MODULATION OF TOTAL CHOLESTEROL AND TRIGLYCERIDE IN *PLASMODIUM BERGHEI* INFECTED MICE BY AQUEOUS CRUDE EXTRACT OF *ANDROGRAPHIS PANICULATA*

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ABSTRACT:

During malaria infection in erythrocytes, critical markers including total cholesterol and triglyceride are markedly increased. Hence, finding new medicinal plants to have homeostatic effect on these markers during malaria infection are urgently needed. *Andrographis paniculata* is a medicinal plant and widely cultivated in Southeast Asia. This plant exhibits antioxidant property and is used to treat liver disorder and control lipid profile. In this study, we assessed changes in plasma total cholesterol and triglyceride levels accompanying *Plasmodium berghei* ANKA infection in mice treated orally with aqueous crude extract of *A. paniculata* at 100, 300, and 600 mg/kg. Significant increases of total cholesterol and triglyceride levels in plasma were observed in infected untreated mice compared to normal control. However, treatment with 300 and 600 mg/kg of the extract produced significant reduction in plasma total cholesterol and triglyceride compared to untreated mice. Moreover, prolonged survival time was found in infected mice treated with this extract. It can be concluded that the extract has anti-hyperlipemic property during malaria infection that can be explored for the management of malaria.

Keywords: *Andrographis paniculata*, *Plasmodium berghei*, Cholesterol, Triglyceride

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INTRODUCTION

Malaria is a serious public health problem worldwide and kills 1-2 million people every year, mostly children residing in Africa. Moreover, malaria is the most lethal parasitic disease in the world, annually affecting approximately 500 million people mostly in African sub-Saharan countries [1]. It is caused by the parasite *Plasmodium* and transmitted by female *Anopheles* mosquito. The malaria parasite has been observed to have a tremendous requirement for lipids during erythrocytic stage development, especially total cholesterol and triglyceride [2, 3]. It has been reported that plasma lipids including total cholesterol and triglyceride were markedly increased during malaria infection in erythrocytes [4]. This is the most important independent predictor of a fatal outcome in both adults and children. This has prompted research towards the discovery and development of new, safe, and affordable drugs to maintain total cholesterol and triglyceride concentration in plasma during malaria infection. In this respect, medicinal plants are potential targets for research and development of alternative drugs. Although up to 80% of Thai population uses traditional medicine especially plant remedies for management of diseases, plants are not yet fully explored [5-8].

Andrographis paniculata is a medical plant

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with an extremely bitter taste of family Acanthaceae [9]. It is widely cultivated in Southern Asia including Thailand. It contains diterpenoids, flavonoids, and polyphenols as the major bioactive compounds [10-12]. This plant exhibits antioxidant, free radial scavenging, anti-inflammation, anti-cancer, anti-bacterial, anti-malarial properties, and used to treat liver disorders, bowel complaints of children, colic pain, common cold and upper respiratory tract infection [13-18]. Moreover, it has been reported hypoglycemic and lipid lowering activities in diabetic patients [19, 20]. It has also been reported the hepatoprotective effect of *A. paniculata* extract on *P. berghei* infection in mice. In this report showed that aspartate aminotransferase and alanine aminotransferase levels were maintained into normal levels in infected mice treated with *A. paniculata* extract [21]. However, effect of *A. paniculata* extract on plasma total cholesterol and triglyceride levels during malaria infection has not yet been reported. Hence, this study was aimed to investigate the effect of aqueous crude extract of *A. paniculata* on total cholesterol and triglyceride levels during malaria infection using *P. berghei* infected mouse model.

MATERIALS AND METHODS

Plant material

The commercial dried leaves of *A. paniculata* were used in this study. They were grounded, and 500 g of *A. paniculata* dry powder was subsequently extracted with 1000 ml of distilled water by stirring 72 h at room temperature to ensure exhaustive extraction. The extract was filtered through Whatman No.1 filter paper, and concentrated in a rotary evaporator at 55^{o}C under vacuum, followed by removal of residual water at 40^{o}C in water bath [12]. Aqueous crude extract yield of 70 g was obtained, and stored at -20^{o}C. For treatment, the dry extract was dissolved in water at chosen doses for administration orally by gavage.

