REDUCTION OF PLASMA TOTAL CHOLESTEROL, ASPARTATE AMINOTRANSFERASE, AND ALANINE AMINOTRANSFERASE LEVELS BY GREEN TEA EXTRACT DURING *PLASMODIUM BERGHEI* INFECTION

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ABSTRACT: Malaria is still a major problem around the world, especially tropical and subtropical areas with estimated one million deaths annually. During malaria infection in erythrocytes, oxidative stress was developed and organ damage was then occurred, especially hepatocyte damage. Therefore, finding new medicinal plant extract to protect and reduce oxidative damage to the vital organ was urgently needed. Green tea extract has been reported to have antioxidant property and can protect hepatocytes from oxidative damage. Hence, effect of green tea extract on the reduction of plasma total cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels during *Plasmodium berghei* infection was investigated. Dried leaves of green tea were extracted using hot water method, and total polyphenolic content was then determined. For *in vivo* study, ICR mice were infected with $1x10^7$ parasitized erythrocytes of *P. berghei* ANKA by intraperitoneal injection, and treated orally with green tea extract (3000 mg/kg), twice a day for 6 consecutive days. Parasitemia was then monitored by microscopy as well as levels of total cholesterol, AST, and ALT in plasma was also measured. It was found that levels of total cholesterol, AST, and ALT in plasma were increased significantly ($p < 0.01$) during *P. berghei* ANKA infection and the highest levels were observed on day 6 after infection. However, liver damage was decreased and protected by green tea extract treatment as indicated by decreasing of plasma total cholesterol, AST, and ALT levels in green tea extract treated group. These finding can be concluded that liver damage from oxidative stress during malaria infection can be protected by green tea extract.

Keywords: Alanine aminotransferase, Aspartate aminotransferase, Green tea extract, *Plasmodium berghei*, Total cholesterol

INTRODUCTION

Malaria caused by *Plasmodium* parasites is a major public health problem around the world, especially tropical and subtropical areas, where it is most prevalent, with estimates of more than 70-80 million cases annually [1]. Liver injury resulting from oxidative stress induced by malaria infection has been reported in several areas. It occurs between 1-4% of hospitalized patients with a mortality that can reach more than 50% [2, 3]. Liver function test has been reported and usually perform in routine work in hospital including total cholesterol, AST, and ALT levels in plasma.

Cholesterol is synthesized by liver cells as well as AST and ALT are abundant liver enzymes. During liver damage and inflammation, levels of all markers are increasing and can be used as critical

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markers for liver function. Oxidative stress and inflammation developed during malaria infection are caused by hemolysis and low antioxidant enzyme activities followed by liver cell damage and death [3]. Affordable alternative treatments for liver injury during malaria infection are urgently needed. Green tea (*Camellia sinensis*) and its active compound, catechins, have been shown to regulate a number of biological responses.In addition to its antioxidant, anti-inflammation anti-cancer, and anti-microbial effects, green tea has been shown to protect vital organ damage, especially liver [4, 5]. We hypothesized that green tea would similar property in protection of liver injury during malaria infection. This study was undertaken to determine the protective effect of green tea extract on liver damage during *Plasmodium berghei* infection in mice.

MATERIALS AND METHODS

Green tea material

Fresh leaves of green tea (*Camellia sinensis*) were obtained at the Royal Project shop, Chiang Mai province, Thailand, and a voucher specimen has been deposited in the Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. The plant was air dried at room temperature and then powdered. Dried leaves of green tea (10 g) were used to prepare crude extract with 100 ml of water using hot water method [6].

Experimental animals

Female ICR mice, 6-8 weeks obtained from National Laboratory Animal Center, Mahidol University, Bangkok, Thailand were used in this study. They were kept in the animal room with temperature control between 22-25^{o}C and given mouse pellet diet and clean water *ad libitum*. All animal experiments were ratified and approved by Animal Ethic Committee from Faculty of Medical Technology, Western University, Thailand.

Rodent malaria parasites

Chloroquine-sensitive strain of *Plasmodium berghei* ANKA (PbANKA) was used in this study. Glycerol stock in liquid N_2 of this parasite was warmed at 37^{o}C and subsequently inoculated by intraperitoneal (IP) injection into naïve female ICR mice. Parasitemia was daily monitored using microscopic examination of Giemsa-stained thin blood smear and used formula below to calculate.

$$\text{Percent parasitemia} = \frac{\text{Number of infected erythrocytes} \times 100}{\text{Number of all erythrocytes}}$$

For mechanical passage, blood was collected by cardiac puncture from the infected mice with a parasitemia of 10-15%, and IP injection of 200 µl containing $1x10^6$ infected erythrocytes was performed into a naïve mice using phosphate buffer saline (PBS) as solvent.

