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Effects of Bayluscide on *Bithynia siamensis goniomphalos*, the first intermediate host of the human liver fluke, *Opisthorchis viverrini*, in laboratory and field trials

S. Tesana [a,*], P. Thapsripair [b], C. Thammasiri [c], S. Prasopdee [a], A. Suwannatrai [a], S. Harauy [d], S. Piratae [a], P. Khampoosa [a], J. Kulsantiwong [a], C. Donthaisong [a], P. Chalokepanrat [e], V. Viyanant [f], J.B. Malone [g]

[a] *Food-Borne Parasite Research Group, Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen province 40002, Thailand*
[b] *Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen province 40002, Thailand*
[c] *Faculty of Nursing, Ratchathani University Udonthani Campus, Udonthani province 41000, Thailand*
[d] *Community Health Program, Faculty of Public Health, Ubon Ratchathani Rajabhat University, Ubon Ratchathani province 34000, Thailand*
[e] *Department of Fishery, Faculty of Agriculture, Khon Kaen University, Khon Kaen province 40002, Thailand*
[f] *Graduate Program in Biomedical Sciences, Faculty of Allied Health Sciences, Thammasat University, Pathumthani 12121, Thailand*
[g] *Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, USA*

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ABSTRACT

The molluscicidal effects of Bayluscide (niclosamide) were investigated on *Bithynia siamensis goniomphalos*, the first intermediate host of human liver fluke, *Opisthorchis viverrini*. Lethal concentrations of 50% (LC_{50}) and 95% (LC_{95}) against young and adult males were 0.38 and 0.80, 0.42 and 0.86 ppm, respectively. The LC_{50} and LC_{95} against young and adult females were 0.42 and 0.86, 0.46 and 0.97 ppm, respectively. No significant differences in mortality rate between sexes or snail size ($p>0.05$) was detected. Bayluscide-related tissue damage in *B. siamensis goniomphalos* included detachment of cilia of the epithelial layer of the digestive tract and decreased number of calcium cells. In tests of lethal concentrations of Bayluscide on non-target animals, no lethal effect was observed on *Filopaludina martensi martensi* (Viviparous snail) but high mortality rates were recorded in *Puntius gonionotus* fingerling, Ricefish (*Oryzias mekongensis*) and shrimp (*Macrobrachium lanchesteri*), but lower in guppy fish (*Poecilia reticulata*) after 24 h exposure. For field trials, sufficient Bayluscide was sprayed in 3 roadside ditches to result in final concentrations of 5, 10 or 20 ppm, with mortality rates on *B. siamensis goniomphalos* of 10.94, 20.00 and 31.25%, respectively. Non-target snails died in small numbers but no effect was observed in other aquatic vertebrate animals. Field trials of Bayluscide on *B. siamensis goniomphalos* revealed low mortality rates, suggesting the need for application methods of higher efficacy or that Bayluscide is not suitable for application to operculate snails or snails which are able to escape by burying in mud.

1. Introduction

Bithynia siamensis goniomphalos is the most important snail intermediate host of the human liver fluke, *Opisthorchis viverrini* in northeast Thailand and neighboring countries including Lao PDR, Cambodia and South of Vietnam [1]. Praziquantel is an effective, widely available anthelmintic drug for treatment of *O. viverrini* infection but the prevalence of infection in many areas remains high, ranging from 2.1% to 70.8% in different districts of Khon Kaen province, and the infection is correlated with the incidence of cholangiocarcinoma [2]. Snail hosts acts as amplifiers of the parasite by asexual reproduction, liberating large numbers of free-swimming cercariae that infect cyprinoid fish. The maximum number *O. viverrini* cercariae shed from an infected *B. siamensis goniomphalos* is as high as 1728 cercariae daily, with a grand total production from one snail host of up to 27,692 cercariae [3]. However, the prevalence of *O. viverrini* infection in snail hosts in endemic areas is remarkable low. A report by Brockelman and colleagues [4] revealed that prevalence rates of *O. viverrini* in *B. siamensis goniomphalos* in a water body in an endemic area of Khon Kaen province was 0.11–0.63% during a 12 month period [4] and that monthly infection rates fluctuated from 0.03–0.36% [5]. In Kalasin Province, a neighboring province of Khon Kaen, prevalence rates were slightly higher in Yang Talat district (1.3%), and in Kamalasai district (0.61%) [6]. There is a far greater chance of second intermediate hosts, cyprinoid fish, acquiring high infection rates, reaching as high as 25–28% in some endemic areas [7] with the first intermediate host snails having the main role in propagation of this parasite.

B. siamensis goniomphalos is a fresh water snail in the family Bithyniidae. It is of small size, 10.2–14.9 mm in length, operculate and has separate snail sexes. The shell morphology is subovate conic with an eroded apex and reddish brown color. This snail species is widely

* Corresponding author. Tel.: +66 43 348387; fax: +66 43 202475.
E-mail address: smarn_te@kku.ac.th (S. Tesana).

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distributed in northeast Thailand in endemic areas limited by salinity levels of water bodies [8,9].

Molluscicide has been widely used for schistosomiasis control in many endemic countries as part of strategic control programs. Bayluscide (niclosamide), UPAC name of 5 chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide is one of the most effective molluscicides and there are many reports of its efficacy in decreasing the prevalence of schistosome infection. It was known as anti-cestode infection by the effect of respiratory blocking. For control programs for opisthorchiasis in northeast Thailand treatment of human infections with praziquantel have not been fully successful, and there is a need to employ a combination of methods in control strategies, including targeted first intermediate host population control by molluscicide as in control of schistosomiasis [10,11]. The objective of the study reported here was to determine the effects of Bayluscide on *B. siamensis goniomphalos* in both laboratory test and field trials.

