S. L. J. Tea Sci. 72(1), 9 - 15, 2007, Printed in Sri Lanka

An investigation of *in vivo* antimalarial activity of black tea brew of *Camellia sinensis* L. O. Kuntze in mice

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ABSTRACT

Several traditional medicines and folklore beliefs exist that black tea brew (BTB) from *Camellia sinensis* L. O. Kuntze: (Theaceae) possesses antimalarial activity. This study scientifically investigated the antimalarial potential (in terms of antiparastic activity) of *C. sinensis* using Sri Lankan high grown Dust grade No.1 tea *in vivo* in mice against *Plasmodium yoelii*. 1336 mg/kg of BTB /day (equivalent to 24 cups) or chloroquine or water was orally administered to three different groups (n= 6/group) of mice for 3 consecutive days and the schizonticidal activity determined. The results showed no significant ($P > 0.05$) schizonticidal activity either on early infection or the established malarial infections (measured in terms of parasitaemia, chemosuppresion and mean survival time). However, the number of surviving mice at day 4 post inoculation was higher in the BTB treated group, compared with the vehicle. It is concluded that BTB may not be effective against *P. falciparum* human malaria contrary to the beliefs of traditional medicines and folklore that it is effective.

Key words : *Camellia sinensis*; black tea; antimalarial activity; *Plasmodium yoelii, Plasmodium falciparum*

INTRODUCTION

Malaria is the most important tropical parasitic disease causing great suffering and loss of life. At the end of 2004, 107 countries and territories had areas at risk of falciparum malaria transmission, with 3.2 billion people, about 40% of world's population living in those areas and 300- 500 million clinical attacks including 2-5 million deaths occur annually (World malaria report, 2005). This situation is attributed mainly to the development of resistant strains of *Plasmodium falciparum* and *P. vivax* to chloroquine as well as to other allopathic antimalarial drugs (Phillips, 2001). Thus, there is an urgent need and demand for the development of novel and efficient antimalarial drugs, which can be purchased at affordable prices by the poor, living in malarious areas (Wright, 2005).

In this regard, several plants claimed by traditional practitioners and folklore to have antimalarial properties have been tested as potential antimalarial drugs (Wright, 2005).

Some of these plants tested were shown to possess promising antimalarial activity, which are attributed to alkaloids, flavonoids, saponins or triterpenes (Sanon *et al.*, 2003 Philippe *et al.*, 2005 Wright, 2005). Black tea made from tender shoots, comprising two or three of the topmost, immature leaves and the buds of tea plants, *Camellia sinensis* L. O. Kuntze is yet another plant which is claimed to have antimalarial properties (Anon, 2005). Disappearance of malaria in Japan between fourteenth and fifteenth centuries, and in Britain in the second half of the eighteenth century exactly coincided with the spread of tea drinking (Stagg and Millin, 1975). However, the validity of this belief has not been scientifically investigated so far. Further, black tea brew is rich in flavonoids (flvanols, flavones, flavanones) and alkaloids (caffeine) and therefore a stray possibility exists that it could have antimalarial potential. Many millions of people drink tea everyday. Thus, investigating the anti-malarial properties of tea is of considerable importance. It is also noteworthy that many of the earlier assumptions regarding the health benefits of tea are found to be correct by scientific experiments (Modder and Amarakoon, 2002).

The aim of this study was to investigate the antimalarial potential of *C. sinensis* leaves. This was tested with Sri Lankan high grown Dust grade No. 1 black tea, a type that is widely consumed, *in vivo* in the *P. yoelii* - murine malaria model.

MATERIALS AND METHODS

Experimental animals

Healthy, adult male ICR mice weighing 20-25 g purchased from the Medical Research Institute, Colombo, Sri Lanka were used in this study.

Animals were housed in plastic cages in the animal house, Department of Zoology, University of Colombo, under standard conditions (temperature 28-31 °C, photoperiod: approximately 12 h natural light per day, relative humidity: 50-55%). The animals were fed with pelleted food (Master feed Ltd, Colombo, Sri Lanka) and clear drinking water *ad libitum*. Except at the time of experimental procedure the animals were handled only during cage cleaning. All the experiments were conducted in accordance with the internationally accepted laboratory animal use and care, and guidelines and rules of the Faculty of Science, University of Colombo, Sri Lanka, for animal experimentations.

Source of tea

Two or three topmost immature leaves and buds of *C. sinensis* plucked from the plantation of St. Coombs tea estate of the Tea Research Institute, Talawakelle, Sri Lanka (1382 m above sea level; high grown) in August 2005, were used to process Dust grade No: 1 black tea by orthodox – rotorvare technique at the estate factory. The

tea samples were packed in triple laminated aluminium foil bags (1kg each) and stored at –20 °C until use. Further, these tea samples selected were pure, unblend and typical to the grade as confirmed by sieve analysis, organoleptic profile and physical and chemical analysis.

