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Effect of Crude Leaf Extract of Bauhinia strychnifolia in BALB/c Mice Infected with Plasmodium berghei

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Abstract

Continuous emergence of antimalarial drug resistant malaria parasites and their rapid spread across the globe warrant urgent search for new antimalarials. Traditional medicinal plants have been the main sources for screening active phytochemicals against malaria. Accordingly, this study was aimed at evaluating the antimalarial activity of crude leaf extract of Bauhinia strychnifolia against Plasmodium berghei infected mice. Aqueous crude leaf extract of B. strychnifolia have been prepared and tested for acute toxicity and antimalarial efficacy in P. berghei ANKA infected BALB/c mice. At three oral doses of 100, 500 and 1,000 mg/kg of extract were safe, chemosuppressive and thus prevented body weight loss, packed cell volume reduction and increased mice mean survival time in a dose-dependent manner compared to the untreated control group. The maximum efficacious extract was found at the dose of 1,000 mg/kg which prolonged mean mouse survival past day 26 of infection with all the mice in this group having the highest parasitemia suppression rate (85%). This study suggests that the crude leaf extract of this plant have promising antimalarial activity against P. berghei in a dose dependent manner, which supports the folkloric use of the plant for treating malaria.

Keywords: Bauhinia strychnifolia; Plasmodium berghei; BALB/c mice

Introduction

Malaria is generally a major public health problem throughout the world, particularly in developing countries. It causes an estimated 0.7-1 million deaths annually. Approximately one-half of the world's population is at risk of contracting malaria. Most cases occur in the Africa region, followed by Southeast Asia and Eastern Mediterranean regions [1]. Malaria is preventable and curable; however, it remains one of the greatest global public health problems, especially in sub-Saharan Africa [2]. Because of increasing resistance to available antimalarial drugs including pyrimethamine, sulfadoxine, and chloroquine by *Plasmodium* malaria parasite, there is broad consensus on the need to develop new antimalarial drugs. Antimalarial drug development can follow several strategies, which range from minor modifications of existing agents to the design of novel agents that act against new targets [3]. Natural products and plant extracts have been important sources of different drugs currently available to treat severe malaria [4,5]. Quinine and derivatives of artemisinin are the two most important products of plants useful in clinical practice. In the case of artemisinin, relatively simple chemical modifications of the natural parent compound have led to a series of highly potent antimalarials [6]. The development of these two important drugs from natural sources and the utilization of many plants traditionally in various parts of the world trigger the conduction of *in vitro* and *in vivo* studies because natural products can be a source of new antimalarial drugs.

Bauhinia strychnifolia is known in Thai as Yanang Dang. For traditional medicine, the stem and root have been used to treat cancer (breast and colon cancers), fever, alcoholic toxication and allergy [7]. The leaves or a stem in boiling water has been used as a tonic. It has been reported that aqueous leaf extract of *B. strychnifolia* presented potent antioxidant, anti-inflammation, anti-microbial activities [8]. However, antimalarial activity of this plant extract has not yet been reported. Hence, this study was aimed to determined antimalarial activity of the crude leaf extract of *B. strychnifolia* against *Plasmodium berghei* infection in mice in order to obtain the scientific support for its traditional use.

Materials and Methods

Preparation of crude leaf extract of *Bauhinia strychnifolia*

B. strychnifolia leaves were collected at the Suan Ya Thai Thongnoppakhun herbal garden in Chonburi province and were identified by a Thai traditional doctor, Mr. Sraupsin Thingnoppakhun. A voucher specimen is now kept at the Department of Clinical Chemistry, Faculty of Medical Technology and Western University, Thailand. A dried powdered of *B. strychnifolia* leaves was extracted with distilled water (20 g%) using microwave at 360 W for

5 min and incubated at room temperature for 24 h, then filtered. The filtrate was evaporated to dryness on a boiling water bath to yield dried leaf aqueous extract and stored at 4°C. Before using, the dried leaf aqueous extract was dissolved in distilled water at the chosen doses [7].

