WALAILAK JOURNAL
http://wjst.wu.ac.th Health Sciences

Anti-hemolytic, Hepatoprotective, and Nephroprotective Effects of Black Tea Extract against *Plasmodium berghei* Infected Mice

Somrudee NAKINCHAT[1], Sukanya CHACHIYO[1], Somdet SRICHAIRATANAKOOL[2], Chairat UTHAIPIBULL[3] and Voravuth SOMSAK[1,*]

[1]Department of Clinical Chemistry, Faculty of Medical Technology, Western University, Kanchanaburi 71170, Thailand
[2]Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
[3]National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathumthani 12120, Thailand

([*]Corresponding author's e-mail: voravuthsomsak@gmail.com)

Received: 10 February 2016, Revised: 25 July 2016, Accepted: 24 August 2016

Abstract

Malarial associated hemolysis and liver and renal injuries are associated with mortality in adult patients with the severe form of this disease. Hence, we aimed to investigate the effects of black tea extract on hemolysis and liver and renal injuries induced by *Plasmodium berghei* ANKA infected ICR mice. Aqueous crude extract of black tea was prepared using the hot water method. For efficacy test *in vivo*, a standard 4-day test was carried out. Groups of ICR mice (5 mice of each) were inoculated intraperitoneally with 10^7 infected red blood cells of *P. berghei* ANKA. They were then treated orally by gavage with 100, 500, and 1,000 mg/kg of extract for 4 consecutive days. Parasitemia, hematocrit, alanine aminotransferase (ALT), and creatinine levels were measured. The results showed that black tea extract exerted dose-dependent anti-hemolysis and protection from liver and renal injuries induced by malaria, especially at doses of 500 and 1,000 mg/kg. However, 100 mg/kg of this extract did not show any effect. Additionally, there were no any toxicity in hemolysis or liver or renal injuries in normal mice treated with the highest dose of black tea extract. It can be concluded that aqueous crude extract of black tea presented anti-hemolytic, hepatoprotective, and nephroprotective activities against *P. berghei* ANKA infected mice.

Keywords: Anti-hemolytic, hepatoprotective, nephroprotective, black tea, *Plasmodium berghei*

Introduction

Malaria caused by the *Plasmodium* malaria parasite is a major public health problem in tropical and subtropical areas, with estimates of more than 70 - 80 million cases annually. Moreover, approximately 800 thousand people die from the severe form of malaria [1]. The causes of death from malaria include cerebral malaria, severe anemia, acute hemolysis, hypoglycemia, multiple organ failure, and metabolic acidosis [2]. In particularly, hemolysis and liver and renal injuries induced by malaria have been described. The pathogenesis of malarial associated acute hemolysis has been suggested to involve cytoadherence of infected red blood cells (RBC) and inflammatory responses, as well as oxidative stress through generation of reactive oxygen intermediates by host cells [3-5]. Moreover, parasite invasion and subsequent RBC rupture also contributes to pathogenesis of hemolysis [4]. In addition, liver and renal injuries induced by malaria are multifactorial and not well characterized; however, it is hypothesized that involvement of oxidative stress during malaria infection damages the vital organs especially the liver and kidneys [5,6]. For screening of liver and renal injuries, increased enzyme alanine aminotransferase (ALT)

and creatinine in plasma can be used as critical markers, respectively. This has prompted research towards the discovery and development of compounds to protect or reduce liver and renal injuries during malarial infection. In this respect, plant resources are potential targets for the research and development of alternative malarial drugs as supplements or when used in combination with standard antimalarials.

The effective antimalarial properties of the 2 plant-based drugs, quinine and artemisinin, has generated much interest in exploring other plant resources for their possible antimalarial efficacy. Black tea (*Camellia sinensis*) is a widely consumed beverage throughout the world. It has attracted large amounts of attention recently, both in the scientific community and in public opinion, for its pronounced health benefits towards a variety of disorders, from cancer to weight loss [7]. It has been suggested that the activities of black tea polyphenol are mostly caused by their powerful scavenging and antioxidant activity. Antioxidant tea components are reported to have beneficial protective effects against cancers and pathogenic microorganisms. It has been reported that black tea in both crude and pure substance extracts have many properties that protect and reduce hemolysis and vital organ damage induced by oxidative stress [8-10]. According to this, the main focus of this study was to evaluate the efficacy of black tea extract on the protection of hemolysis and liver and renal injuries during malaria infection using a *Plasmodium berghei* infected mouse model.

