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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 pharmacological properties. The pharmacological properties of A. paniculata leaf extract are well documented and several in vitro and in vivo studies describe its antioxidant, anti-inflammatory, anti-cold, anti-hepatotoxicity, anti-nephrotoxicity, antimalarial, antimicrobials, 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. Sakaewon Ounjaijeen, 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 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 1x10⁷ 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 PbANKA infection, standard 4-day suppressive test was carried out as previously described [14]. ICR mice were infected with 1x10⁷ 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 this extract. 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.
infection has also been described [20]. Furthermore, hyperinsulinemia and hypoglycemia during malaria fold increase in glucose utilization when compared with uninfected process of glycolysis. This is accompanied with approximately 100-facilitated hexose transporter and is in turn metabolized through the is rapidly taken up across the parasite plasma membrane through a could be due in part to the fact that during malaria infection, glucose and renal damage [18]. For hypoglycemia during malaria infection, this major factor in the iron-induced lipid peroxidation resulting in liver [17]. In addition, heme-derived oxidative stress is considered to be a 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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References