Experimental mice

ICR female mice, 6-8 weeks old, weighting 20-35 g obtained from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand were used throughout the study. They were kept at room temperatures between 22 and 25^{o}C and a 12-h light/ 12-h dark cycle. All mice were fed on standard pellet diet (CP diet 082, Perfect Companion Company, Bangkok, Thailand) and drinking water *ad libitum*. Procedures of all animal experiments were ratified by the Ethical Committee on Animal Experimentation, Faculty of Medical Technology, Western University, Thailand.

Acute toxicity test

The acute toxicity of the aqueous crude extract of *A. paniculata* was tested on mice using 3 doses including 600, 1800, and 5400 mg/kg administered orally [22]. Control mice were kept under the same condition without any treatment. The mice were routinely inspected for appearances or signs of toxicity such as tremors, weakness and refusal of feeds, falling of hair, coma or death within 48 h.

Parasite strain and infection of animal

P. berghei strain ANKA (PbANKA) was used. Parasitized erythrocytes were obtained from an infected mouse donor by cardiac puncture. Mice were inoculated intraperitoneally with infected blood suspension (0.2 ml) containing 6×10^{6} parasitized erythrocytes. Parasitemia was monitored daily by microscopy of Giemsa stained thin blood smear. Parasite was maintained by serial passage of blood from mouse to mouse.

Determination of total cholesterol and triglyceride in plasma

Mouse blood was collected from tail vein into heparinized hematocrit tube, and centrifugation was subsequently performed at 10,000 g for 10 min. Plasma was collected into a new 1.5-ml microcentrifuge tube and used for measurement of total cholesterol and triglyceride with commercial kit (BioSystem S.A. Costa Brava, Barcelona, Spain) following the manufacturer's instruction.

Efficacy test *in vivo*

Efficacy test *in vivo* of aqueous crude extract of *A. paniculata* was monitored by measuring the levels of cholesterol and triglyceride in plasma from mice that had been infected with PbANKA and subsequently treated with the extract. Mice (5 mice of each) were infected with 6×10^{6} parasitized erythrocytes of PbANKA and the first drug dose was administered orally 3 h after infection and every 24 h subsequently for a further 3 days, twice a day, which is comparable to the standard 4-day test [23]. Groups of three mice were treated with 100, 300, and 600 mg/kg of the aqueous crude extracts of *A. paniculata*. For untreated control, mice were given distilled water. On day 10 post-infection, blood was collected and prepared the plasma for measuring total cholesterol and triglyceride. In addition, mean survival time of the mice in each group was also determined.

Statistics

Statistical analysis of the data was performed using GraphPad Prism Software (GraphPad Software, Inc.,