Materials and methods

Plant material and preparation of crude extract

Commercial tea (*Camellia sinensis*), Lipton Yellow Label Black Tea, purchased from a local market, was used in this study. The hot water method was carried out as previously described for crude extract preparation [11]. Dried powdered black tea was extracted in distilled water (10 g%) using a microwave at 360 W for 5 min, allowed to cool down at room temperature, and filtered through Whatman no. 1 filter paper. The filtrate was subsequently dried using lyophilization to obtain aqueous crude extract of black tea, and stored at 4 °C.

Experimental mice

Female ICR mice used in this study were purchased from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand. The mice were maintained in an animal room with a temperature of between 22 - 25 °C on a 12-h day/12-h night cycle. They were given standard diet pellets (CP082) and clean water *ad libitum*. All animal experiments were ratified by the Animal Ethic Committee, Western University, Kanchanaburi, Thailand (WTU-AE 2558-00068).

Rodent malaria parasite

A drug sensitive strain of *Plasmodium berghei* ANKA (PbANKA), kindly provided by Dr. Chairat Uthaipibull from the National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), was used in this study. The parasite was mechanically maintained in naïve ICR mice by an intraperitoneal injection of 1×10^7 PbANKA infected red blood cells (iRBC), and parasite growth (% parasitemia) was monitored daily by microscopic examination of Giemsa stained thin blood smears.

Measurement of hematocrit

Tail blood was collected into a heparinized microhematocrit tube and subsequently centrifuged at 10,000 rpm for 10 min. The percentage of packed cell volume (%Hematocrit; %Hct) was calculated using the volume of packed erythrocytes divided by the total volume of the blood sample.

Measurements of ALT and creatinine

Mouse blood was collected from the tail vein into a heparinized hematocrit tube. Centrifugation was performed at 10,000 rpm for 10 min, and plasma was then collected into a 1.5 ml microcentrifuge tube. AST, ALT, BUN, and creatinine were measured using a commercial kits (BioSystems S.A., Costa Brava 30, Barcelona, Spain), according to the manufacturer's instruction.

Standard antimalarial drug

Pyrimethamine (PYR) was used as a control for the efficacy test *in vivo*. The drug was freshly prepared in dimethyl sulfoxide (DMSO) at a dose based on ED90 (1 mg/kg) against PbANKA infected mice. The drug was stored at 4 °C.

Efficacy test *in vivo*

A standard Peter's test was carried out to evaluate the efficacy of black tea extract *in vivo* [12]. Groups of ICR mice (5 mice of each) were intraperitoneally inoculated with 1×10^7 iRBC of PbANKA. Two hours after infection, they were given the black tea extract (100, 500, and 1,000 mg/kg) orally by gavage, and treatment was performed every 24 h for 4 consecutive days (day 0 - 3). Control groups were also used, including normal mice treated with or without the extract, untreated, and PYR treated mice. Moreover, combination treatment between PYR and the extract was also investigated. On day 4 of the experiment, all biological markers were measured.

Statistics

Statistical analysis was carried out using GraphPad Prism Software. The one-way ANOVA was used to analyze and compare the results at a 95 % confidence level. Values of $p < 0.05$ were considered significant. All results were expressed as mean ± standard error of mean (SEM).

Results and discussion

Malarial associated hemolysis and liver and renal injuries induced by *Plasmodium berghei* infection in mice

Parasitemia was first detectable on day 1 post-infection, with a parasitemia of 0.5 - 1.5 %, and reached 65 % on day 10 (**Figure 1A**). Next, we observed that %Hct was markedly decreased in infected mice, and the onset of hemolysis came on day 4 post-infection (**Figure 1A**). Anemia and hemolysis are frequently associated with malaria and the usual causes are the destruction of RBC and iRBC, splenic sequestration, dyserythropoiesis, increase in inflammatory cytokines, and nutritional deficiency [13-16]. Moreover, increase of oxidative stress and metabolic acidosis have also been reported as causes of hemolysis in malaria [17-19]. Additionally, marked increases of ALT and creatinine levels in infected mice were also found, especially on day 4 to 10 (**Figure 1B**). Hence, liver and renal injuries were developed during malaria infection in the blood stage, as indicated by increases of ALT and creatinine levels. The pathogenesis suggested that malarial associated liver and renal injuries were a consequence of parasite adhesion and exacerbated immune response against oxidative stress products during infection. Therefore, proinflammatory molecules and products of oxidative stress have a central role in the development of the pathogenesis of malarial associated liver and renal injuries [20-23]. The extent of reactive oxygen species-induced oxidative damage can be exacerbated by decreased efficiency of antioxidant and cytoprotective defense mechanisms. Moreover, modifications in the permeability of the renal vascular endothelium decreased O_2 delivery to cells and tissues and contributed to increased hypoxic microenvironments [24].

