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Antimalarial Activities of Moringa Oleifera Leaf Extract Against Plasmodium Berghei ANKA Infection in ICR Mice

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Abstract: *- Malaria caused by Plasmodium malaria parasite is a major public health problem in tropical and subtropical areas. However, the recent reports on malaria parasite resistant to antimalarial drugs highlight the need for new approaches to antimalarial chemotherapy. Hence, the study was aimed to investigate the antimalarial activity of M oleifera leaf extract against P. berghei infection in mice. Aqueous crude extract of M. oleifera leaves was freshly prepared, and acute toxicity test was then carried out in mice. For efficacy test in vivo, suppressive and curative tests were used to evaluate antimalarial activity in P. berghei ANKA infected mice administered orally of the extract (100, 1,000, and 2,000 mg/kg). Percent parasitemia and mean survival time were subsequently monitored. The results showed that the aqueous crude extract of M oleifera leaves at doses of 100, 1,000, and 2,000 mg/kg exhibited suppressive and curative activities with significant ($p<0.05$) at dose-dependent manners. Moreover, 1,000 and 2,000 mg/kg of the extract treated mice lived longer than the corresponding untreated control with significant ($p<0.05$). It could be concluded that the aqueous crude extract of M. oleifera leaves demonstrated suppressive and curative antimalarial activities against P. berghei ANKA infection in mice.*

Keywords*: - *Antimalarial activity; Moringa oleifera; Plasmodium berghei.

Introduction

Malaria is the most important parasitic infection and it remains a major public health in Africa, South America, and Asia [1]. The present global situation indicates an insurgence in the severity of the disease, and malaria could still be described as one of the most important communicable diseases, with an annual incidence of 300-500 million cases and a death tool of 1-2 million people [2, 3]. Even though a malarial vaccine is the best malarial control, however current research on development of vaccine is still at preclinical stage [4]. Therefore, the strategy for malaria mainly focuses on the drugs capable of reducing or eliminating malaria parasites. Unfortunately, the emergence of antimalarial drug resistant Plasmodium parasite is causing not only the spread of malaria to new endemic areas but also its re-mergence in areas where it had previously been eradicated [5]. In this respect, medicinal plants are a potential source of new, effective and affordable antimalarials. However, most of these plants are not explored chemically and can constitute lead molecules for new antimalarial development [6]. Hence, antimalarial discovery from such plants is currently more targeted because histories proved that plants are richest sources of antimalarial phytochemicals [7]. Moreover, quinine and artemisinin have been provided by Cinchona species and Artemisia annua traditionally used for malarial treatment for a long period of times, respectively. Therefore, medicinal plants have made and continue to make a great contribution to antimalarials as they contain molecules with a great variety of activities.

Moringa oleifera Lam. is a miracle tree that grows widely in many tropical and subtropical countries including India, Africa, South and Central

America, and throughout Asia and Southeast Asia. It is postulated to have the potent antioxidant content in food and also has a remarkable range of medicinal uses [8]. Recently, several therapeutic effects of M. oleifera such as anti-cancer, anti-microbial, antioxidant, anti-inflammatory, anti-diabetic, anti-dyslipidemic, and anti-hypertensive effects have been investigated [9]. It has also been reported that the leaves of this plant provide a rich source of polyphenols, flavonoids, carotanoids, alkaloids, quercetin, kaempferol, apigenin, and many other phytoconstituents that offer essential and disease preventing nutrients to humans [10]. However, antimalarial activity of M. oleifera, especially in mouse model has not yet been reported. Considering all the above since there is no previous describe, experimental-based evidence for its antimalarial effectiveness is found essential. This study is designed to evaluate the antimalarial activity of M. oleifera leaf extract against P. berghei infection in mice.

Materials and Methods

Collection and preparation of plant materials

Fresh leaves of M. oleifera were collected in May, 2016 from Suphanburi province, Thailand. The plant was identified and authenticated by Dr. Sakaewan Ounjaijean of Department of Pharmacology, Faculty of Pharmacy, Payap University, where a voucher specimen (No. 101A) was maintained. The leaves were cleaned and dried in hot-air oven at 50oC for overnight. The dried leaves were then ground into fine powder using an electric grinder and kept in a tightly closed brown bottle until used for extraction.

Preparation of crude extract

Aqueous crude extract of M. oleifera leaves was prepared by a microwave-associated water extraction method [11]. Briefly, the plant material was extracted by dissolve with distilled water (1:10) and put in microwave at 360 W for 5 min. Incubation at room temperature with continuous stirring for overnight was subsequently performed. The mixture was first filtered using cotton, and the filtrate was then passed through Whatman no. 1 filter paper. The aqueous extract was freeze-dried using centrifugal freeze dryer. The extract was stored at -20oC until used. Before experiment, the powdered extract was dissolved in distilled water completely to obtain appropriate doses for using in the in vivo experiment.

