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Research Article

### **Antimalarial and anti-hypoglycemic effects of *Moringa oleifera* leaf Extract in *Plasmodium berghei* infection in mice**

Voravuth Somsak\*, Somrudee Nakinchat

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

\* Voravuth Somsak: voravuthsomsak@gmail.com

#### **Abstract**

The emergence and spread of antimalarial drug resistance of *Plasmodium* parasites as well as malaria-associated hypoglycemia and deaths 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 was 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 its *in vivo* efficacy test. Six groups of ICR mice (5 mice of 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 the doses of 100, 500, and 1000 mg/kg for four-consecutive days. Parasitemia and plasma glucose levels were subsequently measured. The results showed that *M. oleifera* leaf extract showed significant ( $p < 0.001$ ) inhibition of parasitemia at a dose-dependent manner. Moreover, this extract also exerted anti-hypoglycemia in infected mice with a dose-dependent manner. The highest activities were found at the 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* infected mice.

**Keywords:** medicinal plants, antiprotozoa, pharmacology

#### **Introduction**

Malaria is an important 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 estimated 1 million deaths annually (WHO 2009). Most of the deaths occur among children living in Africa where a child dies every minute from malaria (Hotez, Bottazzi et al. 2015).



Control and treatment of these *Plasmodium* infection have been complicated due to widespread resistance to the available antimalarials such as chloroquine (Antony and Parija 2016). Moreover, malaria-associated hypoglycemia, one of the most causes of death in *P. falciparum* and *P. vivax* severe malaria, occurs between 2-6% of hospitalized patient with a mortality that can reach up to 45% (Ali, Elhassan et al. 2011). Hence, there is an urgent requirement for the search of alternative antimalarials to combat the resistance of the existing antimalarials as well as to treat and protect hypoglycemia induced by malaria. In this respect, natural products are the promising sources for biologically active compounds and have potential for the development of novel antimalarial and anti-hypoglycemic compounds, which are generally safer to human (Mojab 2012). The potential of chemotherapeutic compounds against malaria have been proved with the examples such as quinine from cinchona species, and artemisinin from *Artemisia annua* (Duffy, Wade et al. 2012). *Moringa oleifera* is a highly valued plant, distributed in 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 vegetable, for spice, cooking, cosmetics, and medicinal properties (Hussain, Malik et al. 2014). Important medicinal properties of this plant include antioxidant, anti-inflammation, anti-pyretic, anti-bacterial, anti-fungal, anti-diabetic, anti-hypertensive, and anti-cancer (Abdull Razis, Ibrahim et al. 2014). Moreover, *M. oleifera* has been used to treat malaria (Prabhu, Murugan et al. 2011). It has been reported that the leaf extract of *M. oleifera* presented important active compounds such as polyphenols, flavonoids, terpenoids, quercetin, and kaempferol (Stohs and Hartman 2015). However, it has not yet been studied the effect of *M. oleifera* leaf extract on hypoglycemia induced by malaria parasite. Hence, the aim of the present study was to investigate the anti-hypoglycemic effect of aqueous crude extract of *M. oleifera* leaves as well as antimalarial activity against *P. berghei* infection in mice.

## Materials and Methods

**Plant material:** Dried leaves of *Moringa oleifera* were purchased from the Royal Project shop at Suphanburi province. This plant was the identified by Dr. Sakaewan Ounjaijean from the Faculty of Pharmacy, Payap University. The voucher 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, microwave-assisted water extraction method was carried out (Rodriguez-Perez, Gilbert-Lopez et al. 2016). Dried plant material was ground to obtain the powder using electric blender. Ten grams of powdered dried plant material was dissolved in 100 ml of distilled water, and heated in microwave at 360 w for 5 min. Incubation at room temperature for 3 h was subsequently done, and then filtered through Whatman no. 1 filter paper to collect the filtrate. Freeze-dry was performed to remove the solvent, and the aqueous crude extract of *M. oleifera* leaves (MOE) was then kept at -20°C.

**Mice:** Female, 4 weeks old ICR mice, weighting 25-30 g used in the present study were purchased from the National Laboratory Animal Center, Mahidol University. They were kept at the animal room with temperature control of 25-28°C. They were freely given the standard diet pellet and clean water *ad libitum*. All experiments associated with the mice were ratified by the Animal Ethics Committee, Western University (WTU-AE-04-2017).