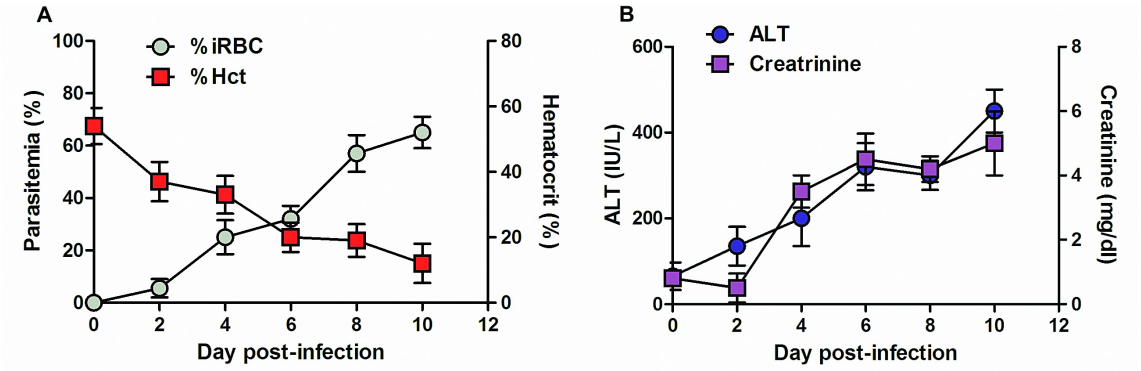

Figure 1 Malarial associated hemolysis and liver and renal injuries induced by *Plasmodium berghei* ANKA infection in mice. ICR mice (5 mice) were inoculated with 10^7 iRBC by intraperitoneal injection. Parasitemia, hematocrit, alanine aminotransferase (ALT), and creatinine levels were measured daily as described in the Materials and methods section. Results are expressed as mean ± SEM.

Effects of black tea extract on hemolysis and liver and renal injuries during *Plasmodium berghei* infection in mice

In order to evaluate the effects of aqueous crude extract of black tea against PbANKA infected mice, a standard Peter's test was carried out. We observed that aqueous crude extract of black tea exerted dose-dependent anti-hemolytic, hepatoprotective, and nephroprotective effects against PbANKA infection in mice (**Figures 2A - 2C**). This extract caused significant effects at doses of 500 and 1,000 mg/kg when compared to the untreated control, which showed a significant ($p < 0.01$) decrease in %Hct and an increase in ALT and creatinine, compared to normal mice. The highest effects were observed in infected mice treated with black tea extract at a dose of 1,000 mg/kg. However, significantly, hemolysis and liver and renal injuries were found in infected mice treated with 100 mg/kg of extract. Additionally, there was no effect on %Hct, ALT, or creatinine levels in normal ICR mice treated with this extract at the maximum dose of 1,000 mg/kg. The participation of the products generated by oxidative stress in the development of malarial associated hemolysis and liver and renal injuries have been reported [5,25]. It can be suggested that catechins and theaflavins, major components in black tea extract, showed antioxidant and anti-inflammation properties, which can reduce and protect organ damage from oxidative stress [26]. Moreover, it has been reported that catechins and theaflavins in black tea extract increased total antioxidant capacity, especially catalase, glutathione peroxidase, and superoxide dismutase [27,28]. This study supports black tea extract as an effective dietary component during malaria infection.