Animals

Female ICR (Institute of Cancer Research) mice, weighting 25-30g and 4-6 weeks old obtained from the National Laboratory Animal Center, Mahidol University, Thailand were used in this study. All mice were housed in plastic cages in the animal house, Department of Clinical Chemistry, Faculty of Medical Technology, Western University under standard conditions (temperature 25-28oC, photoperiod: approximately 12 h natural light per day and relative humidity: 50-55%). The mice were fed with food pellets and drinking water ad libitum. Procedures of the animal experiments were ratified by the Ethical Committee on Animal Experimentation, Western University.

Acute toxicity study of the extract

The acute toxicity of the aqueous leaf extract of M. oleifera was tested to determine the safety of the agent using method as previously described [12]. Groups of ICR mice (5 mice/group) were orally administered with the extract at the doses of 100, 1,000, 2,000, 4,000, and 6,000 mg/kg. The mice were observed for signs of toxicity which includes paw licking, salivation, stretching of the entire body, weakness, respiratory distress, coma and death for 72 h, and up to 1 week.

Malaria parasites

Chloroquine sensitive strain of Plasmodium berghei ANKA (PbANKA) obtained from Dr. Chairat Uthaipibull, National Center for Genetic

Engineering and Biotechnology (BIOTEC), National of Science and Technology Development Agency (NSTDA) was used in this study. This parasite was maintained by continuous re-infestation in mice. Infected erythrocytes were obtained from a donor infected mouse by cardiac puncture. Parasitemia and erythrocyte count were then determined, and diluted with normal saline. Each mouse was inoculated intraperitoneally with infected blood suspension (0.2 mL) containing 1x107 infected erythrocytes of PbANKA.

Parasitemia determination

Thin blood smears of blood were made from tail of mice on microscopic glass slides and allowed to dry at room temperature. Then, they were fixed with 100% methanol for 10s and stained with 20% Wright stain at pH 7.2 for 30 min. The stained slides were washed with tap water and allowed to dry at room temperature. The parasitemia was determined by counting the number of infected erythrocytes under light microscope with 100x oil immersion lens. Percent parasitemia was subsequently calculated by using the following formula.

$$\% \text{ Parasitemia} = \frac{\text{Number of infected erythrocytes} \times 100}{\text{Total number of erythrocytes}}$$

Standard antimalarial drug

Chloroquine diphosphate salt (CQ) was used as standard antimalarial drug in this study. The drug at a dose of 10 mg/kg was freshly prepared in distilled water and treatment orally by intragastric gavage.

Suppressive antimalarial test

The standard 4-day suppressive test was employed for the study [13]. After standard parasite inoculum, 1x107 infected erythrocytes of PbANKA, 25 mice were randomly divided into 5 groups with 5 mice each as above mentioned in grouping of animals. Treatment was started after 3 h of infection on day 0, and was continued daily for 4 consecutive days (day0-3) with 100, 1,000, and 2,000 mg/kg of the extract, CQ (10 mg/kg), and distilled water, all treatment was administered orally by gavage. On the fifth day, parasitemia was determined, and percent suppression was then calculated using the following formula.

$$\% \text{ Suppression} = \frac{(\text{Parasitemia in untreated group} - \text{Parasitemia in treated group}) \times 100}{\text{Parasitemia in untreated group}}$$

Curative antimalarial test

Evaluation of curative potential of M. oleifera leaf extract was carried out according to the method described previously [14]. Groups of mice (5 mice of each) were passed intraperitoneally with standard inoculum of 1x107 infected erythrocytes of PbANKA. After 72 h, the mice were treated with 100, 1,000, and 2,000 mg/kg of the extract, CQ (10 mg/kg), and distilled water, all treatment was administered orally by gavage for 4-consecutive days. Parasitemia and percent suppression were consequently calculated.

Determination of mean survival time

Mortality was monitored daily, and the number of the days from the time of infection up to death was recorded for each mouse in the treatment and untreated groups. Mean survival time (MST) was then calculated for each group by using the following formula [15].

$$\textbf{MST} = \frac{\text{Sum of survival time of all mice in a group}}{\text{Total number of mice in that group}}$$

Data analysis

The data of the study were presented as mean + SEM (standard error of mean). Statistical

significance was determined by one way ANOVA and Tukey post-hoc test using GraphPad Prism software. For all the data obtained, statistical significance was set at $p<0.05$.

