

Effect of Black Tea Extract on Hypoglycemia Induced by *Plasmodium berghei* ANKA Infection in Mice

Muthita Sihabud, Puangphaka Yongthawee, Palatip Chutoam, Suwit Klongthalay and Voravuth Somsak*

Department of Clinical Chemistry, Faculty of Medical Technology, Western University, Kanchanaburi 71170, Thailand

Abstract

Hypoglycemia was found as one of all causes of death in malaria disease and urgently needed to find new drugs to treat this condition. Hence, the present study was aimed to evaluate the effect of black tea extract on hypoglycemia induced by *Plasmodium berghei* infection in mice. Aqueous crude extract of black tea was freshly prepared using hot water method and used for efficacy test in vivo. For in vivo test, *P. berghei* ANKA infected mice were given orally by gavage with 500, 1000, and 2000 mg/kg of black tea extract for 4 consecutive days. Blood glucose levels were then measured. It was found that aqueous crude extract of black tea exerted dose-dependent anti-hypoglycemic activity, especially at a dose of 2000 mg/kg showed the highest activity. Although, pyrimethamine treated group showed significantly ($p < 0.01$) decreasing of blood glucose levels, combination treatment with black tea extract could protect and maintain blood glucose to normal level. It can be concluded that aqueous crude extract of black tea presented anti-hypoglycemic activity against *P. berghei* infection in mice.

Keywords: Black tea; Hypoglycemia; *Plasmodium berghei*

Introduction

Malaria remains a major public health problem in tropical and sub-tropical areas such as Africa, North and South America, Asia and Southeast Asia including Thailand. It is estimated that 700-800 million people are at risk and 1 million deaths annually [1]. This disease is caused by protozoa parasite in genus *Plasmodium*, and transmitted by female *Anopheles* mosquito. During malaria infection in blood stage, it can cause of death including cerebral malaria, severe anemia, acute hemolysis, and organ failure [2]. Moreover, malaria-associated hypoglycemia is one of all causes of death in *P. falciparum* and *P. vivax* severe malaria. It occurs between 1-5% of hospitalized adult with a mortality that can up to 45% [3]. Activation of glucose uptake into infected red blood cells, oxidative stress, and hemolysis has been described to cause hypoglycemia during malaria infection [4]. This has prompted research towards the discovery of new drugs with anti-hypoglycemic property. In this respect, plant extracts are targets for research of the alternative drugs. Tea (*Camellia sinensis*) is one of the most widely consumed beverages in the world. Because of ~80% of the tea produced in the world is consumed as black tea and only 20% as green tea, research has focused mainly on black tea [5]. During the manufacture of black tea, green tea catechins undergo oxidation to form the complex product theaflavins by a process commonly known as fermentation [6]. It has been indicated that theaflavins in black tea and catechins in green tea are equally effective antioxidants [5]. In one study, the black tea extract scavenged hydrogen peroxide more potently than those from green tea [7]. The recent reports have been described that black tea and green tea showed the effect to maintain and control blood glucose in diabetes patients [8]. Additionally, green tea extracts in malaria have been studied [9]. However, black tea extract in malaria researches and hypoglycemia protection during malaria infection have not yet been studied. Hence, this study was aimed to investigate anti-hypoglycemic effect of black tea extract against *P. berghei* infected mice.

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. For crude

extract preparation, hot water method was carried out as previously described [10]. Dried powder black tea was extracted in distilled water (10 g%) using microwave at 360 W for 5 min allowed to cool at room temperature and filtered through Whatmann no. 1 filter paper. Filtrate was then dried using freeze drying to obtain aqueous crude extract of black tea, and stored at 4°C.

Experimental mice

Female ICR mice purchased from the National Laboratory Animal Center, Mahidol University, Bangkok, Thailand were used. The mice were maintained in animal room with temperature between 22-25°C and 12 h light-dark cycle. They were given standard diet pellet (CP082) and clean water *ad libitum*. All animal experiments were approved by the Animal Ethic Committee, Western University, Kanchanaburi, Thailand.

Rodent malaria parasite

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

*Corresponding author: Voravuth Somsak, Department of Clinical Chemistry, Faculty of Medical Technology, Western University, Kanchanaburi 71170, Thailand, Tel: +66898009939; E-mail: voravuthsomsak@gmail.com

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Measurement of blood glucose

Mouse blood was collected from tail vein into heparinized hematocrit tube. Centrifugation was performed at 10,000 g for 10 min, and plasma was then collected into new a 1.5 ml micro-centrifuge tube. Blood glucose was measured using a commercial kit (Bio-Systems S.A. Costa Brava 30, Barcelona, Spain), according to the manufacturer's instruction.

Standard antimalarial drug

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

Efficacy test *in vivo*

Standard 4-day suppressive test was carried out to evaluate the efficacy of black tea extract *in vivo* [11]. Groups of ICR mice (5 mice of each) were infected with 1×10^7 iRBC of PbANKA by intra-peritoneal injection. Two hours later after infection, they were given orally by gavage with black tea extract (500, 1000, and 2000 mg/kg), and treatment was performed every 24 hrs for 4 consecutive days. The 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 6 after infection, blood glucose levels were measured.

Statistics

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

Results

Hypoglycemia induced by PbANKA infection

As showed in Figure 1A, there was a markedly increase in level of parasitemia from day 2 to 10 in PbANKA infected ICR mice, and survival time was 10 days (Figure 1B). Interestingly, blood glucose levels presented a progressive decrease in the correlation to the presence of parasitemia, and significance ($p < 0.05$) was found on day 6 after infection (Figure 1C). In addition, strong negative correlation ($R^2 = 0.8564$) between parasitemia and blood glucose levels was found (Figure 1D).

