

## A Pilot Study on Antimalarial Effects of *Moringa oleifera* Leaf Extract in *Plasmodium berghei* Infection in Mice

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### Abstract

The emergence and spread of antimalarial drug resistance of *Plasmodium* parasites, as well as hypoglycemia, during malaria infection, and subsequent death, are critical problems in malaria-endemic areas. Hence, finding new compounds, especially plant extracts having antimalarial and anti-hypoglycemic activities, are urgently needed. The present study aimed to investigate the antimalarial and anti-hypoglycemic effects of *Moringa oleifera* leaf extract in *Plasmodium berghei* infection in mice. Aqueous crude extract of *M. oleifera* leaves was freshly prepared and used for an efficacy test *in vivo*. Groups of ICR mice (5 mice in each) were infected with  $1 \times 10^7$  infected red blood cells of *P. berghei* ANKA by intraperitoneal injection and given the extract orally with doses of 100, 500, and 1000 mg/kg for 4 consecutive days. Parasitemia and plasma glucose levels were subsequently measured. The results showed that *M. oleifera* leaf extract presented significant ( $p < 0.001$ ) inhibition of parasitemia in a dose-dependent manner. Moreover, this extract exerted anti-hypoglycemia effects in infected mice in a dose-dependent manner. The highest degrees of activity were found at a dose of 1000 mg/kg of the extract. Additionally, no effect on plasma glucose was found in normal mice treated with this extract. It can be concluded that aqueous crude extract of *M. oleifera* leaves exerted antimalarial and anti-hypoglycemic effects in *P. berghei* infection in mice.

**Keywords:** Antimalarial, anti-hypoglycemic, *Moringa oleifera*, *Plasmodium berghei*

### Introduction

Malaria is a parasitic disease which is widely prevalent in the world, especially in tropical and sub-tropical zones. According to the 2015 world malaria report, there were about 2 million cases of malaria, and an estimated 1 million deaths, annually. Most of the deaths occur among children living in Africa, where a child dies every minute from malaria [1]. The control and treatment of these *Plasmodium* infections has been complicated by widespread resistance to the available antimalarials, such as chloroquine [2]. Moreover, hypoglycemia during malaria infection is a well-recognized complication of *P. falciparum* and *P. vivax* malaria, occurring in between 2 - 6 % of hospitalized patients, with a mortality that can reach up to 45 % [3]. Hence, there is an urgent requirement to search for alternative antimalarials to counter resistance to existing ones, as well as to treat and protect against hypoglycemia induced by malaria. In this respect, natural products are promising sources for biologically active compounds, and have the potential to be developed as novel antimalarial and anti-hypoglycemic compounds, which are generally safer to humans [4]. The potential of chemotherapeutic compounds against malaria have been proven, with such examples as quinine from the cinchona species, and artemisinin from *Artemisia annua* [5].

*Moringa oleifera* is a highly valued plant, and is indigenous to and distributed throughout many countries of the tropical and sub-tropical regions, such as Africa, Asia, and Southeast Asia, including

Thailand. This tree is cultivated and used as a vegetable for spice, cooking, cosmetics, and medicinal properties [6]. Important medicinal properties of this plant include antioxidant, anti-inflammatory, anti-pyretic, anti-bacterial, anti-fungal, anti-diabetic, anti-hypertensive, and anti-cancer effects [7]. Moreover, *M. oleifera* has been described to have larvicidal and repellent potential against malaria vector *Anopheles stephensi* [8]. It has been reported that the leaf extract of *M. oleifera* contains important active compounds, such as polyphenols, flavonoids, terpenoids, quercetin, and kaempferol [9]. However, the antimalarial effect of *M. oleifera* leaf extract, as well its protection against hypoglycemia during malaria parasite infection, has not yet been studied. Hence, the aim of the present study was to investigate the antimalarial activity of aqueous crude extract of *M. oleifera* leaves, as well as its anti-hypoglycemic effect against *P. berghei* infection in mice.

## Materials and methods

### Plant material

Dried leaves of *M. oleifera* were purchased from the Royal Project shop in Suphanburi province. This plant was identified by Dr. Sakaewan Ounjaijean from the Faculty of Pharmacy, Payap University. The identified specimen was deposited at the Faculty of Medical Technology, Western University.

### Preparation of aqueous crude extract of *M. oleifera* leaves

For preparation of aqueous crude extract of *M. oleifera* leaves, the microwave-assisted water extraction method was carried out [10]. Dried plant material was ground to obtain the powder using an electric blender. Ten grams of powdered dried plant material was dissolved in 100 ml of distilled water, and heated in a microwave at 360 watts for 5 min. Incubation at room temperature for 3 h was subsequently done, and the resulting mixture filtered through Whatman no. 1 filter paper to collect the filtrate. Freeze-drying was performed to remove the solvent, and the aqueous crude extract of *M. oleifera* leaves (MOE) was then kept at  $-20\text{ }^{\circ}\text{C}$ .