Antimalarial drug

Antimalarial drug used in this study was chloroquine diphosphate salt (CQ) for study *in vivo* drug susceptibility of PbANKA. The drug was freshly prepared in distilled water (DW) and administered orally by gavage [7]. 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 sub-curative dose of this drug on PbANKA infected mice.

Assessment of liver function

One hundred µl of tail blood was collected in heparinized hematocrit tube. Centrifugation was then performed at 10,000 g for 10 min, and plasma was subsequently collected into a new 1.5-ml microcentrifuge tube. Levels of total cholesterol, AST and ALT in plasma were measured using commercial kit (BioSystem S.A.Costa Brava 30, Barcelona, Spain), according to manufacturer's instruction. For total cholesterol measurement, 10 µl of plasma was mixed with 1 ml of cholesterol reagent containing appropriate enzymes and cofactors. After incubation at 37^{o}C for 10 min, absorbance was then measured at OD 500 nm, and calculated using standard cholesterol provided by commercial kit. For AST and ALT measurement, 50 µl of plasma was mixed with 1 ml of working reagent for AST and ALT. Absorbance at OD 340 nm was then measured at 1 min intervals thereafter for 3 min. Average absorbance per min was then used to calculate enzyme activity.

In vivo test

For efficacy test of green tea extract *in vivo*, the experiment was based on standard 4-day suppressive test [8]. ICR mice (5 mice in each group) were inoculated by IP injection of $1x10^6$ infected erythrocytes of PbANKA, and given green tea extract (3,000 mg/kg) orally by gavage twice a day for 6 consecutive days. Parasitemia was daily monitored and liver function test including total cholesterol, AST, and ALT were also measured as previously described (Figure 1). The control groups were used; the uninfected and infected controls were treated daily with DW, and the drug treated control was given sub-curative dose of CQ (7.5 mg/kg).

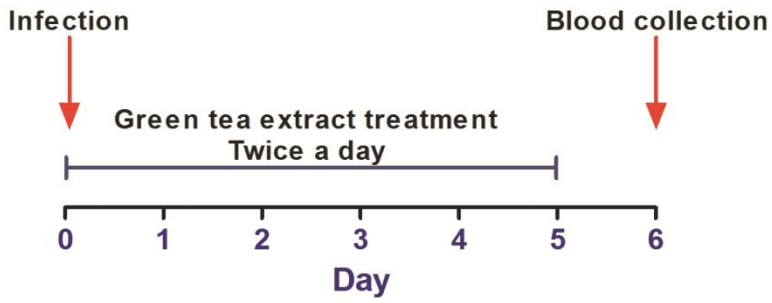

Figure 1 Diagram for *in vivo* experiment using green tea extract for treatment during *P. berghei* ANKA infection. Naïve ICR mice, 5 mice of each, were inoculated with $1x10^6$ infected erythrocytes of PbANKA and subsequently treated orally for 6 consecutive days, twice a day. On day 6 after infection and treatment, blood was collected for further analysis.