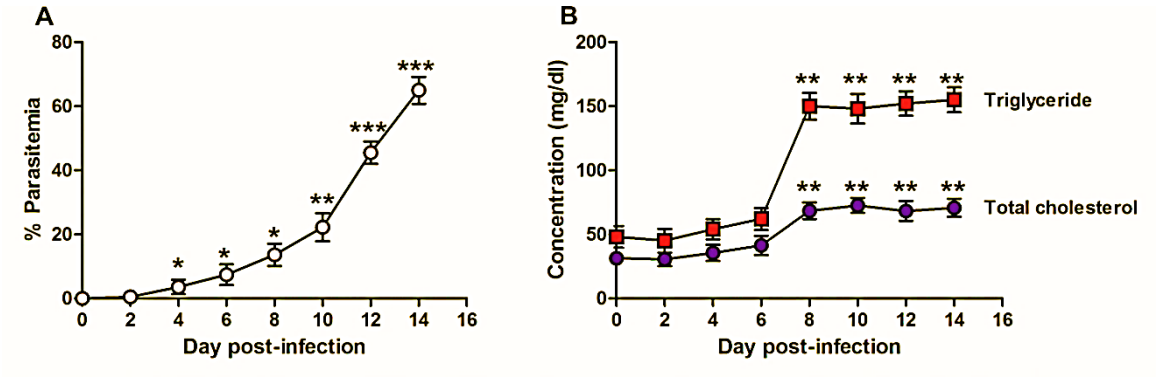


Figure 1 Increasing of total cholesterol and triglyceride levels during *Plasmodium berghei* ANKA infection. ICR mice (5 mice of each) were inoculated with 6×10^6 parasitized erythrocytes of PbANKA by intraperitoneal injection. **(A)** Parasitemia was daily monitored by Giemsa stained thin blood smear under light microscope with oil immersion lens. **(B)** Total cholesterol and triglyceride levels in plasma were also measured using commercial kit. Results were presented as mean ± SE. * p<0.05, ** p<0.01, and *** p<0.001 compared to day 0 post-infection.

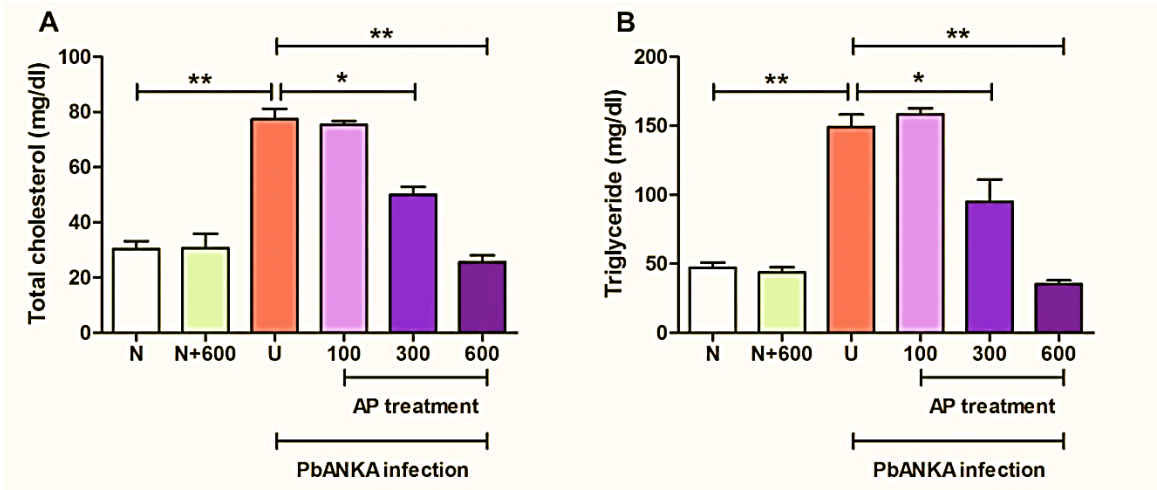


Figure 2 Modulation of plasma total cholesterol and triglyceride levels during *Plasmodium berghei* ANKA infection by aqueous crude extract of *Andrographis paniculata.* Groups of ICR mice (5 mice of each) were intraperitoneally injected with 6×10^6 parasitized erythrocytes of PbANKA. They were given with 100, 300, and 600 mg/kg of the extracts orally for 4-consecutive days. On day 10 post-infection, (A) plasma total cholesterol and (B) triglyceride levels were measured. Results were expressed as mean ± SE. N; normal, U; untreated, AP; *A. paniculata* extract. * p<0.05 and ** p<0.01.

US). A one way ANOVA test 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).

RESULTS

Acute toxicity test

Acute toxicity test was conducted for *A. paniculata* extract. Behavioral signs of toxicity observed in mice given 1800 mg/kg and above include; paw licking, salivation, stretching and reduce activity. There was however no mortality at all the dose levels used.

Increasing of total cholesterol and triglyceride during malaria infection

Propagation of PbANKA *in vivo* and increasing of total cholesterol and triglyceride were presented in Figure 1. Parasitemia was first detectable on day 2 (0.5%) and reached 65% on day 14 post-infection (Figure 1A). The parasitemia increased daily until the death of the mice which was also observed in other studies. Next, we observed that total cholesterol and triglyceride levels in plasma were markedly increased in infected ICR mice (Figure 1B).

