ANTIMALARIAL AND ANTIHYPOGLYCEMIC ACTIVITIES OF SHALLOT (*ALLIUM ASCALONICUM*) EXTRACT IN *PLASMODIUM BERGHEI* INFECTED MICE

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Abstract:

The present study investigated antimalarial and antihypoglycemic activities of aqueous crude extract of shallot (*Allium ascalonicum*) in *Plasmodium berghei* infected mice. Groups of ICR mice were treated orally with shallot extract (500, 1000, and 2000 mg/kg) after infection with *P. berghei* ANKA. Parasitemia and blood glucose levels were determined. At these doses, shallot extract inhibited parasitemia in dose-dependent manner, and could be used as combination treatment with pyrimethamine. In addition, antihypoglycemic activity was observed in dose-dependent fashion in infected mice treated with shallot extracts. In particular, the highest activities of shallot extract were found at dose 2000 mg/kg. These results indicated that aqueous crude extract of shallot has antimalarial and antihypoglycemic activities in *P. berghei* ANKA infected mice.

Keywords: *Plasmodium berghei,* Antimalarial, Antihypoglycemic, shallot, *Allium ascalonicum*

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INTRODUCTION

Malaria is an infectious disease with ravaging effects in the world, especially tropical and sub-tropical areas. The World Health Organization (WHO) has reported statistics that reveal half the world's population is at risk of malaria and that 1-2 million annual deaths can be attributed to malaria alone [1]. Malaria is caused by protozoa in genus *Plasmodium* that transmitted by female *Anopheles* mosquito. Five species of malaria parasites including *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* are responsible for human infection, although the majority of fatal cases are caused by *P. falciparum* [2]. Although an effective vaccine is the best long term control for malaria, current research on vaccine development is still in the laboratory. Therefore, global strategy for malaria mainly focuses on drug treatment. However, antimalarial drug resistant malaria parasites are causing not only the spread of malaria to new areas but also its re-emergence in areas where it had previously been eradicated [3]. In addition, malaria-associated hypoglycemia has been reported during malaria parasite infection [4-6]. This has prompted research towards the discovery and development of new antimalarial drugs with antihypoglycemic properties. In this respect, medicinal plants are potential targets for research and development of the alternative drugs.

Shallot (*Allium ascalonicum*) is a member of the Alliaceae family, which has been used mainly as a spice traditionally from the ancient times. Many different benefits of shallot have been described including antibacterial, antifungal, antioxidant, free radical scavenging, inhibit growth of tumors, and hypoglycemic effect on diabetes mellitus [7-11]. As far as we searched, there is no

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previous trial study showing the antimalarial activity of shallot extract, effect on blood glucose level during malaria infection. Hence, the aim of this study was to investigate the antimalarial activity of aqueous crude extract of shallot, and effect on blood glucose level during *P. berghei* infection in mice.

MATERIALS AND METHODS

Plant material

The bulbs of shallot (*A. ascalonicum*) were purchased from local vegetable market at Suphanburi province, and authenticated by Dr. Sakaewan Ounjaijean, Faculty of Pharmacy, Payap University.

Preparation of crude extract

Aqueous crude extract of shallot was performed as previously described [11]. In brief, the fresh bulbs of shallot were mixed with deionized water in an equal ratio (w:v). Homogenization was subsequently done using electric blender, and stirred overnight at room temperature to complete extraction. Then, it was filtered through a cheese cloth and centrifuged at 16,000 g, 4^{o}C for 30 min. After complete extracting and drying the shallot extract (SE) by freeze dryer, it's the yield of which was about 28% as compared to original bulb weight.

Experimental mice

Healthy ICR mice (female, 6-8 weeks old weighting between 30-35 g) obtained from National Laboratory Animal Center, Mahidol University, Thailand were used. The mice were conveniently housed under standard environmental condition at 22-25^{o}C with a 50% relative humidity and a 12 hr light/dark cycle. 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, Western University.

Rodent malaria parasite

Chloroquine-sensitive *P. berghei* ANKA strain (PbANKA) was used and maintained in our laboratory by weekly serial passage of 6×10^6 infected red blood cells in naïve mice. Parasitemia was daily monitored by Giemsa stained thin blood smear under light microscope with 100× oil immersion lens.