Preparation of black tea brew (BTB)

BTB was made by adding 2g of tea sample to 100 ml boiling water and brewed for 5 min (Anon, 1980) (Yield: 43.7 % (w/w)). For oral treatment of mice supraphysiological dose of 1336 mg/ml, equivalent to 24 cups was used. The volume of 1 cup is considered as 170ml.

***In vivo* antimalarial drug susceptibility testing**

Parasite isolates

Chloroquine sensitive, 17 XL (lethal strain) *Plasmodium yoelii* was used to assess the *in vivo* antimalarial activity of BTB. The parasite strain was maintained in the rodent model by serial passage of blood.

Parasite inoculation

The inoculum consisted of 10^7 *P. yoelii* parasitized red blood cells (RBCs)/ ml of blood. This was prepared by determining both parasitaemia and the erythrocyte counts of the donor mouse and diluting the blood with isotonic saline (0.2 ml).

Evaluation of blood schizonticidal activity on an early infection

The 4-day suppressive assay was used to evaluate the blood schizonticidal activity of BTB (Peters, 1967). Eighteen mice were randomly assigned in to 3 equal groups (n = 6/ group) and were inoculated intraperitoneally with 10^7 (in 0.2 ml isotonic saline) infected RBC of *P. yoelli* (day 0). These mice in-group 1 were daily orally treated with BTB equivalent to 24 cups, group 2 with 10mg/ kg dose of chloroquine phosphate (State Pharmaceutical Corporation, Colombo, Sri Lanka) and group 3 with 1 ml water for 3 consecutive days starting from day 0. On day 4, blood was obtained from the tip of the tail of each mouse using aseptic conditions and then blood smears were made on glass slides. These slides were stained with Giemsa Stain (Fluka Chemie AG CH-9470, Buchs, Switzerland) and the number of parasitized RBC were counted out of at least 3000 erythrocytes in random fields under oil immersion. The degree of parasitaemia was then calculated (%). The average percentage chemosuppression was also calculated as (A-B) / A x 100, where A is the average percentage of parasitaemia in the control group, and B average parasitaemia in the BTB treated group (Okokon *et al.*, 2006).

Evaluation of blood schizonticidal activity in BTB established infection (Curative or Rane test)

Eighteen mice were randomly divided in to 3 equal groups (n = 6/ group) and injected intraperitoneally with 10^7 *P. yoelli* infected RBC (day 0). Seventy two hours following inoculation the mice in group 1 were orally administered daily with BTB equivalent to 24 cups, group 2 with 10mg / kg of chloroquine phosphate and group 3 with 1ml of water for 5 consecutive days or until the death of mice. (BTB for 3 days post inoculation, control for 2 days post inoculation and chloroquine 5 days post inoculation). Blood smears were prepared daily until the animals survive or day 7 post inoculation. Further, the mean survival time for each group of mice were determined arithmetically by finding the average survival time (days) of the mice (post inoculation) in each group over a period of 14 days (Okokon *et al.*, 2006).

Statistical Analysis

Data are presented as means ± SEM. Statistical comparisons were made using Mann –Whitney U- test and Fisher exact test. Significance was set at $P < 0.05$.

RESULTS

As shown in Figure 1, oral administration of BTB did not significantly alter the parasitaemia in the 4-day suppressive assay compared with the control ($P > 0.05$). Also BTB showed only a negligible chemosuppression. In contrast, the positive drug chloroquine markedly suppressed the parasitaemia (%) significantly ($P < 0.05$) and provided profound chemosuppression. The results obtained in the curative assay are depicted in Table 1. As shown, no alteration in parasitaemia was found in mice treated with BTB compared with the control on days 3 and 4 post inoculation.

On day 4 post inoculation, only 33% of mice survived in the control group while 100% survived in the BTB and chloroquine groups. This effect was marginally significant (Fisher exact test, $P < 0.06$)

However, when mean survival time was calculated there was no significant increase in the BTB group compared to the control. In contrast, there was a significant ($P < 0.05$) increase in the survival time in the chloroquine treated group (Table 2).

DISCUSSION

This study examined the antimalarial potential of BTB of *C. sinensis* using Sri Lankan high grown Dust grade No.1 tea *in vivo* using the *P. yoelii*- murine malaria model. This is a widely used, reliable and sensitive *in vivo* model to detect antimalarial activity of potential drugs effective against *P. falciparum* human parasites (Peters *et al.*, 1975). The results show, for the first time, that black tea brew, even at extremely high oral

dose (equivalent to 24 cups) had no significant schizonticidal activity on early infection, and against established malarial infection (measured in terms of parasitaemia, chemosuppression and mean survival time). It is not advised to drink more than 10 cups of tea per day (Modder and Amarakoon, 2002). However, the number of surviving mice at day 4 post inoculation was higher in the BTB treated group (treated with an equivalent of 24 cups per day), which is not of much practical relevance.