Anti-hypoglycemia of black tea extract against PbANKA infected mice

It was found that hypoglycemia with significant low levels of blood glucose was found in untreated and infected mice treated with 500 mg/kg of extract ($p < 0.001$ and $p < 0.05$, respectively) as showed in Figure 2. Interestingly, the aqueous crude extract of black tea exerted dose-dependent anti-hypoglycemia in the extract treated groups, especially at a dose of 2000 mg/kg showed the highest activity. Surprisingly, hypoglycemia was also observed in PYR treated group. However, normal blood glucose level was observed in combination treatment of PYR and black tea extract. Additionally, no any effects on blood glucose level were found in normal mice treated with this extract.

Discussion

There was a progressive increasing of parasitemia from day 2 to 10

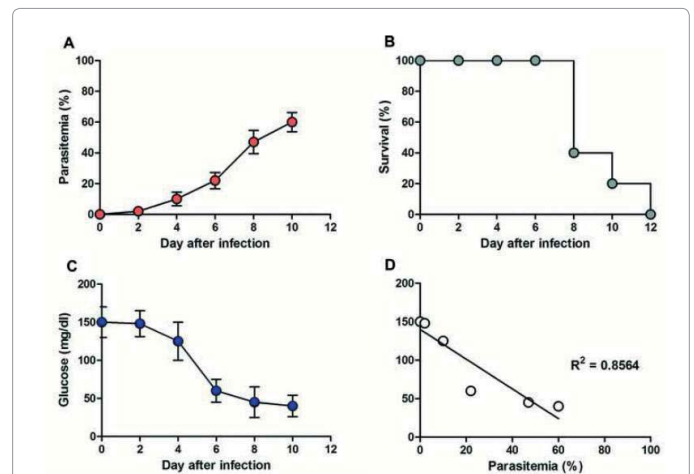


Figure 1: Hypoglycemia induced by *Plasmodium berghei* ANKA infected in mice. ICR mice (5 mice of each) were infected with 1×10^7 iRBC of PbANKA by intraperitoneal injection. (A) Parasitemia, (B) survival time of infected mice, (C) blood glucose levels, and (D) correlation of parasitemia and blood glucose were monitored. Results were expressed as mean+SEM.

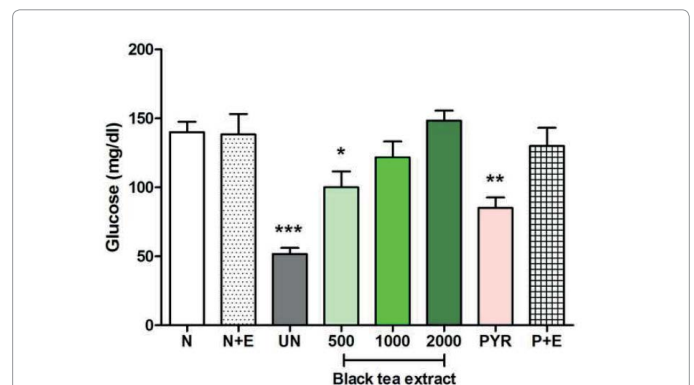


Figure 2: Effect of black tea extract on blood glucose levels in *Plasmodium berghei* ANKA infected mice. Groups of ICR mice (5 mice of each) were infected with 1×10^7 iRBC of PbANKA by intraperitoneal injection. They were given orally with 500, 1000, and 2000 mg/kg of black tea extract for 4 consecutive days. Blood glucose levels were then measured. Results were expressed as mean+SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. N; normal mice, N+E; normal mice treated with 2000 mg/kg of extract, UN; untreated mice, PYR; infected mice treated with 1 mg/kg of pyrimethamine, and P+E; combination treatment of PYR and 2000 mg/kg of black tea extract.

in PbANKA infection in ICR mice with a survival time of 10 days. This is in line with the view that parasitemia increases progressively after infection until the point of death in the absence of suitable treatment. Moreover, hypoglycemia was found in response to the presence of parasitemia. This could be due to the fact that during malaria infection and development in blood stage, 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 for energy supply [12]. It has been described that it is accompanied with approximately 100-fold increase in glucose utilization when compared to uninfected red blood cells so causing a profound hypoglycemia if untreated [13]. Moreover, hyper-insulinemia during malaria infection with a relation to hypoglycemia has also been reported [14]. Several studies have been reported the activity of green tea extract to control

blood glucose level. Knowledge of properties and constituents of black tea extract such as polyphenols, flavonols and sulfur compounds, and its analog with green tea such as theaflavins suggests that biological activity of black tea extract to maintain and control blood glucose level might be similar to green tea extract [15]. Inhibition of glycolysis and hexose transporter of infected red blood cells might be properties of black tea extract on blood glucose levels [16]. Additionally, beneficial effect of black tea extract on insulin may be due to the antioxidant capacity of the extract. It has been also reported that aqueous extract of black tea had significant antioxidant potential [6]. Furthermore, hypoglycemia was also observed in PYR treated group. However, normal blood glucose level was observed in combination treatment between PYR and black tea extract. Hypoglycemia in PYR treatment might be due to the oxidative stress and hemolysis induction by this drug [17], and antioxidant potential of black tea extract might protect and maintain blood glucose level to normal.

It is interesting to note that aqueous crude extract of black tea was found the anti-hypoglycemic activity against PbANKA infection in mice. In addition, combination treatment with standard antimalarial pyrimethamine was also recommended. Even though the bioactive components and mechanism are yet to be studied and compared activity to the crude extract, the results of this study provide the basis for further studies.

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