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Protective effects of Andrographis paniculata leaf extract on liver and renal damage and hypoglycemia during Plasmodium berghei infection

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Abstract

Liver and renal damage are some causes of deaths in malaria disease. Moreover, hypoglycemia and hypoglycemic shock during malaria infection have also been reported. Therefore, finding new plant extracts to have the activities to protect liver and renal damage as well as hypoglycemia during malaria infection is urgently needed. In this study was aimed to investigate the protective effects of *Andrographis paniculata* leaf extract on liver and renal damage as well as hypoglycemia induced by *Plasmodium berghei* ANKA (PbANKA) infection in mice. Aqueous crude extract of *A. paniculata* leaves was freshly prepared using hot water method. For efficacy test, standard 4-day suppressive test was used. ICR mice were inoculated with $1x10^7$ parasitized erythrocytes of PbANKA by intraperitoneal infected, and given the extract (500, 1000, and 2000 mg/kg) orally by gavage for 4 consecutive days. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, blood urea nitrogen (BUN), creatinine, and glucose were measured. It was found that liver and renal damage were developed during malaria infection as indicated by markedly increasing of AST, ALT, BUN, and creatinine, and decreasing of albumin levels. Additionally, hypoglycemia was also found during the infection. Interestingly, *A. paniculata* extract showed protective effects on liver and renal damage, and presented anti-hypoglycemia in a dose-dependent manner against infected mice treated with this extract. The highest effect was found at dose of 2000 mg/kg. Moreover, no any side effects were observed in normal mice treated with the extract. It can be concluded that *A. paniculata* leaf extract can be used as alternative drug to protect liver and renal damage as well as hypoglycemia during malaria infection

Keywords: Protective effects; *Andrographis paniculata*; Liver damage; Renal damage; Hypoglycemia; *Plasmodium berghei*

Introduction

Malaria constitutes one of the biggest health problems in tropical and sub-tropical zones such as Africa, South America, Asia and Southeast Asia including Thailand. It is estimated that 250 million peoples are infected by malaria with about 1 million deaths annually [1]. This disease is caused by protozoa parasite in genus *Plasmodium*, especially *P. falciparum* and *P. vivax* which are major cause of death [1]. For causes of death by malaria including cerebral malaria, hemolysis and severe anemia, metabolic acidosis, multiple organ failure such as liver and renal, and hypoglycemic shock have been reported [2, 3]. Malaria-associated liver and renal damage occur between 2-5% of hospitalized patients with a mortality that can reach up to 45% [4, 5]. The pathogenesis for liver and renal damage induced by malaria is multifactorial and not well characterized, but several hypothesis suggest involvement of cytoadherence of parasitized erythrocytes, proinflammatory response, and damage due to oxidative stress [6]. Moreover, the consumption of hemoglobin by malaria parasites in blood stage of infection gives rise of amounts of free heme that have the ability to induce oxidative stress [7]. In addition, malaria associated hypoglycemia has been reported during malaria infection, and involvement of oxidative stress has also been described [8]. This has prompted research towards the discovery of new drugs with protective effects on organ damage and anti-hypoglycemia. In this respect, plant extracts are potential targets for research and development of the alternative drugs.

In the present study, *Andrographis paniculata* (Acanthaceae) was selected for evaluation of its activities. The pharmacological properties of *A. paniculata* leaf extract are well documented and several *in vitro* and *in vivo* studies describe its antioxidant, anti-inflammation, anti-cold, anti-hepatotoxicity, anti-nephrotoxicity, antimalarial, anti-microbials, and anti-cancer activities [9-11]. Moreover, it has also been reported to have homeostatic effect on blood glucose in diabetic patients [12]. However, *A. paniculata* leaf extract has not yet been studied in its protective effects on liver and renal damage as well as hypoglycemia induced by malaria. Hence, this study was aimed to investigate the protective effects of aqueous crude extract of *A. paniculata* on liver and renal damage, and hypoglycemia during *P. berghei* infection in mice.

Materials and Methods

Preparation of aqueous crude extract of *Andrographis paniculata*:

Leaves of *Andrographis paniculata* were collected from Suphanburi province, Thailand, and identified by Dr. Sakaewan Ounjaijean, Faculty of Pharmacy, Payap University, Thailand. Leaves of this plant were washed with distilled water and dried in hot-air oven at 60°C for overnight. Powdered plant materials were then performed using electric blender, and stored at 4°C until used. For aqueous crude extract preparation, hot water method with microwave was used as previously described [13]. Dried powder plant materials were dissolved in distilled water with a ratio of 1:10 (w/v), then extracted in microwave (360 W for 5 min). Incubation at room temperature was subsequently performed for overnight with continuous stirring to obtain complete extraction.

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Filtration was carried out using Whatman no. 1 filter paper, and filtrate was then dried by freeze drying. Usually, a 2.4% yield of extract was obtain, and stored at 4°C.

