TREATMENT OF STELLANTCHASMUS FALCATUS IN GALLUS GALLUS DOMESTICUS BY NICLOSAMIDE AND SOME ANTHELMINTHIC PLANTS

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Abstract. Treatments of the intestinal trematode (*Stellantchasmus falcatus*) were performed with *Gallus gallus domesticus*, using niclosamide and traditional anthelminthic plants, *Diospyros mollis* Griff. and *Cymbopogon citratus* (DC. ex Nees) Stapf. This study was designed into 4 experimental groups (n = 5 in each group), as follows: control, niclosamide-treated, *D. mollis*-treated, and *C. citratus*-treated groups. Five hundred metacercariae of *S. falcatus* were orally force-fed to chicks individually, followed by the anthelminthic drugs. The results showed that a single dose of niclosamide, given at 40 mg/kg body weight, was able to eradicate the parasite effectively. A similar result was observed in the *D. mollis*-treated group. The numbers of parasites recovered from the small intestines of the chicks in the niclosamide and *D. mollis*-treated groups were significantly different from those of the control group (p<0.05), whereas *C. citratus* had no effect on the number of parasites recovered. It can be concluded that *D. mollis* can be used as an anthelminthic plant, with results comparable to niclosamide.

INTRODUCTION

Humans acquire the intestinal trematode, Stellantchasmus falcatus, by consumption of raw and/ or undercooked freshwater fish containing their infective stages or metacercariae. S. falcatus is one of the heterophyid flukes reported in humans in northern Thailand (Kliks and Tantachamrun, 1974; Radomyos et al, 1998). As for the treatment of heterophyid flukes, niclosamide at a dosage of 6 g over 3 alternate days has been recommended for Heterophyes heterophyes infection (Khalil et al, 1964), or 100-125 mg/kg for Metagonimus yokogawai infection (Rim, 1975), and 40 mg/kg for Haplorchis taichui infection (Sukontason et al, 2000). However, there has been no report on drugs and traditional anthelminthic plants for treating S. falcatus infections, in experimental animals or humans. Therefore, this study was undertaken to investigate the effect of traditional anthelminthic plants on S. falcatus, compared with niclosamide.

The ebony tree (*Diospyros mollis* Griff.) of the family Ebenaceae, is widely found in Thailand. The berries of this plant, popularly known as Maklua, are used extensively to dye black silk since ancient times. Local herbal medicines have used *D. mollis* to treat intestinal diseases (Sadun and Vajrasthira, 1954).

Lemon grass, *Cymbopogon citratus* (DC. ex Nees) Stapf, of the family Gramineae, is an aromatic and medicinal tropical plant with a clumped, bulbous stems that ultimately become leaf blades. The stems and leaves are used for cooking and utilized as traditional medicine such as using against coughing cuts, asthma, and bladder disorders, as a diaphoretic, and to relieve headache and abdominal pain.

MATERIALS AND METHODS

Metacercarial stage *S. falcatus* were immediately collected from half-beaked fish, *Dermogenus pusillus*, by using acid pepsin solution at a ratio of 1 g of fish and 10 ml of acid pepsin solution in a blender. The digested material was incubated in a shaking water bath for $1^{1}/_{2}$ hours at 37°C and subsequently passed through two layers of wet gauze. The digested material was rinsed with 0.85% sodium chloride solution and examined for metacercariae under a stereomicroscope. The identification of metacercariae was carried out by morphological examination based on Scholz *et al* (1991) and Wongsawad *et al* (2000) under a compound microscope.

There were four experiments in this study. Each experiment contained five three-day-old chicks (*Gallus gallus domesticus*) weighing 60-70 g. Each chick was intragastrically inoculated with 500 encysted active metacercariae via a polyethylene tube (feeding tube), then kept in a feeding cage and fed with commercial chick food for 4 days. All experiments were done simultaneously, as follows; experiment 1 was the control group; experiment 2, niclosamide-treated (Yomesan tablets; Bayer, Germany) at a single dose of 40 mg/kg body weight; experiment 3, *D. mollis*-

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treated, at the single dose of 1.6 ml/kg body weight; and experiment 4, *C. citratus*-treated, at the single dose of 350 ml/kg body weight.

Chicks were sacrified at day 2 after drug administration. The chick's intestines were resected and scraped, then rinsed with 0.85% sodium chloride solution. The sediment was examined under a stereomicroscope for adult *S. falcatus*. The number of worms recovered in the treated and control groups were compared by Mann-Whitney *U* test.

RESULTS

The result of treating *S. falcatus* in *Gallus gallus domesticus* is shown in Table 1. A single dose of niclosamide given at 40 mg/kg body weight, was able to eradicate the parasites effectively. A similar result was observed in the *D. mollis*-treated group. The mean worm recoveries in niclosamide and *D. mollis*-treated groups were 20 and 22, respectively, which was significantly different from the control group (p<0.05), whereas *C. citratus* had no effect on the number of parasites recovered.

