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Short Communication

Hypoglycemia induced by *Plasmodium berghei* infection is prevented by treatment with *Tinospora crispa* stem extract

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ABSTRACT

During *Plasmodium* malaria parasite infection in a human, the intraerythrocytic stages lead to the clinical manifestations of the disease, especially hypoglycemia. Hypoglycemia is a recognized feature of severe malaria and linked with a high risk of mortality for children. Hence, the present study aimed to investigate the protective effect of *T. crispa* stem extract on hypoglycemia induced by *P. berghei* infection tested with a mouse model. ICR mice were inoculated with 1×10^7 parasitized erythrocytes of *P. berghei* ANKA (PbANKA) by intraperitoneal injection and given 50, 100, and 200 mg/kg of ethanolic extract for 4-consecutive days. The results showed that *T. crispa* stem extract exerted a protective effect (100%) on hypoglycemia induced by PbANKA infection at doses of 100 and 200 mg/kg. A significantly ($p < .05$) prolonged mean survival time (28.0 ± 1.9 days) of the extract treated mice was also observed. Additionally, no effect on blood glucose levels was seen in normal mice treated with all doses of extract. It can be concluded that *T. crispa* stem extract may have beneficial properties in protecting against hypoglycemia, and in increasing survival time during malaria infection.

Plasmodium spp., the causative agent of malaria, is a prominent cause of human morbidity and mortality for about 40% of world's population, and it kills about 1 million children under 5 years of age annually [1]. During *Plasmodium* infection in a human, the intraerythrocytic stages lead to the clinical manifestations of the disease, including hypoglycemia and hypoglycemic shock [2]. Hypoglycemia is often associated with severe malaria, especially in children and pregnant women [3–5]. Growing evidence suggests that the depletion of critical gluconeogenic substrates by the malaria parasite may play an important role in malaria-associated hypoglycemia [6,7]. This has prompted research towards the discovery of new and affordable substances to lessen the impact of malaria-associated hypoglycemia. Approximately 80% of the world's population depends on traditional and herbal medicines as a source of disease treatment [8], and the use of traditional and herbal remedies seems to be an alternative choice for malaria treatment.

Tinospora crispa (L.) Hook. f. & Thomson is a medicinal plant belongs to the genus *Tinospora* of the Menispermaceae family. It is common in primary rainforests of Southeast Asia and Africa, including Thailand, Malaysia, and Indonesia [9]. It has been traditionally used for the treatment of rheumatism, jaundice, intermittent fever, and urinary disorders. Moreover, a number of studies investigating the anti-inflammatory, antioxidant, anti-bacterial, anti-parasitic, anti-filarial, anti-proliferative, analgesic, atherosclerosis inhibitory, cardioprotective, anti-hyperglycemic, and immunomodulatory activities of *T. crispa* extract have been carried out [10]. Additionally, an antimalarial activity of *T. crispa* extract has been reported [11–13]. However, any effect of extracts of this plant on hypoglycemia induced by *P. berghei* in mouse model has not yet been reported. The present study therefore aimed to investigate the protective effect of *T. crispa* stem extract on hypoglycemia induced by *P. berghei* infection in mice.

T. crispa was collected in Kanchanaburi province, Thailand. The stems were cut into small pieces and oven-heated (Memmert UF55) at 60 °C for 72 h. The dried plant material was ground into a powder using an electric blender. Then 300 g of powdered sample was extracted by maceration in 70% ethanol for 72 h. After filtration through Whatman no. 1 filter paper the filtrate was evaporated to dryness by a rotary vacuum evaporator (Buchi R-100) under reduced pressure and stored at -20 °C until use [12]. Before the experiment, dried crude extract of *T. crispa* stem was dissolved in 20% Tween-80 to obtain an appropriate dose, with a total volume not exceeding 1 ml. *Plasmodium berghei* ANKA (PbANKA) used in this study were kindly provided by Dr. Chairat Uthaipibull from the National Center for Genetic Engineering and Biotechnology (BIOTEC), NSTDA. The parasite was maintained by

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mechanical passage into ICR mice. Naïve mice were infected with 1×10^7 parasitized erythrocytes of PbANKA by intraperitoneal (IP) injection. Parasitemia was monitored daily under a light microscope by observation of Giemsa stained thin blood smears. The mice were used in accordance with NIH Guidelines for the care and use of laboratory animals; NIH Publication (No. 83–23) revised (1985) NIPRD-Standard Operation Procedures (SOPs). All experiments involving animals were ratified by the Animal Ethics Committee, Western University (WTU 23/2017). For *in vivo* experiments, a standard 4-day test was carried out as previously described [14]. Groups of ICR mice were randomly divided into 6 groups (5 mice per group). ICR mice were infected with 1×10^7 parasitized erythrocytes of PbANKA by IP injection. Two hours later, doses of 50, 100, and 200 mg/kg of extract were administered by oral gavage, followed by administration on four consecutive days (days 0–3). Normal and toxic controls were established by administering 10 ml/kg of 20% Tween-80 and 200 mg/kg of extract by oral gavage, respectively. Moreover, untreated controls were given only 10 ml/kg of 20% Tween-80. On day four, blood glucose and MST were measured. All results of this study are presented as mean ± standard error of mean (SEM) using an available commercial statistic program (GraphPad Prism version 5.01). Significance was considered at a 95% confidence, p-value < .05 using one-way ANOVA with post-hoc Tukey analysis.