Results

Acute toxicity study of M. oleifera extract

The aqueous crude extract of M oleifera leaves administered orally in a single dose of up to 2,000 mg/kg showed no lethal effect within 72 h and up to 1 week of observation. Physical and behavioral observation revealed no visible signs of toxicity including urination, hair erection, lacrimation, and reduction of feeding activity. In addition, these mice were physically active. However, at the dose of 4,000 mg/kg, hair erection and sleepy activities were observed within 24 h although the mice became active from that time onwards. Moreover, at dose of 6,000 mg/kg, all mice were dead within 24 h.

Suppressive antimalarial test

The aqueous crude extract of M oleifera leaves exhibited a dose-dependent activity at different employed. Doses of 100, 1,000, and 2,000 mg/kg caused 40%, 80%, and 85% inhibition of parasitemia, respectively. The effect of the extract was significantly ($p<0.05$), compared to the untreated control. The standard antimalarial, CQ (10 mg/kg), caused 90% suppression (Figure 1).

Figure 1

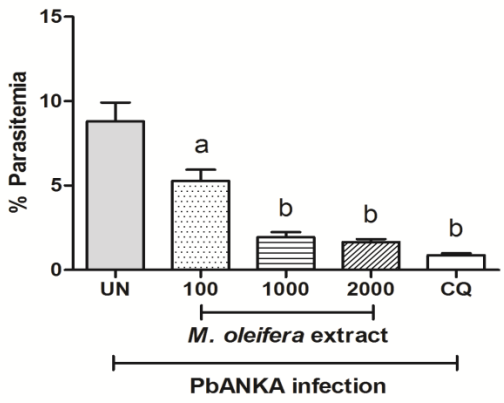

Figure Legends

Figure 1 Suppressive effect of M. oleifera leaf extract. Groups of ICR mice (5 mice of each) were inoculated with 1x107 parasitized erythrocytes of PbANKA by intraperitoneal injection. They were then given with 100, 1,000, and 2,000 mg/kg of the extract orally by gavage for 4-consecutive days. Parasitemia was measured by microscopic examination. $^{a}p<0.05$ and $^{b}p<0.01$, compared to untreated control. UN; untreated control, CQ; PbANKA infected mice treated with 10 mg/kg of chloroquine.

Curative antimalarial test

It was found that the aqueous crude extract of M. oleifera leaves produced dose-dependent reduction of parasitemia in the extract treated groups. The percent inhibition of parasitemia of the extract treated groups were 25%, 65%, and 70% for the 100, 1,000, and 2,000 mg/kg of the extract, respectively. While that of the CQ treated group was 80%. The effect of the extract was significantly ($p<0.05$), compared to the untreated control. However, no significance was observed in the extract treated group at s dose of 100 mg/kg (Figure 2).

Figure 2

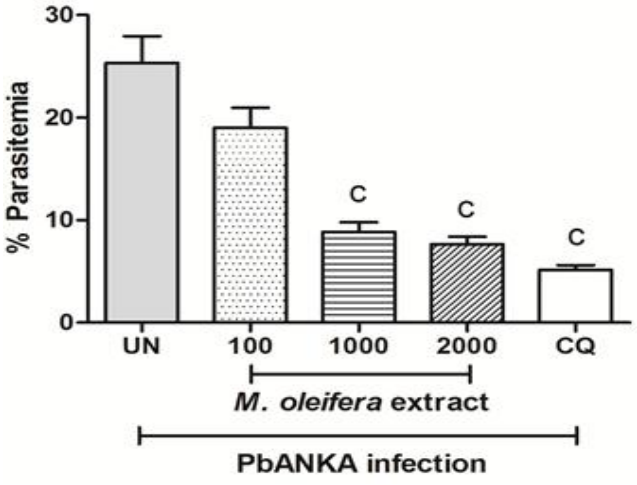

Figure 2 Curative effect of M. oleifera leaf extract. Groups of ICR mice (5 mice of each) were inoculated with 1x107 parasitized

erythrocytes of PbANKA by intraperitoneal injection for 4 days. They were then given with 100, 1,000, and 2,000 mg/kg of the extract orally by gavage for 4-consecutive days. Parasitemia was measured by microscopic examination. [c]p<0.001, compared to untreated control. UN; untreated control, CQ; PbANKA infected mice treated with 10 mg/kg of chloroquine.