### Mice

Fifty female, 4 week old ICR (Imprinting Control Region) mice, weighing 25 - 30 g were used in the present study, purchased from the National Laboratory Animal Center, Mahidol University. They were kept in an animal room with a controlled temperature of 25 - 28  $^{\circ}\text{C}$ . They were freely given the standard diet of pellets and clean water, *ad libitum*. All experiments associated with the mice were ratified by the Animal Ethics Committee, Western University (AE-WTU-042016).

### Rodent malaria parasite

*Plasmodium berghei* strain ANKA (PbANKA) was used in the present study. This parasite was maintained in the ICR mice by an intraperitoneal (IP) injection of  $1 \times 10^7$  infected red blood cells (iRBC). Propagation of parasites in the mice was monitored by microscopic examination of Wright-Giemsa stained thin blood smears under a light microscope with a 100 $\times$  oil immersion lens. Percent of parasitemia was then calculated using the formula below:

$$\% \text{ parasitemia} = \frac{\text{Number of iRBC} \times 100}{\text{Total number of RBC}} \quad (1)$$

### Measurement of plasma glucose

Tail blood was collected from the ICR mice into a heparinized hematocrit tube, and centrifuged at 20,000 g for 5 min. Plasma was subsequently collected for measurement of plasma glucose using a commercial kit (BioSystems S.A., Costa Brava, Barcelona, Spain), according to the manufacturer's instructions.

### Efficacy test *in vivo*

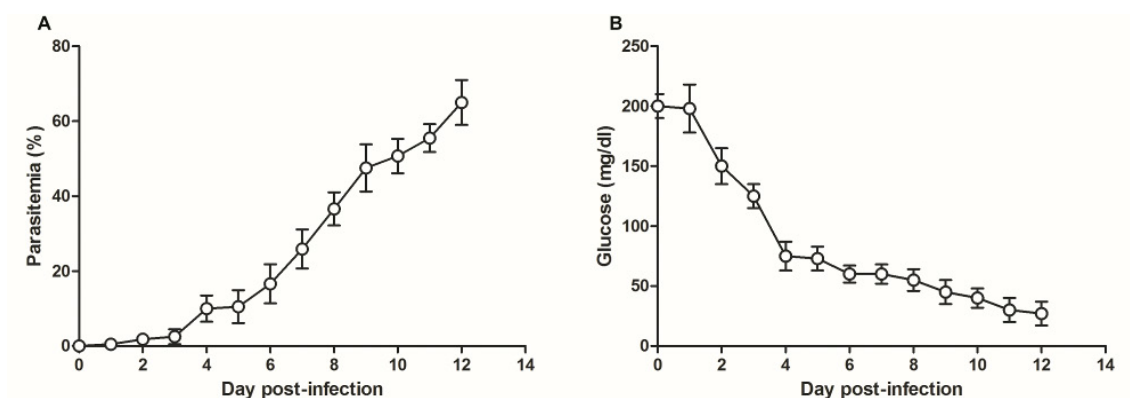
For evaluation of efficacy of MOE in mice, a standard 4-day was carried out [11]. Seven groups of ICR mice (5 mice in each) were infected with  $1 \times 10^7$  iRBC of PbANKA by IP injection. They were subsequently administered with MOE at doses of 100, 500, and 1000 mg/kg, by oral gavage twice a day for 4 consecutive days. On day 5 of the experiment, tail blood was collected for measurement of plasma glucose as previously described above, as well as for percent of parasitemia. Additionally, 3 controls were used: normal mice, normal mice treated with 1000 mg/kg of MOE, and infected mice without treatment (untreated mice).

### Statistics

The results were analyzed by GraphPad Prism version 5.01 (GraphPad Prism Software, Inc., US), and expressed as mean  $\pm$  standard error of mean (SEM). A significant level was considered to be at 95% confidence, and  $p < 0.05$  was analyzed by one-way ANOVA with Tukey post-hoc test.