Acute toxicity test

Fifteen BALB/c mice were used by randomly dividing them into 5 groups of 5 mice per cage. The mice were given orally 100, 500, 1,000, 4,000 and 6,000 mg/kg in single dose volume of 0.2 ml of the extract, respectively. Then, the mice were monitored continuously for 1 h,

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intermittently for 4 h and for a period of 24 h for any gross behavioral changes such as rigidity, sleep, mortality and other signs of acute toxicity manifestations, and the follow-up continued for 28 days [9].

Mice

BALB/c female mice, 4-6 weeks old, weighting 1 20-30 g at the time of the primary infection obtained from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand were used throughout the study. They were kept in a room with temperatures between 22-25°C and a 12-h light/12-h dark cycle. All mice were fed on pelleted diet (CP diet 082, Perfect Companion Company, Bangkok, Thailand) and sterile-filtered tap water *ad libitum*. Procedures of the animal experiments were ratified and approved by the Ethical Committee of Animal Experimentation, Faculty of Medical Technology and Western University.

Rodent malaria parasite

Plasmodium berghei ANKA (PbANKA) was used in this study. Naïve BALB/c mice were infected with $1x10^7$ infected erythrocytes of PbANKA by intraperitoneal (IP) injection. Blood stage propagation (% parasitemia) was daily monitored by microscopy of Giemsa stained thin blood smear. The parasite was maintained by serial passage of blood from infected mice to non-infected one on a weekly basis.

$$\% \text{ parasitemia} = \frac{\text{(number of infected erythrocytes)}}{\text{Total number of erythrocytes}} \times 100$$

Standard antimalarial drug

For antimalarial activity test, chloroquine (CQ) was used. 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) on PbANKA infected mice [10].

Antimalarial activity

The standard 4-day suppressive test was used 1 in screening of the plant extracts [11]. Experimental BALB/c mice infected with 1×10^7 infected erythrocytes of PbANKA by IP injection were randomly divided into 6 groups (5 mice of each). They were treated orally with the extracts at doses of 100, 500 and 1,000 mg/kg. Untreated and the normal control groups was treated with distilled water while the positive control group was given 5 mg/kg of CQ. Treatment was started after 3 h of infection on day 0 (D0) and was continued daily for four days (D0 to D3). On the fifth day (D4), blood sample was collected from the tail vein and % parasitemia was subsequently measured. Moreover, percentage of suppression (% suppression) was calculated using the formula below:

$$\% \text{ suppression} = \frac{\text{(parasitemia in untreated control group – parasitemia in treated group)}}{\text{Parasitemia in untreated control group}} \times 100$$

Moreover, body weight change, packed cell volume and mean survival time were also determined.

Determination of body weight change

The body weight (BW) of each mouse in all groups was measured by using a sensitive digital weighing balance and mean BW per group was calculated using the formula:

$$\text{Mean BW} = \frac{\text{mean BW of mice in a group}}{\text{Total number of mice in that group}}$$

Determination of packed cell volume

Packed cell volume (PCV) was determined using blood collection from tail vein of each mouse in heparinized micro hematocrit capillary tubes and centrifugation was then performed at 10,000 rpm for 5 min [12]. PCV was subsequently calculated using the formula:

$$\text{PCV} = \frac{\text{(volume of total packed erythrocytes)}}{\text{Total blood volume}} \times 100$$

Determination of mean survival time

Mortality was monitored daily and the number of days from parasite inoculation up to death was recorded for each mouse throughout the follow-up period. The mean survival time

(MST) was calculated using the formula below:

$$\text{MST} = \frac{\text{Sum of survival time (days) of mice in a group}}{\text{Total number of mice in that group}}$$

Statistical Analysis

The results were presented as mean+ standard error of mean (SEM). The one way ANOVA was used to analyze and compare the results at a 95% confidence level. Values of $p<0.05$ were considered significant.