During PbANKA infection of mice, parasitemia was first detectable on day 1 post-infection, with a parasitemia of lower than 1% and parasitemia subsequently reached 45% at day 14 post-infection (Fig. 1a, left axis). Moreover, hypoglycemia was found in PbANKA infected mice as indicated by markedly decreased blood glucose (Fig. 1a, right axis). Additionally, a negative correlation ($R^2 = 0.8142$) between parasitemia and blood glucose during PbANKA infection was observed (Fig. 1b). This could be due to the fact that utilization of blood glucose and the host's glucose stores by *Plasmodium* malaria parasites affects hypoglycemia during infection [15]. Moreover, it has been shown that blood glucose is rapidly taken up across the malaria parasite membrane facilitated by the hexose transporter, and is metabolized through the glycolysis pathway approximately 100-fold faster as compared to normal erythrocytes, therefore causing hypoglycemia if untreated [16,17]. In addition, high levels of tumor necrosis factor (TNF) has been reported to be associated with severity and mortality of malaria by causing hypoglycemia *via* induction of hyperinsulinemia [18–21].

For the efficacy of *T. crispa* stem extract on hypoglycemia induced by PbANKA infection, this extract of *T. crispa* stem exerted a protective effect on hypoglycemia induced by PbANKA at a dose of 100 (158 ± 18.2 mg/dl) and 200 mg/kg (164 ± 12.9 mg/dl) of the extract, resulting in glucose levels similar and insignificant ($p > .05$) to those in uninfected mice (176 ± 16.4 mg/dl) (Fig. 2a). The highest protective effect was observed at a dose of 200 mg/kg. However, a dose of 50 mg/kg of extract did not protect against hypoglycemia as indicated by a significantly ($p < .01$) decrease blood glucose (102 ± 11.5 mg/dl), compared to uninfected control. Moreover, *T. crispa* stem extract also showed antimalarial activity at the doses of 100 and 200 mg/kg (Fig. 2b). In addition, no effect on blood glucose level was seen in normal mice treated with 200 mg/kg of extract (178 ± 25.1 mg/dl). The *T. crispa* stem extract, the 100 (27.0 ± 2.0 days) and 200 (28.0 ± 1.9 days) mg/kg extract treated groups lived longer than the corresponding untreated control (13.6 ± 1.1 days) ($p < .001$). There were no significant differences in MST among these extract treated groups. For the 50 mg/kg extract treated mice, however, the MST was not prolonged (13.8 ± 1.3 days) significantly as compared to untreated control as shown in Table 1. Additionally, the toxicity control group survived throughout the experiment. Several studies have shown that different plant extracts can contain antioxidant activity that controls blood glucose levels [22–25]. The constituents and the properties of *T. crispa* such as borapetoside, borapetol, and apigenin suggests that the mechanism of action of *T. crispa* stem extract in control of blood glucose levels might be similar to that of other plant extracts [9,26–28]. Furthermore, the ability of *T. crispa* to affect blood glucose levels might be explained by the inhibition of the glycolysis pathway or the hexose transporter of infected erythrocytes [9,29]. It has been reported that the hepatoprotective and beneficial effects on insulin due to the antioxidant activity of *T. crispa* stem extract may, in part, play a critical role in improving blood glucose levels and get them back to the normal range [30,31]. Accordingly, a plant extract that can prolong the MST of infected mice compared to the untreated controls is considered an active agent against malaria. In the present study, the infected mice treated with 100 and 200 mg of the extract had significantly longer survival times than the untreated controls. This might be due to the anti-hypoglycemic activity of the extract and suggests the presence of bioactive compounds in the extract.

All the results obtained in the present study show that the *T. crispa* stem extract exerts an anti-hypoglycemic effect during PbANKA infection in mice, and prolonged MST were also observed. According to its potent activities, along with the lack of toxicity of this extract, it can be a potential medicinal remedy for malaria treatment. However, the finding is not sufficient to pinpoint the mechanism of action. The identification of the active compounds and exploration of the mechanism responsible for the observed anti-hypoglycemia effect are recommended.