Experimental animals

Female ICR mice, 4-6 weeks old, weighing 25-30 g, obtained from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand were used in this study. They were kept in animal room at stable temperature of 22-25°C with 12 h light-dark cycle, and given pellet diet CP082 and clean water *ad libitum*.

Plasmodium berghei parasites

Chloroquine-sensitive strain *Plasmodium berghei* ANKA (PbANKA) obtained from BIOTEC, NSTDA was used. Parasites were maintained in ICR mice by intraperitoneal (IP) injection of $1x10^7$ parasitized erythrocytes of PbANKA. Parasitemia was daily monitored by microscopic examination of Giemsa stained thin blood smear. When the infected mice showed a parasitemia of 15-20%, mechanical passage was then performed into naïve mice.

Assessment of liver and renal function, and blood glucose

Blood was collected into heparinized vacuum tube and then centrifuged at 10,000 g for 10 min. Plasma was subsequently collected into a new tube and used for measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, blood urea nitrogen (BUN), creatinine, and glucose levels by automate analyzer for blood chemistry.

Efficacy test *in vivo* of *Andrographis paniculata* extract

For assessment of *A. paniculata* extract to protect liver and renal damage, and hypoglycemia during *P. berghei* infection, standard 4-day suppressive test was carried out as previously described [14]. ICR mice were infected with $1x10^7$ parasitized erythrocytes of PbANKA by IP injection. Infected mice were randomly divided into 3 groups (5 mice of each), and 2 h after infection, they were given orally by gavage of the extracts (500, 1000, and 2000 mg/kg) and every 24 h for 4-consecutive days (day 0-3). The control groups were also used including normal mice treated with or without the extract and untreated mice treated with distilled water. On day 4 of the experiment, blood was collected, and AST, ALT, albumin, BUN, creatinine, and blood glucose levels were then measured.

Statistics

Data were analyzed using GraphPad Prism software. All results were expressed as mean±standard error of mean (SEM). Moreover, the one-way ANOVA was used to analyze and compare the results at a 95% confident levels. Values of $p<0.05$ were considered significant.

Results

Malaria-associated liver and renal damage, and hypoglycemia during PbANKA infection

After PbANKA infection in ICR mice, parasitemia was first detectable on day 1 with a parasitemia of <1%, and reached 65% on day 10 (Figure 1A). Next, we observed that AST and ALT levels were markedly increased while albumin was decreased in infected mice (Figure 1B-C). Moreover, renal damage was also observed in infected mice as indicated by progressive increasing of BUN and creatinine levels (Figure 1D-E). Additionally, we found a decrease of blood glucose in infected mice (Figure 1F).

Protective effects of *A. paniculata* extract on liver and renal damage induced by PbANKA

Aqueous crude extract of *A. paniculata* leaves exerted a dose-dependent protective effects on liver and renal damage during PbANKA infection as indicated by normal levels of AST, ALT, albumin, BUN, and creatinine in infected mice treated with the extract (Figure 2A-E). The highest activity of the extract was found at dose of 2000 mg/kg. Moreover, no effects on liver and renal function in normal mice treated with 2000 mg/kg of the extract.

Anti-hypoglycemia of *A. paniculata* extract against PbANKA infection

Hypoglycemia was found in untreated mice as indicated by significant decreasing of blood glucose, compared to normal. However, *A. paniculata* extract showed anti-hypoglycemia in a dose-dependent manner against infected mice, the highest activity was found at dose of 2000 mg/kg of the extract (Figure 2F). No side effect on blood glucose was found in normal mice treated with this extract.

Discussion

Impairment of liver and renal function as well as hypoglycemia during malaria infection have been reported, and they are important life-threatening complication of malaria infection that goes beyond the classical clinical symptoms of malaria [6, 8]. The onset of liver and renal damage, and hypoglycemia in PbANKA infected mice come out from day 4 after infection. Organ damage during malaria infection is proposed to be a consequence of parasite adhesion as well as exacerbated immune response against products of oxidative stress released during infection [15, 16]. The hemolysis during blood stage infection accumulates high levels of toxic free heme that has ability to induce oxidative stress from production of hydroxyl radicals via the Fenton/Haber-Weiss reaction