DISCUSSION

The numbers of parasites found in the control and *C. citratus* groups were highly variable between chicks. Sukontason *et al* (2000) also found this phenomenon in studies of *Haplorchis taichui* in mice, similar to this study of *S. falcatus* in chicks, with significant differences from niclosamide and *D. mollis*-treated groups (p<0.05). It is generally noted that the number of flukes recovered was much smaller than the number of cysts administered.

Niclosamide has previously been reported in the treatment of intestinal cestode infection (Katz, 1986); it has also been reported as a treatment for trematodes such as *Fasciolosis buski* in humans (Suntharasamai

et al, 1974). As for minute intestinal flukes, this drug was effective against *H. heterophyes* and *M. yokogawai*, but higher dosages were required (Rim, 1975). Niclosamide at a dosage of 40 mg/kg body weight has eradicated *H. taichui* from the intestines of mice (Sukontason *et al*, 2000). However, this same property may be useful in eradicating simultaneously, with the same or nearly the same dose, other small flukes in the intestines.

Praziquantel, at the same dosage used to treat *O. viverrini* infection (single dose 40 mg/kg body weight), has also been proven to eradicate both *O.viverrini* and minute intestinal flukes (Radomyos *et al*, 1998). Low doses of praziquantel may be effective against not only minute intestinal flukes but also *O. viverrini*, as indicated by the 44% cure in *O. viverrini*-infected patients treated with a single dose of 25 mg/kg body weight (Bunnag and Harinasuta, 1981).

D. mollis as a single dose of 2-4 g/kg body weight in humans and dogs was able to eradicate *Ancylostoma duodenale*, *Necator americanus*, *Enterobius vermicularis*, *Strongyloides stercoralis*, *Fasciolopsis buski*, *Taenia saginata*, and *T. solium* (Mokkhasmit and Harinasuta, 1960). With preliminary studies, the crude extract of *D. mollis* at a single dose of 1.6 ml/kg body weight was able to eradicate *S. falcatus* in chicks.

This is the first report of niclosamide and traditional anthelminthic plants being used against *S. falcatus*. This information is particularly important for public health care, due to the high prevalence of this parasite in human populations in northern Thailand (Radomyos *et al*, 1998).

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 Table 1

 Number of adult Stellantchasmus falcatus recovered from Gallus gallus domesticus after treated with niclosamide, Diospyros mollis, and Cymbopogon citratus.

Treatments	Regimes	Number of recovered worms range(mean)
Control	-	132-274 (229)
Niclosamide	40 mg/kg	4-40 (20)
Diospyros mollis	1.6 ml/kg	9-41 (22)
Cymbopogon citratus	350 ml/kg	157-192 (180)

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REFERENCES

- Bunnag D, Harinasuta T. Studies on the chemotherapy of human opisthorchiasis: III. Minimum effective dose of praziquantel. *Southeat Asian J Trop Med Public Health* 1981; 12: 413-7.
- Katz M. Anthelmintics: Current concepts in the treatment of helminthic infections. *Drugs* 1986; 32: 358-71.
- Khalil HM, Rifaat MA, Koura M. A further trial of "Yomesan" in *Heterophyes heterophyes* infection. *Trop Med Hyg* 1964; 67: 286-7.
- Kliks M, Tantachamrun T. Heterophyid (trematoda) parasites of cats in North Thailand, with notes on a human case found at necropsy. *Southeast Asian J Trop Med Public Health* 1974; 5: 547-55.
- Mokkhasmit D, Harinasuta T. Studies on the anthelmintic activity of Maklua. *Bull Dept Sci, Thailand* 1960; 2: 153-79.
- Radomyos P, Wansaroj T, Wilairatana P, et al.

Opisthorchiasis and intestinal fluke infections in northern Thailand. *Southeast Asian J Trop Med Public Health* 1998; 29: 123-7.

- Rim HT. Studies on the chemotherapy of trematode infections in Korea. *Korean Med J* 1975; 2: 455-7.
- Sadun EH, Vajrasthira S. The effect of maklua (*Diospyros mollis*) in the treatment of human hookworm. *J Parasitol* 1954; 40: 49-53.
- Scholz T, Ditrich O, Giboda M. Differential diagnosis of opisthorchiid and heterphyid metacercariae (Trematoda) infecting of flesh cyprinid fish from Nam Ngum Dam Lake in Laos. *Southeast Asian J Trop Med Public Health* 1991; 22(suppl): 171-3.
- Sukontason K, Sukontason K, Muangyimpong Y, Piangjai S. Treatment of *Haplorchis taichui* in *Mus musculus* mice. *Exp Parasitol* 2000; 94: 48-50.
- Suntharasamai P, Bunnag D, Teiavanij S, et al. Comparative clinical trials of niclosamide and tetrachlorethylene in the treatment of *Fasciolopsis buski* infection. *Southeast Asian J Trop Med Public Health* 1974; 5: 556-9.
- Wongsawad C, Rojanapaibul A, Mhad-arehin N, et al. Metacercaria from freshwater fishes of Mae Sa stream, Chiang Mai, Thailand. Southeast Asian J Trop Med Public Health 2000; 31(suppl 1): 54-7.