2. Materials and methods

2.1. Snail preparation

B. siamensis goniomphalos was collected from a natural water body in Muang District, Khon Kaen province, northeast Thailand. The snails were maintained in the laboratory and provided with artificial snail food [12] for few days before examination for trematode infection by the cercarial shedding method twice over a one week period. The infected snails were excluded from the study.

2.2. Laboratory tests for the effect of Bayluscide on B. siamensis goniomphalos

Snail samples which were free from trematode infection were separated by sex and divided into groups of young (size 5.0–8.0 mm in length) and adult snails (size 9.0–12.0 mm in length). Snails were exposed to various concentrations of Bayluscide (Niclosamide 70% wettable powder, Nanjing Essence Fine-Chemical Co., Ltd., China): 0 (control), 0.015625, 0.03125, 0.0625, 0.125, 0.25, 0.375, 0.5, 0.625, 0.75, 0.875 and 1 ppm in a petri dish containing 80 ml of solution, 10 snails each for 24 hours; tests were done in four replicates. After exposure, snails were washed with dechlorinated tap-water three times then observed for movement for a day. Dead snails were recorded based on the criteria of loss of their opercula and no movement. The data were analyzed by SPSS-Probit analyses to estimate 50% (LC_{50}) and 95% (LC_{95}) lethal concentrations of Bayluscide.

2.3. Bayluscide effects on non-target aquatic animals

Aquatic animals which were non-target for lethal effects of Bayluscide were investigated including Viviparous snail (*Filopaludina martensi martensi)*, *Puntius gonionotus* fingerling, guppy fish (*Poecilia reticulata*), shrimp (*Macrobrachium lanchesteri*) and Ricefish (*Oryzias mekongensis*) to test the efficacy of 0, 0.015625, 0.03125, 0.0625, 0.125, 0.25, 0.5 and 1 ppm of 250 ml Bayluscide solution in 500 ml beaker. Each species (total of 20 in 4 replicates) was observed and deaths recorded after 24 h exposure at room temperature.

2.4. Field trials of Bayluscide on B. siamensis goniomphalos

B. siamensis goniomphalos was surveyed by sampling of 10 sites (10 stations on the shore and 10 stations in deep water, 40–100 cm in parallel) by manual collection by two trained persons for 5 min and two sampling of Ekman dredge, respectively each, in 3 roadside ditches for measurement of snail density and size of water body. The volume of water was calculated from length and width of ditches and their depth. The first, second and third ditch had size of water surface area of 160, 110 and 300 m^2 with average water depth 0.5, 1 and 0.5 m and contained water volumes of 80,000; 110,000 and 150,000 L. Sufficient Bayluscide was applied by spraying on the shoreline to reach final concentrations of 5, 10 and 20 ppm, respectively. Applications were done in the evening, to avoid sunlight effects. Snails in water bodies were observed to records effects of Bayluscide at three hours after application and at 1, 7 and 14 days. The death of aquatic non-vertebrate species was recorded and summed 14 days after Bayluscide application. Non-target aquatic animals were also observed for 3–4 h after application and followed up of 1, 7 and 14 days, their behaviors such as swimming or jumping on water surface was observed and recorded number of death.

2.5. Histopathological investigation the effects of Bayluscide on B. siamensis goniomphalos

B. siamensis goniomphalos were exposed to various concentration of Bayluscide of 0, 0.125, 0.25, 0.5 and 1 ppm for 24 h. The snails were washed with de-ionized water, their opercula were removed. Snails were then fixed in Bouin's fixative for an hour and soft tissues were removed from snail shells for further fixation in the Bouin's fixative for 24 h. Snails were washed with distilled water, dehydrated in stepwise series of ethyl alcohol solutions (50, 70, 80, 90, 95% and absolute ethanol), infiltrated with paraffin and embedded. Five micrometer thickness sections were cut and then stained with Hematoxylin and Eosin, mounted with permount on glass slides for examination and photographic recording of pathological changes using a standard compound microscope.

2.6. Statistical analysis

The concentration of 50% and 95% lethal doses of Bayluscide were estimated by SPSS-Probit analyses. The effects of various concentrations of Bayluscide on *B. siamensis goniomphalos* (according to sex and size) and non-target animals tested were analyzed for significance by ANOVA ($p<0.05$).

3. Results

3.1. Laboratory test for effects of Bayluscide on B. siamensis goniomphalos

The effect of Bayluscide was dose dependent, with increased mortality rates with increasing concentration of chemical. Lethal concentrations of 50% (LC_{50}) and 95% (LC_{95}) against young and adult males were 0.38 and 0.80, 0.42 and 0.86 ppm, respectively. The LC_{50} and LC_{95} against young and adult females were 0.42 and 0.86, 0.46 and 0.97 ppm, respectively (Fig. 1). No significant differences on death

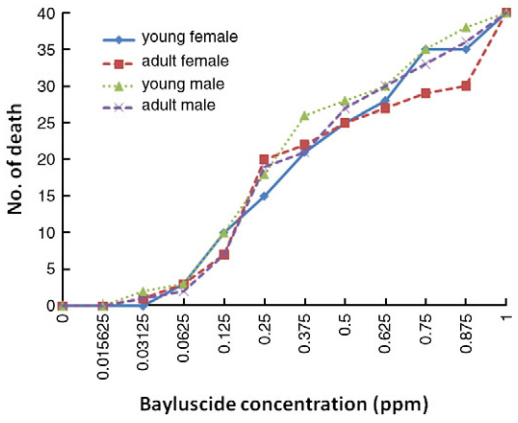

Fig. 1. Graph showing death of *B. siamensis goniomphalos* of males vs. females of young vs. adults after 24 h exposure to various concentrations of Bayluscide.