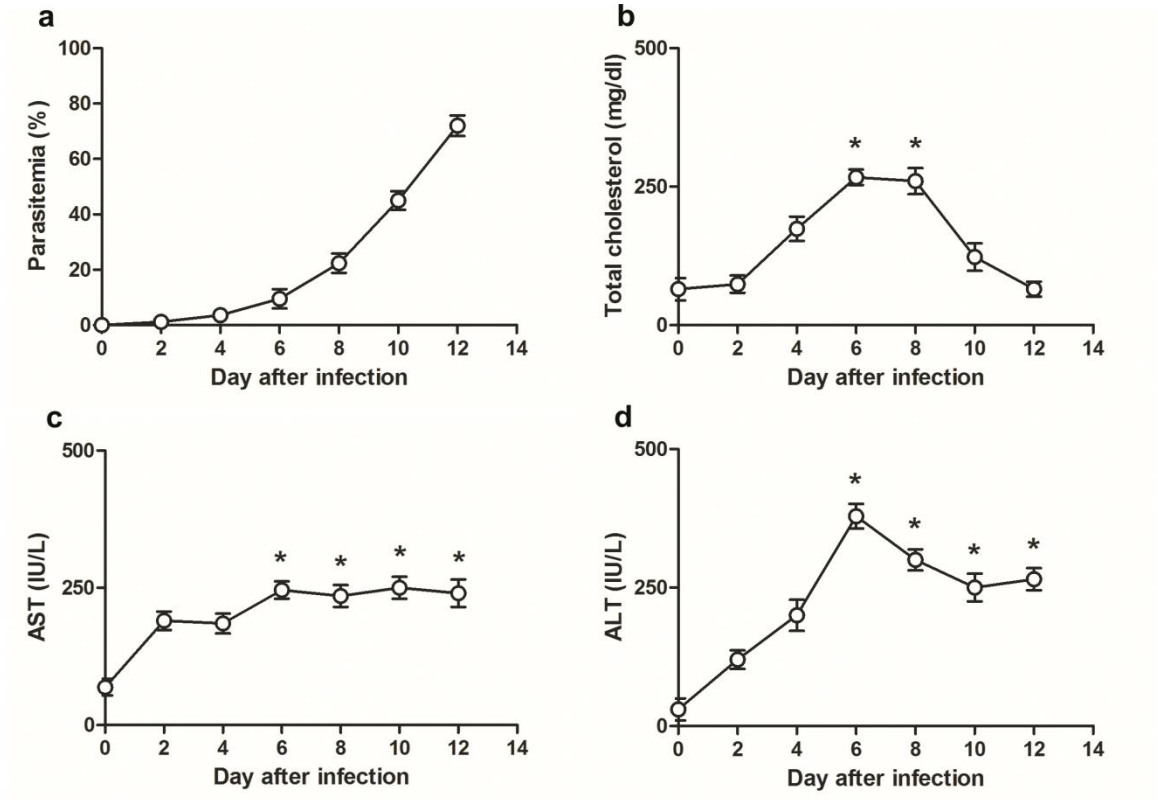

Figure 2 Liver function impairment during *P. berghei* ANKA infection. (a) Parasitemia in mice infected with $1x10^6$ infected erythrocytes by PbANKA. Liver function was assessed by (b) plasma total cholesterol, (c) aspartate aminotransferase (AST) and (d) alanine aminotransferase (ALT) estimated on different days after infection. Results present mean + standard error of mean (SEM). * $p < 0.01$ compare with day 0. Five mice in each group were used.

Statistical analysis

Statistics of the data were performed using GraphPad Prism Software (GraphPad software, Inc., US). The one way ANOVA test was used to analyze and compare the results at a 99% confidence level. Values of $p < 0.01$ were considered significant. Results were expressed as mean + standard error of mean (SEM).

RESULTS

Liver damage during *Plasmodium berghei* infection in mice

In order to evaluate liver damage during malaria infection, parasitemia, total cholesterol, AST, and ALT levels in plasma were measured in PbANKA infected ICR mice. Parasitemia was first detectable on day 2 after infection (< 2%), and

