International Food Research Journal 23(2): 885-888 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Anti-hypoglycemic effect of aqueous leaf extract of Siamese neem tree *(Azadirachta indica)* in *Plasmodium berghei* infected mice

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Article history

Received: 1 June 2015
Received in revised form: 14 September 2015
Accepted: 16 September 2015

Keywords

Anti-hypoglycemia
Siamese neem tree
Azadirachta indica
Plasmodium berghei

Abstract

Hypoglycemia has been reported during malaria infection in blood stage, and can cause of death in malaria endemic countries. Hence, in this study was aimed to evaluate anti-hypoglycemic effect of Siamese neem tree extract in *P. berghei* infected mice. The aqueous leaf extract of Siamese neem tree was freshly prepared and used for orally treatment. Groups of ICR mice were infected intraperitoneally with $6x10^6$ infected erythrocytes of *P. berghei* ANKA, and subsequently given with the extract twice a day for 4 consecutive days. Blood glucose levels were then measured. Normal and untreated mice were used as healthy and disease controls, respectively. The results showed that hypoglycemia was developed during *P. berghei* ANKA infection in mice as indicated by decreasing of blood glucose level. However, blood glucose level in the extract treated mice was similar to normal group. It can be summarized that aqueous leaf extract of Siamese neem tree exhibited anti-hypoglycemic effect against *P. berghei* ANKA infected mice.

Introduction

Malaria caused by *Plasmodium* parasite is a major public health problem in tropical and sub-tropical countries with estimates of 700,000 cases annually (Kar *et al*., 2014). During malaria infection, hypoglycemia is recognized as serious complication malaria and occurred between 1-4% of hospitalized patients with a mortality that can reach up to 45% (Onyiriuka *et al.*, 2012). The glucose metabolism in malaria infection is affected by several factors including drug treatment, fever, parasite metabolism, hormonal changes, cytokines, fasting and gastrointestinal disturbances (Elased *et al*., 1996). This has prompted research towards the discovery and development of new, safe and affordable drugs to protect the hypoglycemia during malaria infection. In this respect, medical plant resources are potential targets for this research.

Siamese neem tree (*Azadirachta indica* A. Juss var. *siamensis* Valeton) is one of two varieties of neem of the family Meliaceae and is found throughout Southeast Asia including Laos, Mynmar, Cambodia and Thailand. This plant is used for the treatment of some pathological conditions related to oxidative disorders such as inflammation and skin diseases, rheumatic, arthritis, fever, and diabetes (Kitdamrongtham *et al*., 2014; Sithisarn *et al*., 2005; Sithisarn *et al*., 2006). However, publications concerning the activity of Siamese neem tree in hypoglycemic condition have not yet been reported. Hence, the aim of this study was to evaluate the anti-hypoglycemic activity of aqueous leaf extract of Siamese neem tree on *P. berghei* infection in mice.

Materials and Methods

Plant material and preparation of crude extract

Leaves of Siamese neem tree *(Azadirachta indica)* were obtained from Kanchanaburi province, and specimen was then verified by Dr. Sakaewan Ounjaijene, Department of Pharmacology, Faculty of Pharmacy, Payap University, Chiang Mai, Thailand. Dried powder leaves (50°C in hot air oven for 30 min) were extracted using hot distilled water (plant:water = 1:20 w/v) for 6-8 h, then filtered. The filtrate was evaporated to dryness on boiling water bath to obtain dried extract (Sithisarn *et al*., 2006).

Experiment mice

Naïve ICR mice weighting 25-30 g, aged 4-6

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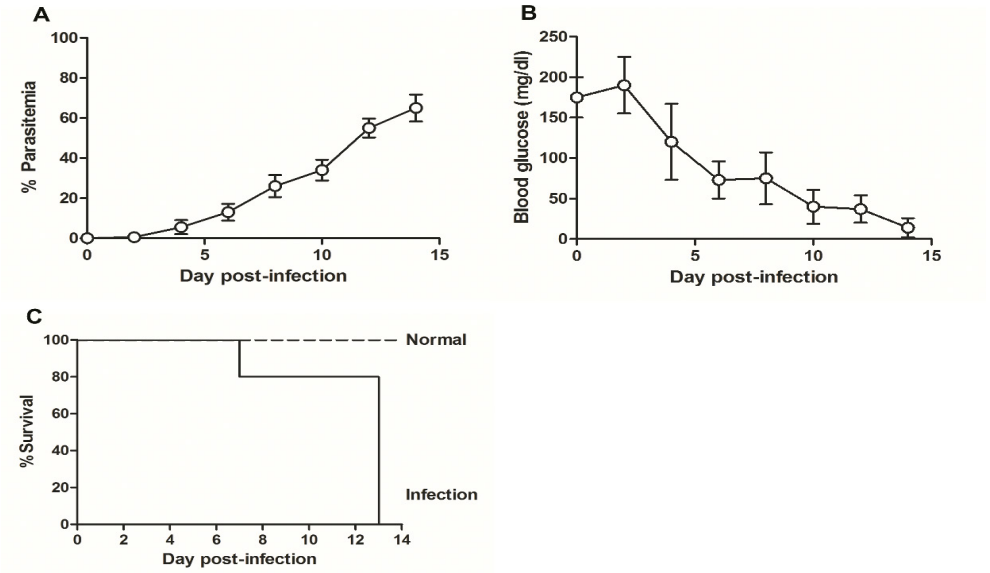

Figure 1. Malaria-associated hypoglycemia development during *P. berghei* ANKA infection. Naïve ICR mice were infected intraperitoneally with $6x10^6$ infected erythrocytes of PbANKA, (A) parasitemia, (B) blood glucose level, and (C) survival of infected mice were daily monitored. Results were expressed as mean+standard error of mean (SEM)

weeks were purchased from National Laboratory Animal Center (NLAC), Mahidol University. They were kept in animal room with temperature 22-25°C, 12 h day/night cycle, and fed with standard pellet and clean water ad libitum. All animal experiments were ratified by Animal Ethical Committee, Western University.