Results

Acute toxicity test

The experimental mice ingested with crude leaf extract of *B. strychnifolia* in all doses did not show any indication of gross physical or behavioral changes such as hair erection, reduction in feeding and motor activities, weight loss, lacrimation, diarrhea, depression or abnormal secretions within 24 h monitoring period. No fatalities occurred within the observation period of four weeks.

Antimalarial activity of crude leaf extract of *B. strychnifolia*

As showed in Figure 1A, the crude leaf extract of *B. strychnifolia* demonstrated significant ($p<0.05$) dose-dependent manner antimalarial activity at a various doses (100, 500 and 1,000 mg/kg) administered with average % parasitemia of 15%, 10%, and 3%, respectively (25%, 50% and 85% suppression, respectively) although no significant reduction of parasitemia was observed at dose of 100 mg/kg. The extract at 1,000 mg/kg performed similarly well as CQ, which produced 90% suppression (2% parasitemia). Moreover, all doses of the extract were correlated with significantly ($p<0.05$) increased MST of mice (14.3, 20.7 and 26.5 for 100, 500 and 1,000 mg/kg, respectively) compared to the untreated control group (MST = 11.2) (Figure 1B). Similar result of increasing MST was observed in CQ treated group.

Effect of crude leaf extract of *B. strychnifolia* on PCV and BW

The crude leaf extract of *B. strychnifolia* showed significant ($p<0.05$) dose-dependent effect on the mean PCV value of PbANKA infected mice (Figure 2A). The maximum effect was observed at a dose of 1,000 mg/kg. In addition, a loss in BW was noticed for PbANKA infected mice. Interestingly, significant ($p<0.05$) protection of BW loss during PbANKA infection was found in the extract treated groups with a dose-dependent manner (Figure 2B). However, significant ($p<0.01$) reduction of PCV and BW were still found in infected mice given 100