**Rodent malaria parasite:** *Plasmodium berghei* strain ANKA (PbANKA) was used in the present study. This parasite was maintained in ICR mice by intraperitoneal (IP) injection of  $1 \times 10^7$  infected red blood cells (iRBC). Propagation of parasite in mice was monitored by microscopic examination of Wright-



Giemsa stained thin blood smear under light microscope with 100x oil immersion lens. Percent parasitemia was then calculated using the formula below.

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

*Measurement of plasma glucose:* Tail blood was collected from ICR mice into heparinized hematocrit tube, and centrifuged at maximum speed for 5 min. Plasma was subsequently collected for measurement of plasma glucose using commercial kit (BioSystems S.A. Costa Brava, Barcelona, Spain), according to manufacturer's instruction.

*Efficacy test in vivo:* For evaluation of efficacy of MOE in mice, standard four days was carried out (Peters 1975). Six groups of ICR mice (5 mice of each) were infected with  $1 \times 10^7$  iRBC of PbANKA by IP injection. They were subsequently administered with MOE at the doses of 100, 500, and 1000 mg/kg by oral gavage, twice a day for 4 consecutive days. At day 5 of the experiment, tail blood was collected for measurement of plasma glucose as previously described above as well as % parasitemia. Additionally, 3 controls were also done including normal mice, normal mice treated with 1000 mg/kg of MOE, and 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). Significant level considered at 95% confidence,  $p < 0.05$  was analyzed by one-way ANOVA with Tukey post-hoc test.

## Results

It has been reported that MOE was safe and non-toxicity was found at the dose up to 4,000 mg/kg of oral administration in mice (Stohs and Hartman 2015). Therefore, the doses of MOE, 100, 500, and 1000 mg/kg were suitable and safe for using in the present study. As showed in Figure 1A, 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 increase progressively after inoculation or infection until the point of death in the absence of suitable treatment (Offeddu, Rauch et al. 2014). Moreover, hypoglycemia was also observed in PbANKA infected mice as indicated by markedly decrease of plasma glucose (Figure 1B). 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 membrane of malaria parasite through a facilitated hexose transporter and is in turn metabolized through the process of glycolysis (Slavic, Straschil et al. 2010). It is accompanied with approximately 100-fold increase in glucose utilization, compared to uninfected RBC thus causing a profound hypoglycemia if untreated. In addition, hyperinsulinemia and hypoglycemia during malaria infection have also been described (Elased, Taverne et al. 1996).

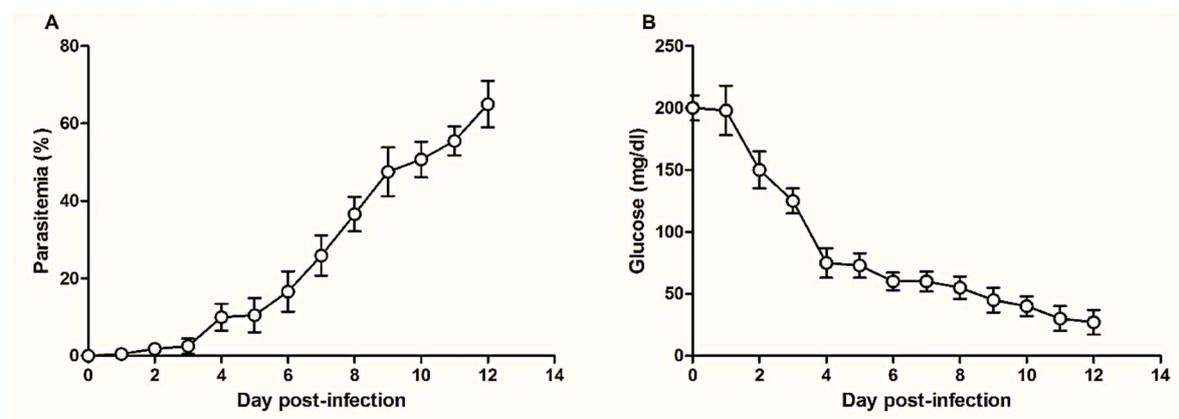


Figure 1. Malaria-associated hypoglycemia. ICR mice were infected with  $1 \times 10^7$  iRBC of PbANKA by IP injection. (A) Parasitemia and (B) plasma glucose were daily monitored by methods as previously described above. Results were expressed as mean  $\pm$  SEM.