In suppressive and curative tests, 1,000 and 2,000 mg/kg of aqueous crude extract of M oleifera leaves treated mice lived longer than the corresponding untreated control with significant (p<0.05). However, the survival times of 100 mg/kg of the extract treated mice did not prolonged significantly, compared to untreated control, shown in Table 1.

Effect of extract on survival times of mice

Table 1 Effect of aqueous crude extract of M oleifera leaves on the mean survival times of PbANKA infected mice in both suppressive and curative tests.

Tests	Treatments	Mean survival time (Days)
Suppressive	Distilled water	10.0 ± 2.0
	100 mg/kg of extract	15.0 ± 5.0
	1,000 mg/kg of extract	21.0 ± 5.6[a]
	2,000 mg/kg of extract	26 ± 4.6[a]
	10 mg/kg of CQ	24.7 ± 5.0[a]
Curative	Distilled water	10.0 ± 2.0
	100 mg/kg of extract	12.3 ± 2.5
	1,000 mg/kg of extract	17.0 ± 5.0[a]
	2,000 mg/kg of extract	20.7 ± 3.1[a]
	10 mg/kg of CQ	23.3 ± 4.2[b]

[a]p<0.05, [b]p<0.01, and [c]p<0.001, compared to PbANKA treated with distilled water

Discussion

Study of the antimalarial activity of aqueous crude extract of M. oleifera leaves was carried out on ICR mice experimentally infected with P. berghei ANKA. Again, the remarkable activity of quinine and the success of artemisinin stimulated the finding for new plant extract derived antimalarial property. The choice of this plant was based on previous reports that showed the presence of alkaloids, flavonoids, quercetin and kaempferol [16]. These constituents have been found in other plant extracts which exhibited antimalarial activity [17-19]. Therefore, the antimalarial activity of aqueous crude extract of M. oleifera leaves could be attributed to the presence of these compounds.

The aqueous crude extract of M. oleifera leaves was well tolerated by the mice up to the dose of 2,000 mg/kg. The LD50 of this extract was estimated to be 5,000 mg/kg far above the highest administered dose of 2,000 mg/kg. It can be indicated that the mice was safe with the different doses of the extract administered to them. Similar result was reported previously [20]. Therefore, the results implied that the aqueous crude extract of M. oleifera leaves was toxic at doses above 5,000 mg/kg causing toxic effects and eventually death of the mice.

Parasitemia in the PbANKA infected mice was monitored using thin blood smear made from the tail vein of the mice. The average of percent parasitemia showed high level of infection after 4

days of inoculation. This result is consistent with previous studies of high percent parasitemia in PbANKA infected mice after 4 days and death of infected mice after 10 days of inoculation [21]. The high level of parasitemia is a critical feature of Plasmodium infection which could result in hemolytic anemia [22, 23].

After administration of the extract in suppressive and curative tests, the results obtained from this study showed that the aqueous crude extract of M. oleifera leaves exhibited significant ($p<0.05$) antimalarial effect against PbANKA infection in mice. A compound is considered as active when percent inhibition is 30% or more, which supports the finding of the present study [3]. In this study, CQ used as a positive control, was observed to significantly ($p<0.05$) decrease the parasitemia in the infected mice at higher rate than the aqueous crude extract of M. oleifera leaves. The antimalarial activity of plant extract is due to the presence of bioactive metabolites. Basically, different secondary metabolites, such as alkaloids, polyphenols, flavonoids, terpenoids, quercetin, and kaempferol, have been reported from the aqueous crude extract of M. oleifera leaves. These metabolites have antimalarial activities [7, 10, 12]. However, the active compounds responsibility for this observed antimalarial activity need to be identified. Hence, the antimalarial activity observed in this extract could have resulted from single or in combination of the above metabolites. The possible mechanisms might be through antioxidant, free radical scavenging, immunomodulatory, intercalation in DNA, inhibition of protein synthesis, interference with the invasion of new erythrocytes by parasites, or by any other unknown mechanisms [11, 24, 25].

Mean survival time is another parameter evaluates the antimalarial of the extracts. Accordingly, the extract which can prolong the survival time of infected mice are considered as active compounds against malaria [15]. This study, the mice treated with 1,000 and 2,000 mg/kg of extract had significantly lived longer than untreated control. However, the survival time of mice treated with 100 mg/kg of extract was not significantly different from untreated control. This might be due to the antimalarial activity of the extract.

From the present study, it can be concluded that the aqueous crude extract of M. oleifera leaves showed potent suppressive and curative antimalarial activities against PbANKA infected mice. Accordingly, with the essence of further studies this plant could serve as the target source of new antimalarial leads and/or drugs for the treatment of malaria.

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