# *IN VIVO* ANTIPARASITIC ACTIVITY OF THE THAI TRADITIONAL MEDICINE PLANT-*TINOSPORA CRISPA*-AGAINST *PLASMODIUM YOELII*

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**Abstract.** We investigated the *in vivo* antimalarial effect of crude extract of *Tinospora crispa*, a Thai traditional medicine plant. Mice were inoculated with *Plasmodium yoelii* then treated with the crude extract of *Tinospora crispa* at doses of 20, 40 and 80 mg/kg. Mice receiving the dose of 20 mg/kg died on average on Day 8. Mice remained alive longer when treated of the dose of 40 mg/kg or even longer under the treatment of the dose of 80 mg/kg. Surprisingly and interestingly, one mouse from the group in which the dose of 80 mg/kg was administrated is still alive and the parasite was cleared from the blood stream. In conclusion, *T. crispa* has an *in vivo* antimalarial effect in dose dependent manner.

## INTRODUCTION

Malaria causes suffering and death to millions of people each year in sub-tropical and tropical countries including Thailand. A major problem with malaria is the increase in resistance to drugs normally used to treat *Plasmodium falciparum*. There is a search for new antimalarial agents. The World Health Organization (WHO) estimates approximately 80% of the world's population uses traditional medicines for health care. We investigated the usefulness of a traditional medicine, *Tinospora crispa*; locally called "*boraphet*" a climber plant, as a treatment for malaria. Thailand is a malaria endemic tropical country with an abundance of diverse plant life widely used by Thais as traditional medicine to treat tropical diseases including malaria. The use of Thai traditional medicine plants to treat malaria has been studied very little. We investigated the antiplasmodial effect of a crude extract of *T. crispa* against *P. yoelii* using ICR mice as subjects.

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## MATERIALS AND METHODS

### Plant material and crude extraction procedure

*Tinospora crispa* was collected from its natural environment in Khon Kaen Province, Thailand. Only the stem was minced into small pieces then dried in sunlight for a day and ground into powder with a traditional grinder. Two hundred, 400 and 800 mg of powder underwent an extraction process in 1 ml of 95% ethanol. The solution was then diluted with distilled water to a final 30% ethanol concentration and stored at -80ºC until use.

### *In vivo* test

Four groups of three, 6-week old female ICR mice were used; one as control and the

other three as experimental groups. On Day 0, all the animals were inoculated intraperitoneally with $10^7$ *P. yoelii* 17X (lethal) strain - infected erythrocytes. Immediately post-infection, 100 µl of *T. crispa* extract was administrated intraperitoneally once a day at doses of 20, 40 or 80 mg/kg, in the experimental groups. The control group received 30% ethanol only. To check the percentage of parasitemia, the mouse blood was collected from tail snip bleeds and thin-smears stained with Giemsa were examined daily.

## RESULTS

*Tinospora crispa* showed an inhibitory effect on the parasite in a dose-dependent manner. Parasitemia was detected in all mice groups by Day 1 and the percent parasitemia increased to 44.80- 45.45% by Day 8 in both the control and 20 mg/kg treated group with the death of all mice in both groups on Day 8. No significant differences were detected between these two groups. In the 40 mg/kg treated group, the percent parasitemia on Day 8 was 22.60% and then increased to 68.09% in two mice and to 71.60% in one mouse by Day 16. Two mice in the 40 mg/kg treatment group died on Day 16 and the third died on Day 17. In the 80 mg/kg treated group, the percent parasitemia increased until Day 14, but never exceeded 53.68% (Table 1). One mouse treated with 80 mg/kg dried on Day 16 (41.44% parasistemia) and one died on Day 19 (43.74%) parasitemia. One mouse in the 80 mg/kg treated group recovered completely with no parasites in the blood after Day 20.

Table 1
Percentage parasitemia *in vivo* in mice treated with *T. crispa*. Each value is the mean of three values ± standard deviation (SD).

| Day(s) | Control % (±SD) | 20 mg/kg % (±SD) | 40 mg/kg % (±SD) | 80 mg/kg %(±SD) |
|---|---|---|---|---|
| 1 | 0.98 (0.56) | 0.96 (0.30) | 0.70 (0.17) | 0.86 (0.28) |
| 2 | 1.81 (0.20) | 1.94 (0.38) | 1.70 (0.45) | 1.88 (0.24) |
| 3 | 3.48 (0.08) | 4.01 (0.28) | 2.87 (0.04) | 2.97 (0.57) |
| 4 | 5.98 (0.37) | 6.12 (0.62) | 3.38 (0.11) | 4.50 (0.93) |
| 5 | 8.13 (0.31) | 10.51 (1.10) | 9.03 (2.07) | 8.31 (0.96) |
| 6 | 15.12 (1.56) | 13.22 (0.97) | 15.62 (0.10) | 12.33 (2.22) |
| 7 | 22.20 (3.26) | 23.57 (1.07) | 19.72 (1.21) | 15.67 (0.34) |
| 8 | 45.45 (3.67) All died | 44.80 (3.75) All died | 22.60 (1.41) | 18.12 (2.24) |
| 9 | | | 28.72 (2.14) | 24.46 (2.02) |
| 10 | | | 31.55 (0.01) | 30.05 (4.44) |
| 11 | | | 35.23 (4.08) | 36.55 (1.21) |
| 12 | | | 39.03 (2.33) | 39.87 (1.34) |
| 13 | | | 40.08 (2.01) | 38.88 (0.59) |
| 14 | | | 43.94 (0.64) | 43.84 (2.11) |
| 15 | | | 55.18 (3.44) | 42.53 (4.18) |
| 16 | | | 68.09 (0.51) (2 died) | 41.44 (5.80) (1 died) |
| 17 | | | 71.60 (All died) | 47.20 (2.47) |
| 18 | | | | 53.68 (0.83) |
| 19 | | | | 43.74 (1 died) |
| 20 | | | | One alive |

## DISCUSSION

To our knowledge this is the first report showing the inhibitory effects of *T. crispa* against *P. yoelii* 17XL in ICR mice. Previous studies were mostly *in vitro* studies (Rahman *et al*, 1999; Ichino *et al*, 2006). In previous report, Rahman *et al* (1999) discovered the methanol extract of *T. crispa* injected intraperitoneally at a dose of 5 mg/kg in mice infected with *P. berghei* strain ANKA could extend the life of the mice only one day compared to control mice. We discovered no differences in the length of time until death in mice between the control group and the group treated with 20 mg/kg. Increasing of the dose of *T. crispa* in our study lengthened the life of the mice. This may be because the dose of 5 mg/kg was too low to inhibit parasite development; probably the methanol affected the parasite growth. Their study used different species of *Plasmodium* from ours. They used *P. berghei* strain ANKA which may have greater virulence than *P. yoelii* since their control mice died earlier than our control mice. Another report showed a suppressive effect of *T. crispa* water extract given orally, against *P. yoelii* yoelii 17X strain in swiss mice and found 53.8% inhibition at a dose of 110 mg/kg (Bertani *et al*, 2005). Their results support ours that *T. crispa* given at higher doses not have toxicity. However, their water extract was different from our ethanol extract, thus using a dose of 110 mg/kg was possible. To compare their findings with ours is difficult because the difference in solvents. Their findings support the use of *T. crispa* against *Plasmodium* infection *in vivo*. Two previous reports show the blockage of protein synthesis in *Plasmodium falciparum* is the main target for antimalarial drugs (Elford, 1986; Rahman *et al*, 1999). It is important to conduct further studies of traditionals medicine as antimalarial drugs with different mixtures; Traore *et al* (2008) reported antiplasmodial activities with various substances.

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