Rodent malaria parasite

Plasmodium berghei strain ANKA (PbANKA) was used in this study. The parasite was maintained in naïve ICR mice by intraperitoneal (IP) injection of $6x10^6$ infected erythrocytes. Parasite propagation was daily monitored as percent parasitemia by Giemsa stained blood smear.

Measurement of blood glucose

Tail blood was collected into heparinized hematocrit tubes. Centrifugation was then performed at 10,000 g for 10 min. Plasma was collected into new 1.5-ml microcentrifuge tube and blood glucose measurement was subsequently done using commercial kit (BioSystems S.A. Costa Brava, Barcelona, Spain).

Efficacy test in vivo

The standard 4-day suppressive test was used to evaluate efficacy of the extract (Peters, 1975). Groups of ICR mice (5 mice of each) were randomly divided, and infected intraperitoneally with 6x106 infected erythrocytes of PbANKA. They were given with 500, 1500, and 3000 mg/kg of the extract twice a day for 4 consecutive days. Blood glucose levels were then measured as previously described. Normal and untreated mice were used as healthy and disease controls, respectively.

Statistical analysis

All data was analyzed using GraphPad Prism (GraphPad Software, Inc., USA). The results were expressed as mean+standard error of mean (SEM). The one-way ANOVA was used to test and compare the results at a 95% confidence. Values of $p<0.05$ was considered significance.

Results and Discussion

Malaria-associated hypoglycemia development during PbANKA infection

In order to determine hypoglycemia during malaria infection, naïve ICR mice were infected intraperitoneally with $6x10^6$ infected erythrocytes of PbANKA. Hypoglycemia was subsequently investigated by measuring blood glucose level. As shown in Figure 1A, there was a progressive increase in line of parasitemia as the days progressed from day 2 to 14 in the infected mice. Parasitemia increased progressively after inoculation until the point of death in the absence of suitable treatment. Next, we observed that blood glucose level was markedly decreased starting at day 6 after infection (Figure 1B), and all infected mice died within 2 weeks (Figure 1C). This could be due in part to the fact that during malaria infection, glucose is rapidly taken up across the parasite membrane through a facilitated hexose transporter and is in turn metabolized through

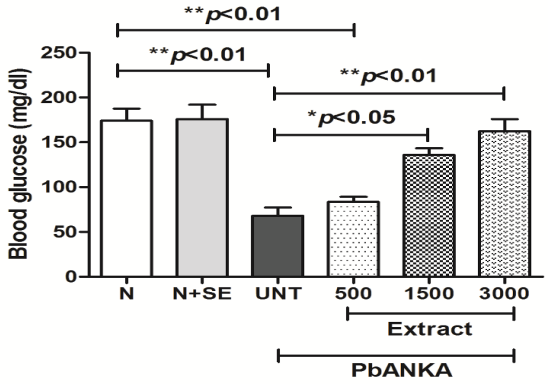

Figure 2. Efficacy of aqueous leaf extract of Siamese neem tree on blood glucose level during *P. berghei* ANKA infection in mice. Groups of naïve ICR mice were infected intraperitoneally with $6x10^6$ infected erythrocytes, and given with 500, 15000, and 3000 mg/kg of the extracts orally by gavage twice a day for 4 consecutive days. Blood glucose levels were then measured. Results were expressed as mean+standard error of mean (SEM). N; normal mice, SE; Siamese neem tree extract, UNT; untreated mice

the process of glycolysis (Tjhin *et al.*, 2013). This is accompanied with approximately 100-fold increase in glucose utilization when compared with uninfected erythrocytes thus causing a profound hypoglycemia (Woodrow *et al*., 2000). Furthermore, impairment of glucose production caused by the inhibition of gluconeogenesis during severe malaria infection has previously been discussed and also leaded to hypoglycemia (Eltahir *et al*., 2010; Geoffrion *et al*., 1985; van Thien *et al*., 2004).

Anti-hypoglycemia of aqueous leaf extract of Siamese neem tree during PbANKA infection

In order to investigate anti-hypoglycemic effect of the extract, PbANKA infected ICR mice were given the extract orally by gavage twice a day for 4 consecutive days. Blood glucose levels were measured and compared to normal and untreated groups. It was found that glucose level was significantly lower ($p<0.05$) in the untreated group compared to the normal control (Figure 2). Interestingly, aqueous leaf extract of Siamese neem tree exerted dose dependent anti-hypoglycemic effect against PbANKA infected mice, especially at the doses of 1,500 and 3,000 mg/kg. This could be due to a fall in glycolysis activity within the cells of mice in these groups (Elased & Playfair, 1994). Moreover, antioxidant properties of Siamese neem extract and its active compound have been reported to protect the cell from oxidative damage and control glucose homeostasis (Sithisarn *et al*., 2005). The result also showed that the extract might have no significant side effects on glucose level in normal mice.

Conclusion

The results of this study showed that aqueous leaf extract of Siamese neem tree exhibited anti-hypoglycemia during PbANKA infection in mice.

Acknowledgements

The authors wish to thank Dr. Sakaewan Ounjaijean for valuable suggestion and discussion about the extract preparation. We like to thank Miss Sukanya Chachiyo for her helping in animal experiments.

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