Table 1 Survival time of *Plasmodium berghei* ANKA infected mice and treatment with aqueous crude extract of *Andrographis paniculata*

Group of *P. berghei* ANKA infected ICR mice	Survival time (Day)
Untreated mice (distilled water)	14
100 mg/kg of extract	15
300 mg/kg of extract	23
600 mg/kg of extract	30

Efficacy of aqueous crude extract of *A. paniculata* on total cholesterol and triglyceride

Modulation of total cholesterol and triglyceride in plasma during PbANKA infection of aqueous crude extract of *A. paniculata* was shown in Figure 2. Plasma total cholesterol and triglyceride levels were observed to be significantly (p<0.05) elevated above normal control in the infected untreated mice. Interestingly, significant decreases (p<0.05) were found in plasma total cholesterol and triglyceride levels in infected mice treated with aqueous crude extract of *A. paniculata* at 300 and 600 mg/kg, compared to infected untreated mice. However, plasma total cholesterol and triglyceride levels were not significantly changed in infected mice treated with 100 mg/kg of the extract. There were no effects on plasma total cholesterol and triglyceride levels in normal mice treated with the extract. Moreover, prolong survival time (29 days) was also observed in infected mice treated with this extract (Table 1).

DISCUSSION

Lipids have been reported to play important roles in pathological changes during malaria infection [24]. The results showed significant increasing of total cholesterol and triglyceride levels during malaria infection. This observation is consistent with reports from earlier studies, which showed increased serum lipoprotein fractions in malarial patients compared with apparently healthy control subjects [25, 26]. Increase in plasma lipids in malaria subjects is consistent with the degree of parasitemia. Plasma lipids in particularly total cholesterol and triglyceride can be elevated by an increase in biosynthesis or by decrease in their removal [27]. Both of these processes appear to contribute to the hyperlipidemia. It has been reported during malaria infection, the liver is commonly enlarged and congested with parasitized erythrocytes in sinusoids and centrilobular veins, and swollen parenchymatous and kupffer cells [28]. Therefore, lipid metabolism is deranged and the resulting lipids synthesized by the liver undergo drastic changes in composition. Moreover, it has demonstrated that increased lipolysis induced by threshold of parasitemia promotes cholesterol and triglyceride synthesis [29]. Thus, with increase in parasitemia there was a progressive increase in total cholesterol and triglyceride. It is also likely that the elevation in total cholesterol and triglyceride may be due to decrease uptake by the infected erythrocytes as a result of increased levels of parasitemia. Recent our finding showed that protection of liver cells by plant extract could maintain lipid levels in plasma. Therefore, liver protection during malaria infection might be a critical point during malaria infection [21]. In addition, anti-inflammatory effect of *A. paniculata* extract by interfering NF-kB might play an important role in order to protect liver damage induced by malaria infection [30-32].

It is evident based on the finding from this study that aqueous crude extract of *A. paniculata* represent potential anti-hyperlipidemia during malaria infection. The activity may be attributed to lactones, sterols, diterpenoids, and flavonoids present in the extract [9]. Andrographolide, active constituent in *A. paniculata* extract, has been showed that total cholesterol and triglyceride were decreased in a dose-dependent manner [33]. In addition, antimalarial activities of *A. paniculata* extract and andrographolide have also been described [34]. Thus, it could be inferred that the antimalarial property of this extract could possibly be mediated by mechanisms involving reduction in total cholesterol and triglyceride. In addition, inactivation of adipose tissue in order to suppress the releasing of triglyceride into blood circulation has been described [35]. For decreasing of total cholesterol and triglyceride during malaria infection, prolong survival time of infected mice treated with the extract was found up to 1 month. In conclusion, results obtained from this study show that aqueous crude extract of *A. paniculata* modulates plasma total cholesterol and triglyceride during PbANKA infection, and prolong survival time of infected mice. This justifies the traditional usage of this plant as malarial remedy.

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