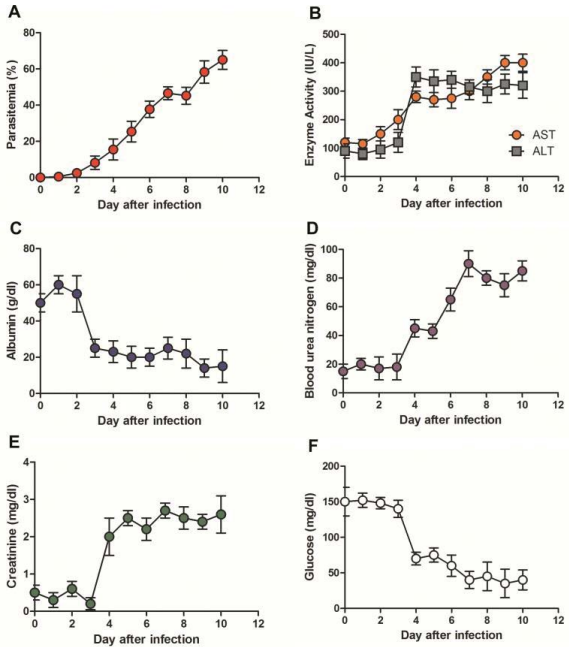

Figure 1: Malaria-associated liver and renal damage and hypoglycemia during *Plasmodium berghei* infection in mice. ICR mice (5 mice of each) were inoculated with $1x10^7$ parasitized erythrocytes of PbANKA by IP injection. (A) Parasitemia, (B) AST and ALT, (C) albumin, (D) BUN, (E) creatinine, and (F) glucose levels were measured. Results were expressed as mean±SEM.

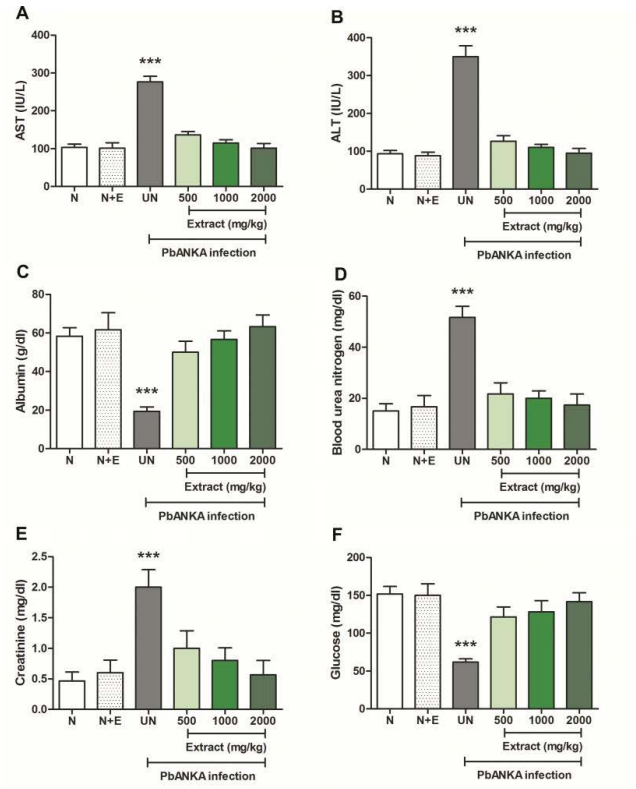

Figure 2: Protective effects of *Andrographis paniculata* extract on liver and renal damage and hypoglycemia during *Plasmodium berghei* infection in mice. ICR mice (5 mice of each) were inoculated with $1x10^7$ parasitized erythrocytes of PbANKA by IP injection. They were given orally by gavage of the extract (500, 1000, and 2000 mg/kg) for 4-consecutive days. (A) AST, (B) ALT, (C) albumin, (D) BUN, (E) creatinine, and (F) glucose levels were subsequently measured. Results were expressed as mean±SEM. *** p<0.001. N; normal mice, N+E; 2000 mg/kg of extract treated normal mice, and UN; untreated mice.

[17]. In addition, heme-derived oxidative stress is considered to be a major factor in the iron-induced lipid peroxidation resulting in liver and renal damage [18]. For hypoglycemia during malaria infection, 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. This is accompanied with approximately 100-fold increase in glucose utilization when compared with uninfected erythrocytes thus causing a profound hypoglycemia if untreated [19]. Furthermore, hyperinsulinemia and hypoglycemia during malaria infection has also been described [20].

Aqueous crude extract of *A. paniculata* leaves showed protective effects of liver and renal damage during malaria infection. It has been reported that leaf extract of *A. paniculata* and its active compound, andrographolide, presented potent antioxidant, anti-inflammation, hepatoprotective, and nephroprotective properties [11]. Additionally, inhibition of lipid peroxidation and increasing of antioxidant molecules has also been described [2]. So, these properties of this extract might play a central role to protect liver and renal damage induced by malaria. Moreover, *A. paniculata* extract exerted anti-hypoglycemia during malaria infection. Andrographolide, polyphenols, and flavonoids containing in this extract have been described to have glucose homeostatic property [15]. Inhibition of glycolysis and hexose transporter of infected erythrocytes might be properties of *A. paniculata* extract on blood glucose levels [21, 22]. In addition, beneficial effect of this extract on insulin may be due to the antioxidant capacity of this extract [23, 24].

It is interesting to note that aqueous crude extract of *A. paniculata* leaves was found the protective effects on liver and renal damage, and anti-hypoglycemic activity against *P. berghei* infected mice. Although the mechanism is yet to be identified, the results of this study provide the basis for further studies.

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