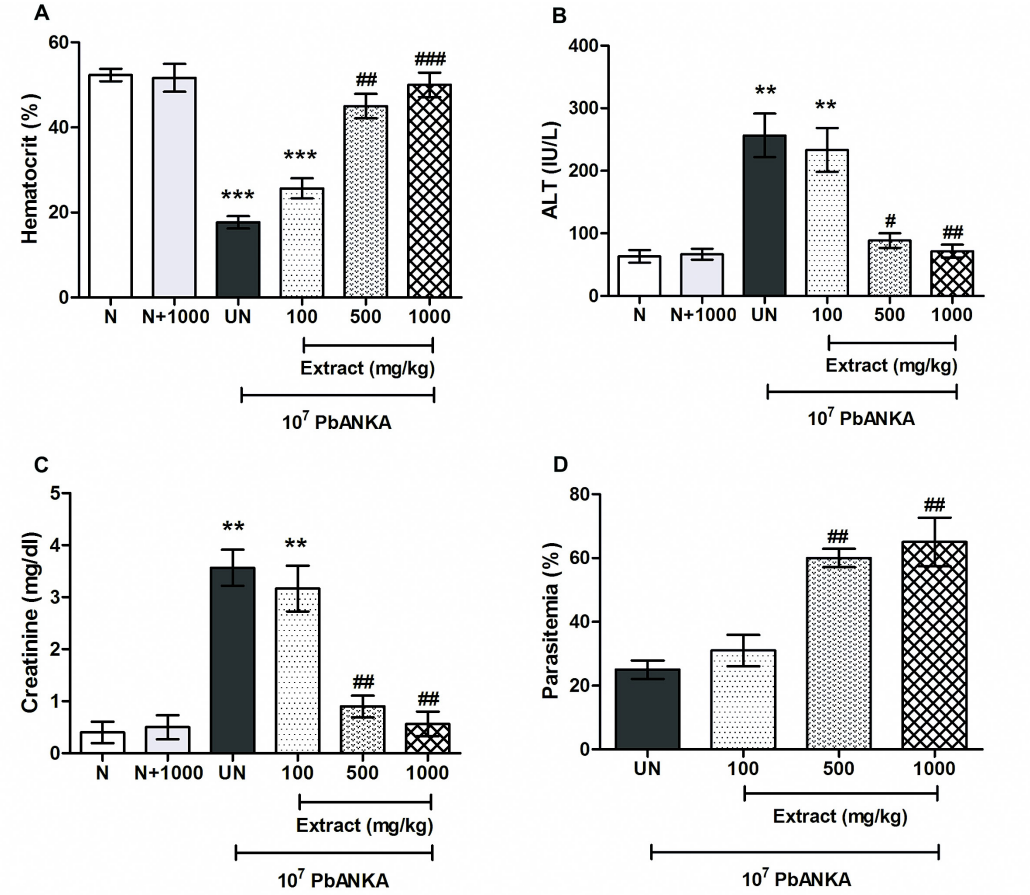

Figure 2 Effects of black tea extract on hemolysis and liver and renal injuries induced by *Plasmodium berghei* ANKA infection in mice. Groups of ICR mice (5 mice of each) were inoculated with 10^7 iRBC of PbANKA by intraperitoneal injection, and subsequently treated orally by gavage with 100, 500, and 1,000 mg/kg of black tea extract for 4 consecutive days. On day 4 of experiment, parasitemia, hematocrit, ALT, and creatinine levels were measured. Results are expressed as mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, compared to normal mice. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, compared to untreated control. N; normal mice, N+1000; normal mice treated with 1,000 mg/kg of extract, UN; untreated mice.

Conclusions

The results obtained in this study showed that aqueous crude extract of black tea exerted dose-dependent anti-hemolytic, hepatoprotective, and nephroprotective activities. It was most effective at a dose of 1,000 mg/kg. This plant can be recommended for use, since it possesses a high effect against malaria.

Acknowledgements

This work was supported by Western University. The authors wish to acknowledge Dr. Sakaewan Ounjaijean for valuable discussions about black tea extraction. The technical assistance of the students in all of the animal experiments is also acknowledged.