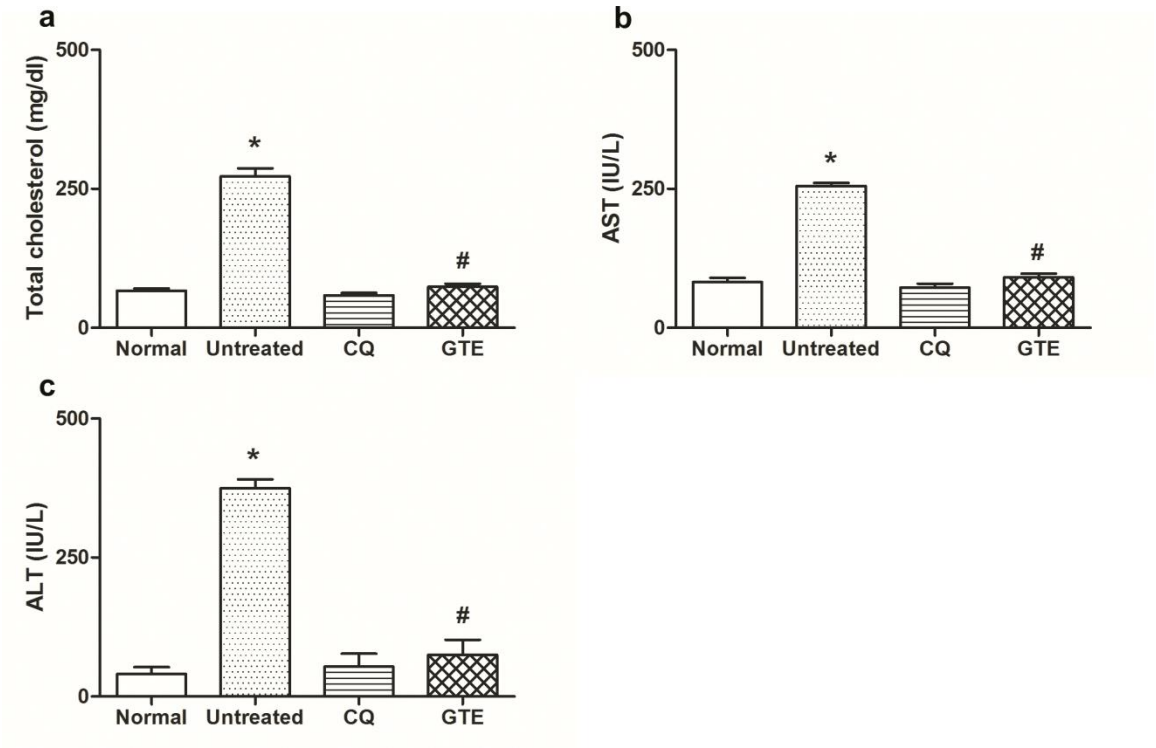

Figure 3 Effect of green tea extract on parameter of liver function during *P. berghei* ANKA infection. ICR mice infected with $1x10^6$ infected erythrocytes of PbANKA and subsequently treated orally for 6 consecutive days with water (Normal and Untreated mice), chloroquine (CQ) and green tea extract (GTE). On day 6, total cholesterol (a), aspartate aminotransferase (AST) (b) and alanine aminotransferase (ALT) (c) levels in plasma were measured. Bar graphs indicate the mean + standard error of mean (SEM). * $p < 0.01$ compared with Normal group. # $p < 0.01$ compared with Untreated group. Five mice in each group were used.

reached about 70% on day 12 (Figure 2a). Moreover, it can be observed that plasma total cholesterol, AST, and ALT levels were markedly increased in infected ICR mice, and the highest levels with significant were found on day 6 after infection (Figure 2b-d).

Effect of green tea extract on reduction of liver function markers during *Plasmodium berghei* infection in mice

In order to investigate the effect of green tea extract on reduction of total cholesterol, AST, and ALT levels in plasma during malaria infection, PbANKA infected ICR mice were treated orally by gavage of green tea extract twice a day for 6 consecutive days. Green tea was extracted using hot water method. Yield of the green tea extract was 42%, and contained 60% of total polyphenols, > 40% of EGCG (epigallocatechin gallate), and < 0.1% of caffeine by HPLC. As shown in Figure 3, levels of total cholesterol, AST, and ALT in plasma were significantly ($p < 0.01$ compared with normal) increased in PbANKA infected mice (Figure 3a-c, Untreated groups). Interestingly, after oral administration of green tea extract during malaria infection, total cholesterol, AST, and ALT levels in plasma declined significantly ($p < 0.01$ compared with untreated group) (Figure 3a-c, GTE treated groups). Moreover, liver damage was not able to observe in CQ treated mice.

DISCUSSION

Impairment of liver function during malaria infection has been reported. It is an important life-threatening complication of malaria infection. In this study, we have demonstrated that liver damage was present during PbANKA infection in mice; however, this damage was protected and maintained by green tea extract treatment. During PbANKA infection in mice, parasitemia was increased until animal died. It has been reported the correlation of high parasitemia, oxidative stress levels, and low hematocrit levels in infected mice [9]. The results showed that during malaria infection in blood stage for 6 days, liver damage was presented as shown by increasing of total cholesterol, AST, and ALT levels in plasma. Importantly, oxidative stress and inflammation during infection with blood stage of *Plasmodium* parasite played a vital role in liver damage. It has been suggested that IL-22 produces during malaria

infection in blood stage and controlled liver damage in order to protect liver damage from oxidative stress [10]. Moreover, during PbANKA infection, large number of parasite-specific $CD8^+$ T cells accumulates in the liver and causes liver damage [11]. From liver damage during malaria infection, animal died during the first 12 days of infection. Interestingly, levels of total cholesterol, AST, and ALT in plasma were decreased when green tea extract was treated orally during malaria infection. Catechins, active ingredient in green tea, have protective effect on several organs especially liver and renal [12, 13]. It has been suggested that catechins have potent antioxidant and anti-inflammation activities, so it can reduce and protect organ damage from oxidative stress [4]. Moreover, green tea extract increases total antioxidant capacity, in particularly glutathione peroxidase, catalase, and superoxide dismutase [14, 15]. It has been reported that 20 cups of tea per day (120 ml of each cup) for drinking tea people in real life could get this effect [15]. This study, green tea extract was supported as an effective dietary component during malaria infection.

CONCLUSION

It can be concluded that green tea extract has potent antioxidant and protective properties to decrease total cholesterol, AST, and ALT induced by oxidative stress during malaria infection. Hence, green tea polyphenols are useful supplements in the prevention and treatment of liver damage in malaria.

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