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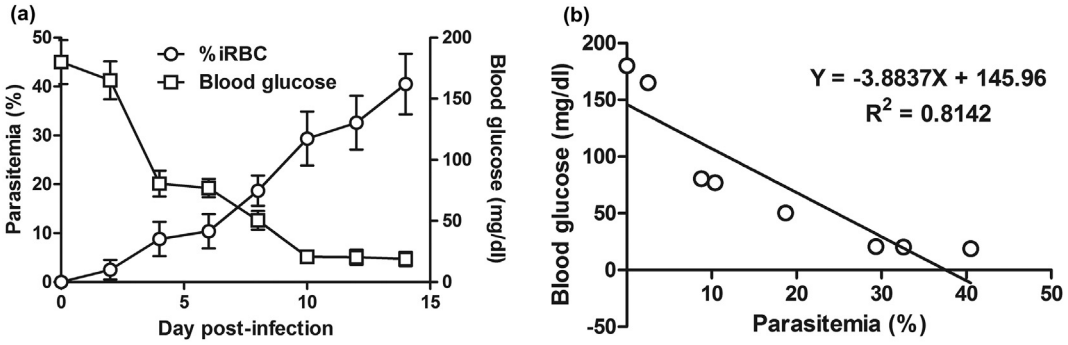

Fig. 1. Hypoglycemia induced by PbANKA infection. ICR mice were infected with 1×10^7 parasitized erythrocytes of PbANKA by IP injection. (a) Parasitemia and (b) blood glucose were daily monitored. Results are expressed as mean ± SEM.

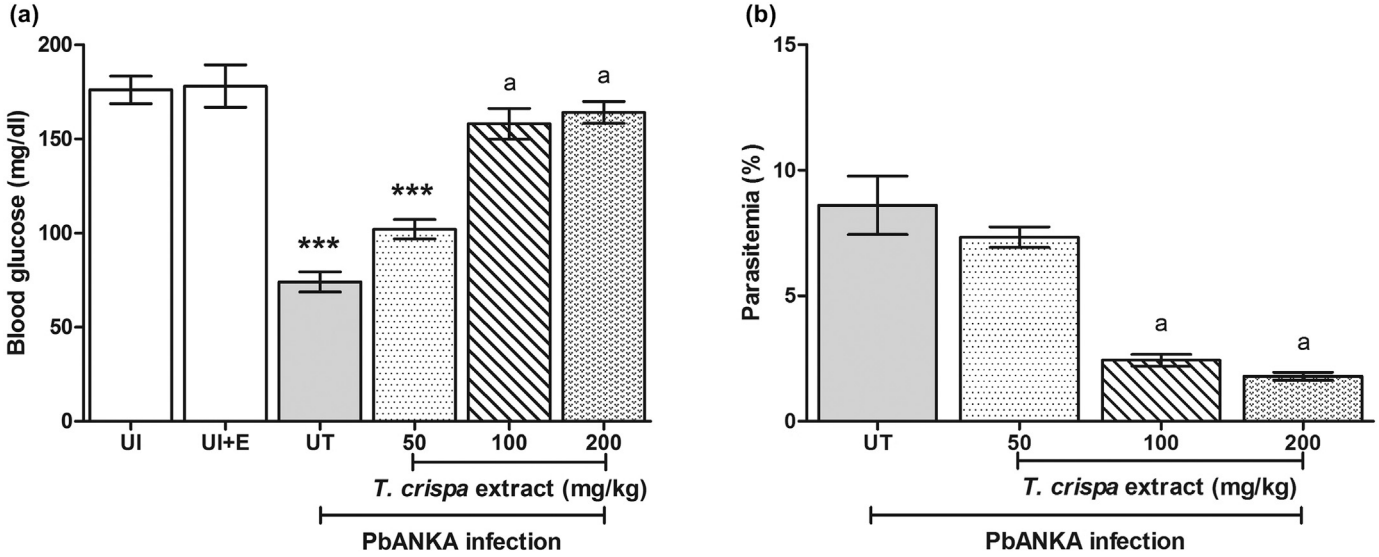

Fig. 2. Efficacy of *T. crispa* stem extract on hypoglycemia induced by PbANKA infection. Groups of ICR mice (5 mice per group) were infected with 1×10^7 parasitized erythrocytes of PbANKA by IP injection. They were administered orally by gavage 50, 100, and 200 mg/kg of extract once a day for four consecutive days (day 0–3). At day four, (a) blood glucose levels and (b) parasitemia were measured. ***$p < .001$, compared to uninfected group, [a]$p < .001$, compared to untreated group. UI; uninfected mice, UI + E; uninfected mice treated with 200 mg/kg of extract, UT; untreated mice, 50,100, and 200; infected mice treated with 50, 100, and 200 mg/kg of extract, respectively. Results are expressed as mean ± SEM.

Table 1
Effect of *T. crispa* stem extract on the MST of PbANKA infected mice.

Groups	Treatment	MST (days)
Untreated group	10 ml/kg of 20% Tween-80	13.6 ± 1.1
T. crispa stem extract treated groups	50 mg/kg of extract	13.8 ± 1.3
	100 mg/kg of extract	27.0 ± 2.0***
	200 mg/kg of extract	28.0 ± 1.9***

Results are expressed as mean ± SEM. ***$p < .001$, compared to untreated group.

Declarations of interest

None.

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