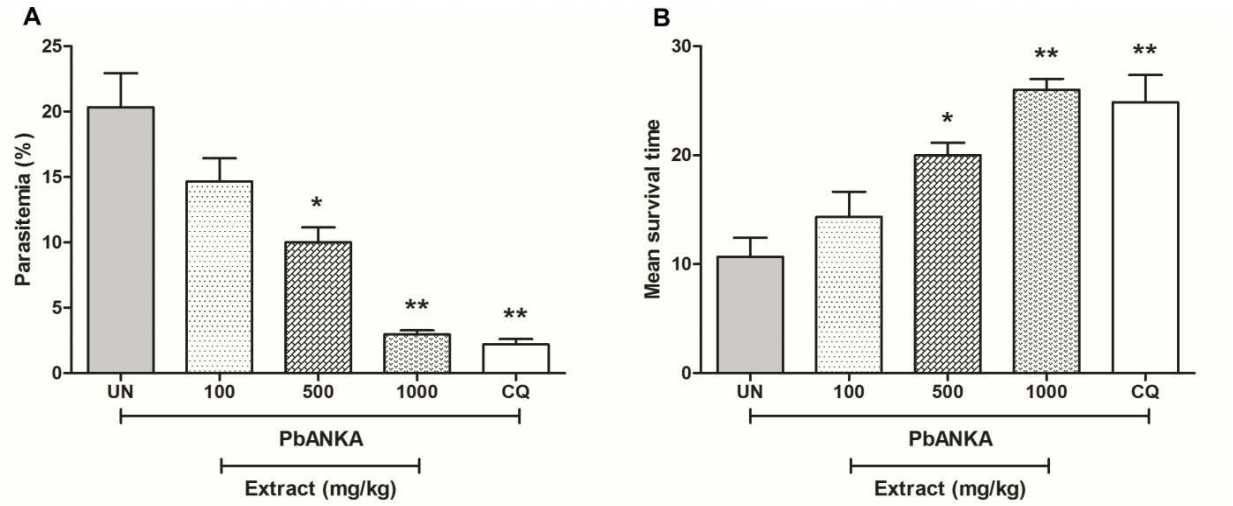

Figure 1: Antimalarial effect of crude leaf extract of *B. strychnifolia* against PbANKA infected mice. Groups of ICR mice (5 mice of each) were inoculated by IP injection of 1×10^7 infected erythrocytes of PbANKA, and subsequently given 100, 500 and 1,000 mg/kg of the extracts orally for 4-consecutive days. On fifth day, (A) parasitemia and (B) mean survival time were monitored. Results were expressed as mean ± SEM. *p<0.05 and **p<0.01 compared to untreated control groups. UN; untreated control group and CQ; 5 mg/kg chloroquine.

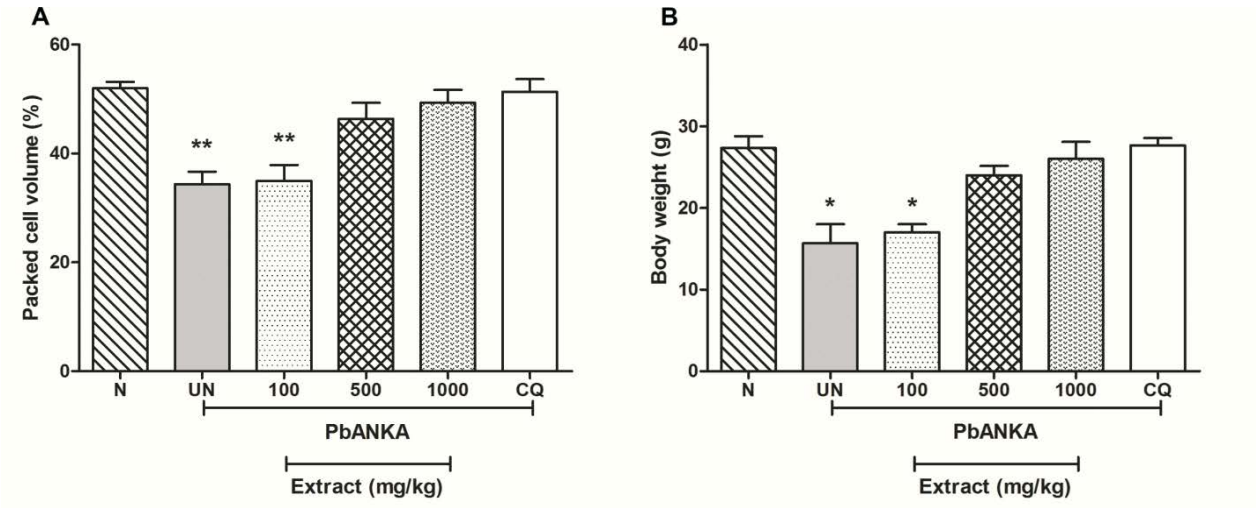

Figure 2: Effect of crude leaf extract of *B. strychnifolia* on PCV and BW in PbANKA infected mice. Groups of ICR mice (5 mice of each) were inoculated by IP injection of 1×10^7 infected erythrocytes of PbANKA, and subsequently given 100, 500 and 1,000 mg/kg of the extracts orally for 4-consecutive days. On fifth day, (A) PCV and (B) BW were monitored. Results were expressed as mean ± SEM. *p<0.05 and **p<0.01 compared to normal control groups. N; normal control group, UN; untreated control group and CQ; 5 mg/kg chloroquine.

mg/kg of the extract.