Acute toxicity test

Acute toxicity of shallot extract was carried out as previously described [12]. Groups of mice (5 mice of each) were given 500, 1000, 2000, and 3000 mg/kg body weight of the extract orally. The mice were then observed for signs of toxicity which include but not limited to paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in first 4 hr and subsequently daily for 7 days.

Measurement of blood glucose level

Tail blood was collected into heparinized microhematocrit tube. The end of tube was sealed with putty and centrifugation was then performed at 10,000 g for 10 min. Plasma was collected into a new 1.5-ml microcentrifuge tube, and used for blood glucose measurement. Blood glucose was measured using a commercial kit (BioSystem S.A. Costa Brava 30, Barcelona, Spain), according to the manufacturer's instruction.

Antimalarial drug

Standard antimalarial drug, pyrimethamine (PYR) was used in this study. The drug was freshly prepared in dimethyl sulfoxide (DMSO) 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 (1.0 mg/kg) on PbANKA infected mice.

Efficacy test *in vivo*

The Peter's 4-day test was employed [13]. Randomly groups of naïve ICR mice (5 mice of each) were inoculated by intraperitoneal injection with 6×10^6 infected erythrocytes of PbANKA, and treated for 4 consecutive days with 500, 1000, and 2000 mg/kg of shallot extracts orally by gavage twice a day (day 0-4). Three control groups were used; the healthy control was given either with normal saline or the extract (2000 mg/kg); the untreated control was given normal saline; the drug treatment control was given 1.0 mg/kg of PYR. Moreover, combination treatment was also carried out using PYR (1 mg/kg) and the extract (2000 mg/kg). On day 8 of the experiment, tail blood was collected to determine parasitemia, and plasma was subsequently used for measurement of blood glucose levels.

Statistics

Statistical analysis of the data was carried out using GraphPad Prism Software (GraphPad software, Inc., CA, USA). 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 were expressed as mean ± standard error of mean (SE).

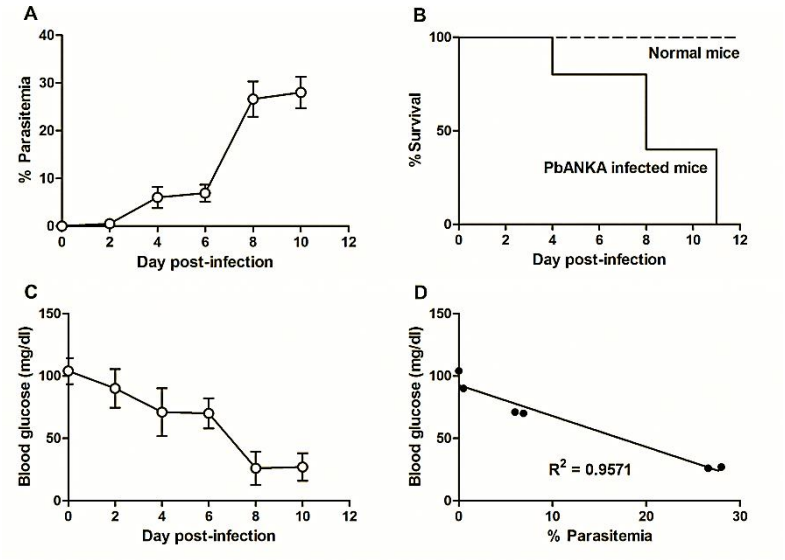

Figure 1 Malaria-associated hypoglycemia during *Plasmodium berghei* ANKA infection. ICR mice (5 mice of each) were intraperitoneally infected with 6×10^6 parasitized erythrocytes of PbANKA. **(A)** Parasitemia **(B)** percentage survival of infected mice, and **(C)** blood glucose levels were daily monitored. **(D)** Correlation between parasitemia and blood glucose level was also investigated. Results were expressed as mean ± standard error of mean (SE).