References

[1] WHO. World Health Organization, World Malaria Report, Available at: http://www.who.int/malaria/world_malaria_report_2009/en, accessed December 2015.
[2] NJ White, S Pukrittayakamee, TT Hien, MA Faiz, OA Mokuolu and AM Dondorp. Malaria. *Lancet* 2014; **383**, 723-35.
[3] F Lang, M Abed, E Lang and M Foller. Oxidative stress and suicidal erythrocyte death. *Antioxid. Redox Signal.* 2014; **21**, 138-53.
[4] S Audomkasok, W Singpha, S Chachiyo and V Somsak. Antihemolytic activities of green tea, safflower, and mulberry extracts during *Plasmodium berghei* infection in mice. *J. Pathog.* 2014; **2014**, 203154.
[5] S Percario, DR Moreira, BA Gomes, ME Ferreira, AC Goncalves, PSOC Laurindo, TC Vilhena, MF Dolabela and MD Green. Oxidative stress in malaria. *Int. J. Mol. Sci.* 2012; **13**, 16346-72.
[6] N Nutham, S Sakulmettatham, S Klongthalay, P Chutoam and V Somsak. Protective effects of *Tinospora crispa* stem extract on renal damage and hemolysis during *Plasmodium berghei* infection in mice. *J. Pathog.* 2015; **2015**, 738608.
[7] MS Butt, A Imran, MK Sharif, RS Ahmad, H Xiao, M Imran and HA Rsool. Black tea polyphenols: A mechanistic treatise. *Crit. Rev. Food Sci. Nutr.* 2014; **54**, 1002-11.
[8] M Fatima, RK Kesharwani, K Misra and SI Rizvi. Protective effect of theaflavin on erythrocytes subjected to *in vitro* oxidative stress. *Biochem. Res. Int.* 2013; **2013**, 649759.
[9] B Szachowicz-Petelska, E Skrzydlewska and Z Figaszewski. Protective effect of black tea on integral membrane proteins in rat liver. *Exp. Toxicol. Pathol.* 2013; **65**, 173-9.
[10] V Sharma and LJ Rao. A thought on the biological activities of black tea. *Crit. Rev. Food Sci. Nutr.* 2009; **49**, 379-404.
[11] E Nkhili, V Tomao, HE Hajji, ESE Boustani, F Chemat and O Dangles. Microwave-assisted water extraction of green tea polyphenols. *Phytochem. Anal.* 2009; **20**, 408-15.
[12] W Peters. The chemotherapy of rodent malaria, XXII: The value of drug-resistant strains of *P. berghei* in screening for blood schizontocidal activity. *Ann. Trop. Med. Parasitol.* 1975; **69**, 155-71.
[13] N Spottiswoode, PE Duffy and H Drakesmith. Iron, anemia and hepcidin in malaria. *Front. Pharmacol.* 2014; **5**, 125.
[14] AR Mawson. The pathogenesis of malaria: A new perspective. *Pathog. Glob. Health* 2013; **107**, 122-9.
[15] KS Akinosoglou, EE Solomou and CA Gogos. Malaria: A haematological disease. *Hematology* 2012; **17**, 106-14.
[16] C Menendez, AF Fleming and PL Alonso. Malaria-related anaemia. *Parasitol. Today* 2000; **16**, 469-76.
[17] S Zougbede, F Miller, P Ravassard, A Rebollo, L Ciceron, PO Couraud, D Mazier and A Moreno. Metabolic acidosis induced by *Plasmodium falciparum* intraerythrocytic stages alters blood-brain barrier integrity. *J. Cereb. Blood Flow Metab.* 2011; **31**, 514-26.
[18] T Planche and S Krishna. Severe malaria: Metabolic complications. *Curr. Mol. Med.* 2006; **6**, 141-53.
[19] M English, R Sauerwein, C Waruiru, M Mosobo, J Obiero, B Lowe and K Marsh. Acidosis in severe childhood malaria. *Q. J. Med.* 1997; **90**, 263-70.
[20] LC Koopmans, ME van Wolfswinkel, DA Hesselink, EJ Hoorn, R Koelewijn, JJ van Hellemond and PJJ van Genderen. Acute kidney injury in imported *Plasmodium falciparum* malaria. *Malaria J.* 2015; **14**, 523.
[21] AS Badiane, K Diongue, S Diallo, AA Ndongo, CK Diedhiou, AB Deme, D Ma, M Ndiaye, MC Seck, T Dieng, O Ndir, S Mboup and D Ndiaye. Acute kidney injury associated with *Plasmodium malariae* infection. *Malaria J.* 2014; **13**, 226.
[22] P Viriyavejakul, V Khachonsaksumet and C Punsawad. Liver changes in severe *Plasmodium falciparum* malaria: Histopathology, apoptosis and nuclear factor kappa B expression. *Malaria J.* 2014; **13**, 106.

[23] P Wilairatana, S Looareesuwan and P Charoenlarp. Liver profile changes and complications in jaundiced patients with falciparum malaria. *Trop. Med. Parasitol.* 1994; **45**, 298-302.
[24] S Ng, S March, A Galstian, K Hanson, T Carvalho, MM Mota and SN Bhatia. Hypoxia promotes liver-stage malaria infection in primary human hepatocytes *in vitro*. *Dis. Model. Mech.* 2014; **7**, 215-24.
[25] N Narsaria, C Mohanty, BK Das, SP Mishra and R Prasad. Oxidative stress in children with severe malaria. *J. Trop. Pediatr.* 2012; **58**, 147-50.
[26] W Luczaj and E Skrzydlewska. Antioxidative properties of black tea. *Prev. Med.* 2005; **40**, 910-8.
[27] SM Khan. Protective effect of black tea extract on the levels of lipid peroxidation and antioxidant enzymes in liver of mice with pesticide-induced liver injury. *Cell Biochem. Funct.* 2006; **24**, 327-32.
[28] KVC Mohan, R Subapriya, Y Hara and S Nagini. Enhancement of erythrocyte antioxidants by green and black tea polyphenols during 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis. *J. Med. Food* 2006; **9**, 373-7.