Discussion

Plant extract are frequently considered to be less toxic and have fewer adverse effects than synthetic ones. A growing number of peoples are therefore turning to alternative therapy, including medicinal plants. The medicinal plants have been used in clinical practice for several countries. However, the compounds and precise mechanisms of most plants remain to be determined. For acute toxicity test, oral administration of crude leaf extract of *B. strychnifolia* did not show changes in general appearance or behavioral pattern of the experimental mice until the end of 28 days. Furthermore, no death was found in the mice receiving the extract up to a dose of 6,000 mg/kg, which is about 10 times the minimum effective dose (500 mg/kg). If a test substance has a lethal dose higher than 3 times the minimum effective dose, it can be a good candidate for further studies [13]. Hence, absence of mortality up to an oral dose of 6,000 mg/kg could indicate that the test extracts were safe and this could explain the routine use of the plant by the local people for traditional management of malaria. The standard 4-day suppressive test is a test commonly used for *in vivo* antimalarial phytochemical screening in which >30% suppression following treatment makes a product to be considered active [13,14].

Accordingly, the crude leaf extract of *B. strychnifolia* which showed 50% suppression at 500 mg/kg and 85% at 1,000 mg/kg can be classified as active. The dose-dependent manner in chemo suppression could be attributed to the low dose of schizocidal compounds in natural products and as such their activity may be undetectable in lower doses. This increased percent suppression of parasitemia with increased dose was observed by other studies on different plant species [15-18]. Alkaloids, polyphenolic compounds, terpenoids, flavonoids and quercetin in this extract could be responsible for its antimalarial activity [19-23]. Moreover, it has been reported that quercetin and terpenoids showed strong antimalarial, anti-microbials and anti-cancer activities [23]. A prolonged MST with significant difference, compared to untreated control group, was observed for mice treated with the extract regardless of dose except for 100 mg/kg implying the role of the extract in control of malaria. Particularly, the extract at maximum dose of 1,000 mg/kg was highly associated with prolonged MST indicating the dominant presence of antimalarial bioactive compounds in this extract.

Anemia and BW loss are the general features of PbANKA infected mice [24]. Therefore, the ideal antimalarial compounds from plant extracts are expected to prevent anemia and BW loss. Despite the fact that significant BW increase among PbANKA infected mice after ingesting 500 and 1,000 mg/kg crude leaf extract of *B. strychnifolia* compared to untreated control group suggests the effect of the extract in preventing malaria-related weight loss. It is well established that BW loss is one feature of rodent malaria. The present result is in agreement with other similar studies that reported mice BW loss using different plant extracts and extraction solvents [25-27]. The absence of significant PCV reduction among extract treated mice at the doses of 500 and 1,000 mg/kg of the crude leaf extract of *B. strychnifolia* may indicate the protective activity of this extract. Moreover, observing a significantly lower PCV reduction among the same groups of mice at the highest dose (1,000 mg/kg) shows the presence of antimalarial compounds in the dose administered. However, it appears that the activity of the extract at dose of 100 mg/kg was not strong enough to significantly prevent PCV reduction among PbANKA infected mice. The influence of malaria on hematological parameters is extensively investigated and PCV reduction is considered a hallmark of both human and rodent malaria [24,28]. Infected mice may suffer from severe anemia because of rapid erythrocyte destruction, either by parasitemia or spleen reticulo endothelial cells [29]. For instance, in one study it was noted that within an estimated 48 h of post-infection rodent PCV was depleted to 43-44%. Further, PbANKA increased erythrocyte fragility and led to subsequent reduction of PCV in infected mice [30-32]. It can be concluded that when oral administered, no adverse effects were noted for the plant extracts ranging from 100-6,000 mg/kg doses signifying the safety of the extract in mice via the oral route. Interestingly, the crude leaf extract of *B. strychnifolia* showed suppressive effect on PbANKA infected mice in a dose-dependent manner and 1,000 mg/kg of the extract was observed to have the strongest activity [33]. The antimalarial activity and lack of toxicity of this extract found in the present study may partly confirm the claim by traditional practitioners about the use of the extract against malaria. However, the finding is only preliminary and thus confirmatory studies followed by isolation and characterization of the active antimalarial compounds of the extract that are responsible for the observed malaria suppression thereby resulting in increased MST, BW loss prevention and PCV reduction in the PbANKA infected mice are recommended.

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