As showed in Figure 2A, hypoglycemia with significant ( $p < 0.001$ ) low level of plasma glucose ( $83 \pm 7.6$  mg/dl) 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 presented the highest activity. Several reports have been described the activity of *M. oleifera* leaf extract to control plasma glucose (Jaiswal, Rai et al. 2013). Inhibition of glycolysis and hexose transporter of iRBC might be properties of MOE on plasma glucose. Moreover, beneficial effect of MOE on insulin may be due to the antioxidant capacity of this extract, as previously described (Olayaki, Irekpita et al. 2015). Additionally, no any effect on plasma glucose was found in normal mice treated with the highest dose (1000 mg/kg) of MOE.

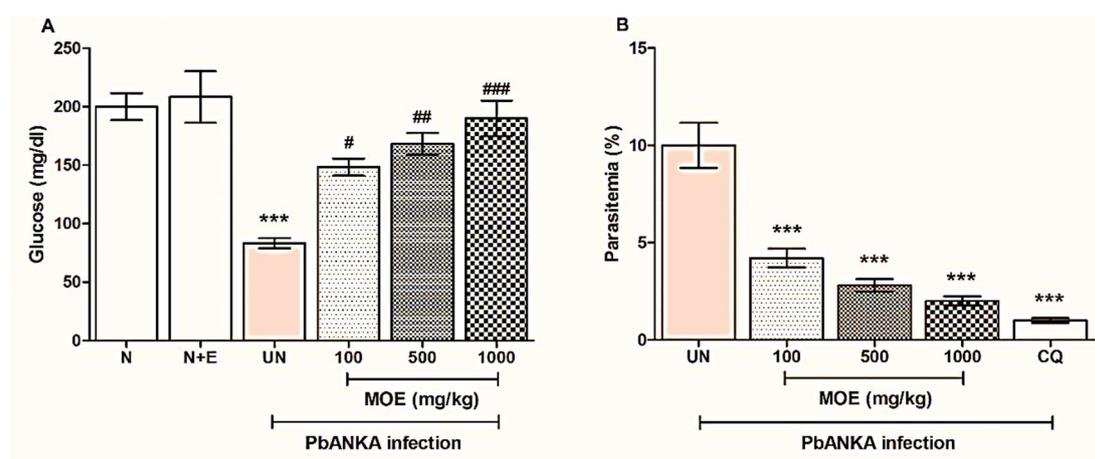


Figure 2. Efficacy of MOE *in vivo*. Groups of ICR mice (5 mice of each) were infected with  $1 \times 10^7$  iRBC of PbANKA by IP injection. They were then given MOE at doses of 100, 500, and 1000 mg/kg orally for 4-consecutive days. (A) Plasma glucose and (B) parasitemia were measured. Results were 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, compared to untreated mice, especially at a dose of 1000 mg/kg showed the highest activity (Figure 2B). The standard antimalarial drug, CQ caused chemosuppression, which was higher than those of the extract treated groups. It has been reported that the antioxidant was related to antimalarial activities in several plant extracts (Memvanga, Tona et al. 2015). Hence, polyphenols and flavonoids in MOE, and its potent antioxidant property might play a central role to inhibit PbANKA growth in mice. Moreover, oxidative damage in order to inhibit malaria parasite of artemisinin has also been reported, and might related to antimalarial activity of MOE. It has been described that quercetin and kaempferol, active compounds in MOE, presented antimalarial activity against *P. falciparum* (Barliana, Suradji et al. 2014; Ezenyi, Salawu et al. 2014). Thus, these compounds in MOE might also play a role to present antimalarial activity. However, the modes of action and other mechanisms should be searched for.

### Conclusion

The present study was shown that aqueous crude extract of *M. oleifera* leaves exhibited a reasonable anti-hypoglycemic activity in *P. berghei* infected mice. Moreover, antimalarial activity was also observed in infected mice treated with this extract. Further studies are recommended on antimalarial activity as well as safety profiles of this extract.

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### Declaration of Conflict of Interest

No conflict of interest associated with this work.

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