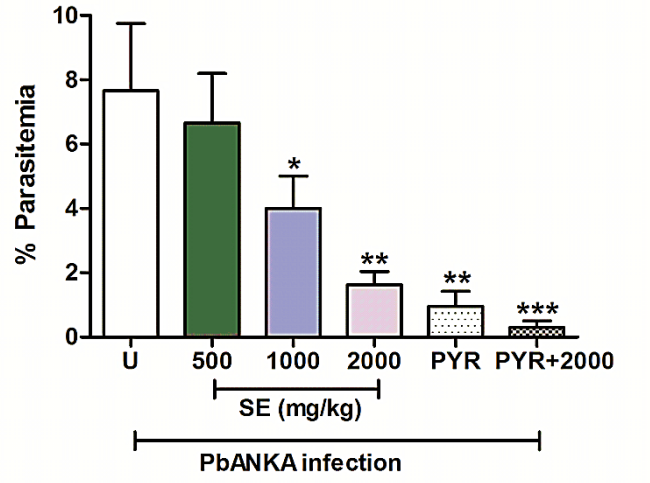

Figure 2 Antimalarial activity of shallot extract against *Plasmodium berghei* ANKA. ICR mice (5 mice of each) were intraperitoneally infected with 6×10^6 parasitized erythrocytes of PbANKA, and given orally 500, 1000, and 2000 mg/kg of the extracts twice a day for 4-consecutive days. Parasitemia was subsequently measured. The results were expressed as mean ± standard error of mean (SE). U; untreated, SE; shallot extract, PYR; pyrimethamine. *p<0.05, **p<0.01, and ***p<0.001 compared to untreated control.

RESULTS

Acute toxicity test

Behavioral signs of toxicity observed in mice given 3000 mg/kg include; paw licking, salivation, stretching and reduce activity. There was however no mortality at all doses used. Therefore, 500, 1000, and 2000 mg/kg were suitable doses for using in this study.

Malaria-associated hypoglycemia during PbANKA infection

There was a progressive increase in level of parasitemia as the days progressed from day 2 to 10 in the PbANKA infected mice (Figure 1A), and survival time was 10 days (Figure 1B). 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. Interestingly, determination of blood glucose levels showed a progressive decrease in the response to the presence of the parasites, which reached significant values on 8 after infection (Figure 1C). Moreover, strong negative correlation (R^2 = 0.9571) between parasitemia and blood glucose was also observed (Figure 1D).

Antimalarial activity of shallot extract against PbANKA

During early malaria infection, the aqueous crude extract of shallot produced a dose-dependent

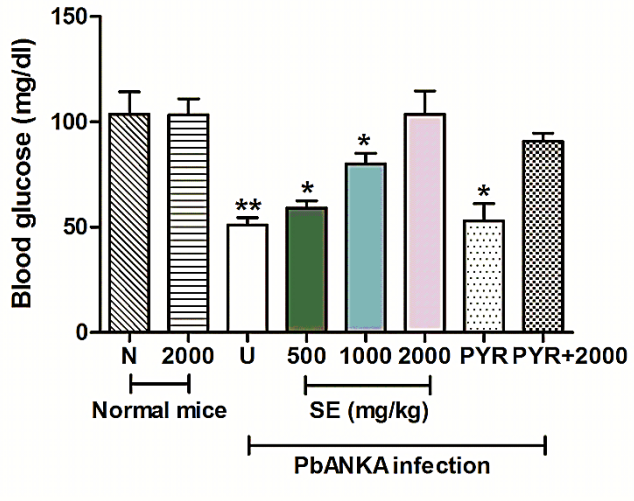

Figure 3 Antihypoglycemic activity of shallot extract against *Plasmodium berghei* ANKA. ICR mice (5 mice of each) were intraperitoneally infected with 6×10^6 parasitized erythrocytes of PbANKA, and given orally 500, 1000, and 2000 mg/kg of the extracts twice a day for 4-consecutive days. Blood glucose levels were then investigated. The results were expressed as mean ± standard error of mean (SE). N; normal, U; untreated, SE; shallot extract, PYR; pyrimethamine. *p<0.05 and **p<0.01 compared to normal control.

antimalarial effect against PbANKA. The extract caused a significant (p<0.05) antimalarial when compared to the untreated control, especially at dose of 2000 mg/kg showed the highest activity (Figure 2). The standard drug, PYR caused chemosuppression, which was higher than those of the extract treated groups. Moreover, combination treatment of PYR and the extract also presented antimalarial activity.

