoculated by intraperitoneal injection with 1x10⁷ infected erythrocytes of PbANKA. The mice were randomly divided into 5 groups (5 mice of each) and treated for 4 consecutive days with 500, 1500, and 3000 mg/kg of the extract orally. Two control groups were used; the positive control was treated daily with 5 mg/kg of CQ while the negative control group was given tween 80. On day 5 of the experiment, blood was collected from tail vein of each mouse and microscopic examination of Giemsa stained thin blood smear was performed to examine parasitemia. Moreover, percentage of inhibition was also calculated as previously described.

Evaluation of curative activity of B. strychnifolia (Rane’s test)

Evaluation of the curative potential of B. strychnifolia leaf extract was carried out according to the method described previously [13]. The mice were injected intraperitoneally with 1x10⁷ infected erythrocytes of PbANKA on the first day (day 0). Seventy two hours later, the mice were divided into 5 groups (5 mice of each). The groups were orally treated with the extract (500, 1500, 3000 mg/kg), CQ (5 mg/kg) was given to the positive control and tween 80 was given to the negative control group. The treatment was carried out once daily for 5 days and parasitemia was then examined by microscopy of Giemsa stained thin blood smear. Moreover, percentage of inhibition was also calculated as previously described.

\[
\text{% inhibition} = \left( \frac{A - B}{A} \right) \times 100
\]

Where \( A \) is the average % parasitemia in negative control group, and \( B \) is the average % parasitemia in the test groups.

% inhibition = (\( A - B \)) / \( A \) × 100
dose dependent suppressive effect against PbANKA and significances were also observed with the doses of 1,500 and 3,000 mg/kg (p<0.05 and p<0.01, respectively) when compared to the negative control with inhibition rate of 64% and 84%, respectively. No suppressive effect was found in infected mice treated with 500 mg/kg of the extract. Moreover, 5 mg/kg of CQ showed significant (p<0.01) suppression at 91% inhibition, which was higher than those of the extract treated groups (Figure 3). However, there was an increase in parasitemia in the negative control group.

Curative activity of ethanolic leaf extract of *B. strychnifolia*

In order to evaluate curative activity of *B. strychnifolia* leaf extract, curative activity test *in vivo* against PbANKA was carried out. It was observed that the ethanolic leaf extract of *B. strychnifolia* reduced in parasitemia levels in the extract treated groups. The significant (p<0.01) reduction in parasitemia level was found in infected mice treated with 3,000 mg/kg of the extract with 68% inhibition, compared to the negative control group (Figure 4). However, no curative effect was observed in infected mice treated with 500 and 1,500 mg/kg of the extract. Moreover, standard antimalarial drug, CQ (5 mg/kg) exerted significantly (p<0.01) curative activity against PbANKA infected mice with 89% inhibition, while increasing of parasitemia was found in the negative control group.

**Discussion**

The in vivo antimalarial activities of the ethanolic crude extract of *B. strychnifolia* leaves were investigated by evaluating the prophylactic, suppressive and curative properties using standard animal model. In vivo models are usually employed in antimalarial studies because they take into account the possible prodrug effect and probable involvement of the immune system in eradication of the pathogen [14]. Plasmodium species that cause human disease are essentially unable to infect
Diterpenoids, flavonoids, saponin, alkaloid and kaempferol classes of metabolites such as polyphenol, flavonoids, diterpenoids, extract of *B. strychnifolia* leaves were reported to contain different solvent for extraction as indicated by high value of total polyphenolic extraction of *B. strychnifolia*, ethanol was found to be an excellent extract exhibited an suppression (3,000 mg/kg), in vivo and curative effects against PbANKA infected mice. At the highest dose presented antimalarial activities including prophylactic, suppressive, and curative activities compared to the extract treated groups and negative control.

For all results, it can be concluded that ethanolic crude extract of *B. strychnifolia* leaves presents antimalarial activities against PbANKA infection in mice. However, it can also be suggested for further studies that pure compounds should be studied in order to compare with crude extract. Furthermore, combination treatment with other standard antimalarial drugs such as pyrimethamine, quinine, and artemisinin should be done for developing this extract to be an alternative drug or supplement in malaria treatment in the future.

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**References**