The lack of antimalarial activity of BTB in this study is contrary to the belief of the traditional practitioners and folklore that it possesses antimalarial properties. *P. yeolii* and several other murine malarial parasites have proved to be analogous to the human parasite, *P. falciparum*, in most essential aspects of structure, physiology and life cycle (Carter and Diggs, 1977). Although, the results of this study indicate that BTB is not effective against falciparum malaria, it is not possible to completely rule out that black tea is not effective against vivax and other forms of human malaria.

Further, black tea produced from other tea growing countries could have antimalarial activity since it is well recognized that composition of tea changes depending on the cultivar of tea, climate, elevation and soil conditions, methods of cultivation, fertilizers applied, nature of shade, type of processing machinery used (Wickramasinghe, 1996; Balentine *et al.*, 1997; Modder and Amarakoon, 2002).

In conclusion, this study shows that oral administration of BTB made from Sri Lankan high grown Dust grade No.1 tea is not effective against *P. yoelii* induced malaria in mice indicating that this tea may not be effective against human *P. falciparum* malaria, rejecting its antimalarial properties claimed by traditional medicine and folklore.

ACKNOWLEDGEMENTS

This investigation received financial support from the National Science Foundation under grant number, NSF / Fellow / 2005 / 01.

Prof Anura Weerasinghe, Professor of Physiology of the University of Kelaniya, Sri Lanka is gratefully acknowledged for providing the murine parasite species and Dr. Ziyad Mohomed Former Director, Tea Research Institute, Talawakelle, for providing the tea sample.

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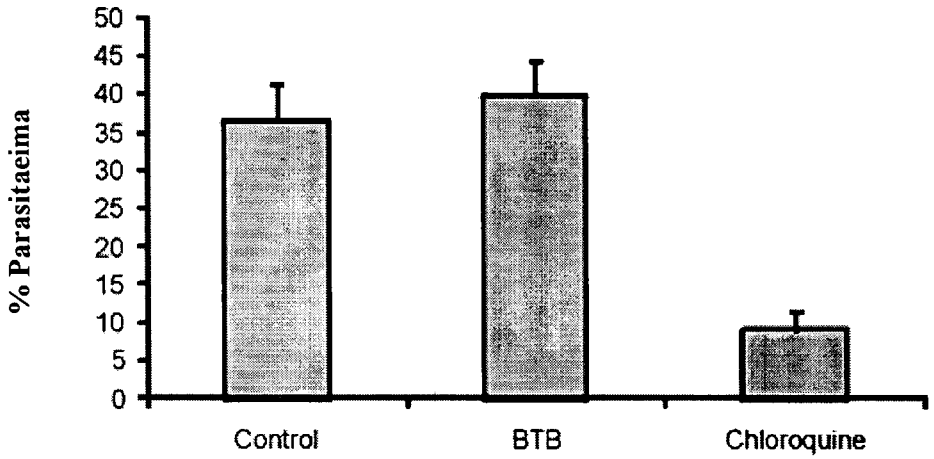

Figure 1. ***In vivo*** **antimalarial activity of black tea brew of** ***Camellia sinensis*** **in the 4-day suppressive assay**

Table 1. ***In vivo*** **antimalarial activity of black tea brew (BTB) of** ***Camellia sinensis*** **in the curative assay**

	% Parasitaemia			
Treatments	**Day 3**	**Day 4**	**Day 5**	**Day 6**
Control (Distilled water)	42.60 ± 4.58	72.3 ± 2.60	100 ±0	100 ± 0
BTB treated	41.90 ±3.85	67.52 ± 4.37	96.48± 3.51	100 ± 0
Chloroquine	38.10 ± 2.92	28.18 ± 1.84*	18.60 ± 1.67*	10.60 ± 1.93*

Values are expressed as Means ± S.E.M (n = 6)
*P < 0.05 as compared with the control (Mann -Whitney U-test)

Table 2. Mean survival time of mice treated with black tea brew of ***Camellia sinensis*** **in the curative assay**

Treatments	Mean Survival time (days)
Control	3.33 ± 0.21
Black Tea Brew	4.16 ± 0.16
Chloroquine	12.33 ± 1.50*

Values are expressed as Means ± S.E.M (n = 6)
*P < 0.05 as compared with the control (Mann -Whitney U-test)