Antihypoglycemic activity of shallot extract against PbANKA

As showed in Figure 3, hypoglycemia with significant (p<0.01) low level of blood glucose was observed in untreated group. Interestingly, the aqueous crude extract of shallot exerted dose-dependent antihypoglycemia in the extract treated groups, especially at a dose of 2000 mg/kg showed the highest activity. Moreover, hypoglycemia was also found in PYR treated group. However, normal blood glucose level was observed in combination treatment between PYR and shallot extract. In addition, no effect on blood glucose level was observed in normal mice treated with this extract.

DISCUSSION

There was a progressive increase in level of parasitemia as the days progressed from day 2 to 10 in the PbANKA infected mice (Figure 1A), and survival time was 10 days (Figure 1B). 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. Interestingly, determination of blood glucose levels showed a progressive decrease in the response to the presence of the parasites, which reached significant values on 8 after infection (Figure 1C). Moreover, strong negative correlation (R^2 = 0.9571) between parasitemia and blood glucose was also observed (Figure 1D). This could be due in part to the fact that during malaria infection, glucose is rapidly taken up across the parasite plasma membrane through a facilitated hexose transporter and is in turn metabolized through the process of glycolysis [14, 15]. This is accompanied with approximately 100-fold increase in glucose utilization when compared with uninfected erythrocytes thus causing a profound hypoglycemia if untreated [16]. Furthermore, hyperinsulinemia and hypoglycemia during malaria infection has also been described [17].

During early malaria infection, the aqueous crude extract of shallot produced a dose-dependent antimalarial effect against PbANKA. The extract caused a significant (p<0.05) antimalarial when compared to the untreated control, especially at dose of 2000 mg/kg showed the highest activity (Figure 2). The standard drug, PYR caused chemosuppression, which was higher than those of the extract treated groups. Moreover, combination treatment of PYR and the extract also presented antimalarial activity. It has been reported the antioxidant potential was related to antimalarial activity in several plant extracts [18-21]. Hence, polyphenolic and sulfur compounds in shallot

extract, and its potent antioxidant activity might play a central role to inhibit PbANKA growth *in vivo*. Moreover, oxidative damage in order to inhibit malaria parasite of artemisinin has also been described, and might related to antimalarial activity of shallot extract. However, the modes of action and other mechanisms should be searched for.

As showed in Figure 3, hypoglycemia with significant (p<0.01) low level of blood glucose was observed in untreated group. Interestingly, the aqueous crude extract of shallot exerted dose-dependent antihypoglycemia in the extract treated groups, especially at a dose of 2000 mg/kg showed the highest activity. Several studies have been reported the activity of garlic extract to control blood glucose level. Knowledge of properties and constituents of shallot such as flavonols and sulfur compounds, and its analog with garlic suggests that biological activity of shallot extract to maintain and control blood glucose level might be similar to garlic extract [11, 22]. Inhibition of glycolysis and hexose transporter of infected erythrocytes might be properties of shallot extract on blood glucose levels. In addition, beneficial effect of shallot extract on insulin may be due to the antioxidant capacity of this extract. It has been also reported that aqueous extract of shallot bulbs had significant antioxidant potential [23]. Moreover, hypoglycemia was also found in PYR treated group. However, normal blood glucose level was observed in combination treatment between PYR and shallot extract. Hypoglycemia in PYR treatment might be due to the oxidative stress and hemolysis induction by this drug [24, 25]. In addition, no effect on blood glucose level was observed in normal mice treated with this extract.

It is interesting to note that aqueous crude extract of shallot (*A. ascalonicum*) was found the antimalarial and antihypoglycemic activities against *P. berghei* infected mice. In addition, combination treatment with standard antimalarial pyrimethamine was also recommended. Although the bioactive components and mechanism are yet to be identified, the results of this study provide the basis for further studies.

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