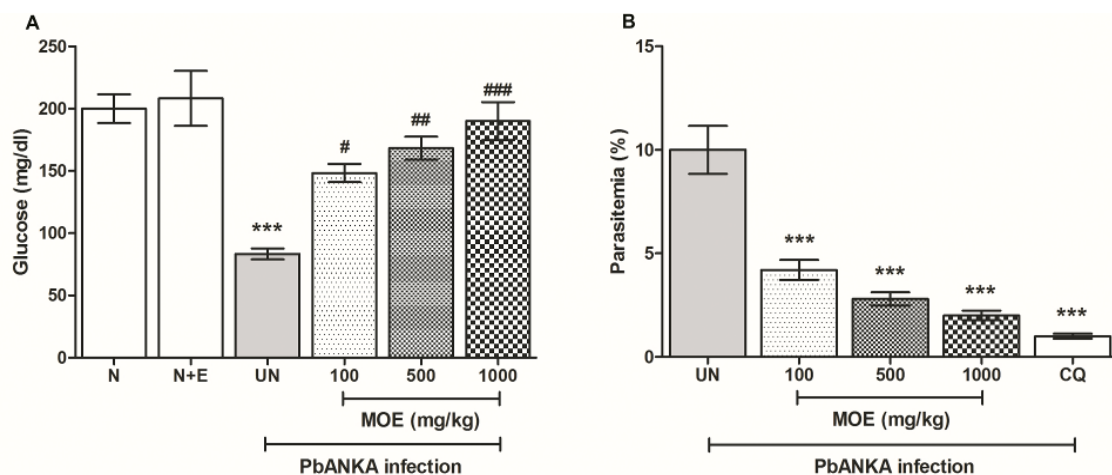
### Results and discussion

It has been reported that MOE is safe and non-toxic up at doses up to 4,000 mg/kg of oral administration in mice [9]. Therefore, the doses of MOE, 100, 500, and 1000 mg/kg were suitable and safe for use in the present study. As shown in **Figure 1(a)**, during PbANKA infection in mice, parasitemia was first detectable on day 1 post-infection, with a parasitemia  $< 1\%$ , and reached to 65% on day 12. The infected mice died within 2 weeks of infection. This is in line with the view that parasitemia increases progressively after inoculation or infection until the point of death in the absence of suitable treatment [12]. Moreover, hypoglycemia was also observed in PbANKA infected mice as indicated by a marked decrease of plasma glucose (**Figure 1(b)**). The onset of hypoglycemia was found on day 4 post-infection. This could be due in part to the fact that, during PbANKA infection, blood glucose is taken up across the plasma membranes of malaria parasites through a facilitated hexose transporter, and is in turn metabolized through the process of glycolysis [13]. This is accompanied with an approximately 100-fold increase in glucose utilization, compared to uninfected RBC, thus causing a profound case of hypoglycemia if untreated. In addition, hyperinsulinemia and hypoglycemia during malaria infection have also been described [14].



**Figure 1** Effect of untreated malaria infection in ICR mice. ICR mice were infected with  $1 \times 10^7$  iRBC of PbANKA by IP injection. (A) Parasitemia and (B) plasma glucose were monitored daily using methods previously described above. Results are expressed as mean  $\pm$  SEM.

As shown in **Figure 2(a)**, hypoglycemia with a significantly ( $p < 0.001$ ) low level of plasma glucose was found in untreated mice (UN). Interestingly, MOE showed dose-dependent anti-hypoglycemic activity in the extract treated mice, especially at a dose of 1000 mg/kg, which presented the highest level of activity. Several reports have described the activity of *M. oleifera* leaf extract in controlling plasma glucose [15]. Inhibition of glycolysis and hexose transporter of iRBC might be properties of MOE on plasma glucose. Moreover, the beneficial effect of MOE on insulin may be due to the antioxidant capacity of this extract, as previously described [16]. Additionally, no effect on plasma glucose was found in normal mice treated with the highest dose (1000 mg/kg) of MOE.



**Figure 2** Efficacy of MOE *in vivo*. Groups of ICR mice (5 mice in each) were infected with  $1 \times 10^7$  iRBC of PbANKA by IP injection. They were then given MOE at doses of 100, 500, and 1000 mg/kg orally for 4 consecutive days (Day 0-3). (A) Plasma glucose and (B) parasitemia were measured on day 4. Results are expressed as mean  $\pm$  SEM. \*\*\*  $p < 0.001$ , compared to normal mice. #  $p < 0.05$ , ##  $p < 0.01$ , and ###  $p < 0.001$ , compared to untreated mice. N; normal mice, N+E; normal mice treated with 1000 mg/kg of MOE, UN; untreated mice, and CQ; 10 mg/kg of chloroquine.

During early malaria infection, MOE produced a dose-dependent antimalarial activity against PbANKA. The extract caused a significant ( $p < 0.001$ ) antimalarial effect, compared to untreated mice, especially at a dose of 1000 mg/kg, which showed the highest level of activity (**Figure 2(b)**). The standard antimalarial drug, CQ, caused chemosuppression, which was higher than those of the extract treated groups. It has been reported the antioxidant is related to antimalarial activities in several plant extracts [17]. Hence, polyphenols and flavonoids in MOE, and their potent antioxidant properties, might play a central role in inhibiting PbANKA growth in mice. Moreover, an oxidative damage effect, inhibiting the malaria parasite, in artemisinin has been reported, and might be related to the antimalarial activity of MOE. It has been described that quercetin and kaempferol, active compounds in MOE, present antimalarial activity against *P. falciparum* [18,19]. Thus, these compounds in MOE might also play a role in present antimalarial activity. However, the modes of action and other mechanisms should be investigated.

## Conclusions

The present study was a pilot study on the antimalarial activity of aqueous crude extract of *M. oleifera* leaves. This extract exhibited a reasonable antimalarial activity in *P. berghei* infected mice. Moreover, protection against hypoglycemia was also observed in infected mice treated with this extract. Further studies are recommended on antimalarial activity, as well as on the safety profiles of this extract.

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