Molluscicidal Effects of Bayluscide (niclosamide) on *Bithynia siamensis goniomphalos*, First Intermediate Host of Liver Fluke, *Opisthorchis viverrini*

ผลของการฆ่าหอยของเบลูไซด์ (นิโคลซาไมด์) ต่อหอยไซ (*Bithynia siamensis goniomphalos*) โฮสต์กลางที่ 1 ของพยาธิใบไม้ตับ *Opisthorchis viverrini*

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ABSTRACT

The effects of Bayluscide were studied on both immature and adults *Bithynia siamensis goniomphalos* for its molluscicidal efficiency. The lethal concentration (LC_{50}) values of immature and adults were 0.28 and 0.17 mg/l, respectively and LC_{95} values were 0.60 and 0.49 mg/l, respectively. The effects on non-target aquatic animals, with no lethal effect to Hoi Kom (*Filopaludina martensi martensi*), slightly effects to guppy fish (*Poecilia reticulata*) but highly effects to Pla Sew Kaosan (*Oryzias mekongensis*). Histological studies of the effects of Bayluscide on *B. siamensis goniomphalos* were performed by paraffin sections and hematoxylin-eosin staining. Separation of epithelial layer of digestive tracts and muscle layers were clearly defined in the treated groups. Cells of digestive glands were swollen, shrinkage of calcium cells and their lumens become narrower while no changes in other tissues compared to the control group.

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บทคัดย่อ

การศึกษาผลของเบลูไซด์ ต่อหอยไซ (Bithynia siamensis goniomphalos) โดยหาประสิทธิภาพการฆ่าหอย ซึ่งความเข้มข้นของสารที่ฆ่าลูกหอย และตัวเต็มวัยได้ร้อยละ 50 (LC_{50}) ที่ 0.28 และ 0.17 และค่าความเข้มข้นที่ฆ่าลูกหอย และตัวเต็มวัยได้ร้อยละ 95 (LC_{95}) ได้แก่ 0.60 และ 0.49 มก/ล ตามลำดับ ผลต่อการมีชีวิตรอดของสัตว์น้ำที่ไม่ใช่เป้าหมาย พบว่าไม่มีผลต่อ หอยขม (*Filopaludina martensi martensi*) มีผลต่อปลาซิวข้าวสารแคระ (*Oryzias mekongensis*) มากที่สุด รองลงมาเป็นปลาหางนกยูง (*Poecilia reticulata*) การเปลี่ยนแปลงของเนื้อเยื่อหอย *B. siamensis goniomphalos* หลังจากสัมผัสสารเคมีโดยเปรียบเทียบกับกลุ่มควบคุม พบว่าเยื่อบุทางเดินอาหารมีรอยแยกกับกล้ามเนื้อ เซลล์ของต่อมย่อยอาหารบวม เซลแคลเซี่ยมเหี่ยว และท่อของต่อมย่อยอาหารตีบแคบลง ไม่พบการเปลี่ยนแปลงที่เนื้อเยื่ออื่น ๆ

Key Words : *Opistrochis viverrini*, Bayluscide, *Bithynia siamensis goniomphalos*, Lethal concentration

คำสำคัญ : พยาธิใบไม้ตับ *Opistrochis viverrini* เบลูไซด์ หอยไซ *Bithynia siamensis goniomphalos* Lethal concentration

Introduction

Bithynia siamensis goniomphalos a fresh-water snail, plays role as first intermediate host of liver fluke, *Opisthorchis viverrini*. Opisthorchiasis is still an important public health problem in many parts of Southeast Asia including Thailand, Lao Peoplēs Democratic Republic, Cambodia and South of Vietnam (Ditrich *et al*., 1990; Jongsuksuntigul and Imsomboon, 1998; Le *et al*., 2006). It was the causative of hepatobiliary diseases such as cholangitis, obstructive jaundice, hepatomegaly, cholecystitis, and cholelithiasis (Sripa, 2003). Furthermore, both experimental and epidemiological evidences strongly implicate that liver fluke infection was the major risk factor of cholangiocarcinoma, the cancer of the epithelial bile ducts (Thamavit *et al*., 1994; Sriamporn *et al*., 2004). Cholangiocarcinoma in Khon Kaen province was remarkable highest incidence as 93.8 to 317.6 per 100, 000 person-years by age-standardized over 35 years of age (Sriamporn *et al*., 2004).

O. viverrini adult worms resided in the intra- hepatic bile ducts rather than in extra-hepatic bile ducts *i.e.* gall bladder and the pancreatic duct of definitive and reservoir hosts. Adult female worms laid eggs and passed with faeces into the external environment, the fresh water reservoir. After mature embryonated eggs were eaten by *Bithynia siamensis goniomphalos*, the first intermediate host in northeast Thailand, miracidia were liberated in the digestive tracts then penetrated snail tissue to form sporocysts. Rediae and cercariae were produced by the asexual reproduction subsequently. Then further developing to be metacercariae after they penetrated cyprinoid fish (Harinasuta and Harinasuta, 1984).

B. siamensis goniomphalos is a member of snail in family Bithyniidae. It is an operculate freshwater snail and its habitat is locally in northeast Thailand. Its shell is conic or ovate-conical in shape with delicate spiral lines but no strong sculpture, reddish-brown colour and highly eroded apex. It is separated sex. Its sizes are 10.2-14.9

mm in length and 5.6-8.5 mm in width (Brandt, 1974).

Bayluscide (Niclosamide) is commonly used to control snail population as molluscicide. The lethal effects are widely ranges on snails, cestodes and *Cercariae* by affecting the respiration and the carbohydrate metabolism. It most likely disturbs oxidation processes by inhibiting oxygen uptake. Widely use for molluscicide on the golden apple snail (*Pomacea canaliculata*) in paddy fields. In public health purpose, it was used for control the snail intermediate hosts of *Schistosoma* spp. The chemical compound is rapidly degraded in water without long-term effect to aquatic organisms. It is also applied to commercial management of fish ponds in order to clean unwanted fish and snails before re-cultivation new batch of fish. Bayluscide is highly toxic to fish however the applications to fish ponds were practical use due to its short half-life in water and rapidly degraded within a few days before rearing of new fish (World Health Organization, 2006).

The objective of this study was to investigate the molluscicidal concentrations of Bayluscide on *B. siamensis goniomphalos*, the effect to non-target organisms and tissue changes after exposure to the chemical compound.

Materials and methods

Preparation of the Bithynia siamensis goniomphalos

B. siamensis goniomphalos was collected from Toong Sarng pond, Muang district, Khon Kaen province. The snails were examined for trematode infection at laboratory of Department of Parasitology, Faculty of Medicine, Khon Kaen University by cercarial shedding both at night and daytime. The non-infected snails were used for the study and were divided into 2 groups as adult snails with shell length 8-15 mm and immature snails with shell length 3-5 mm. (Figure 1). The snails were maintained in laboratory for one week to adapt to laboratory condition and fed with snail food (Kruatrachue *et al.*, 1982) before using.

Figure 1 Shell of *B. siamensis goniomphalos* adult (8-15 mm in length) and immature snails (3- 5 mm. in length)

Preliminary screening of molluscicidal effect of Bayluscide on *B. siamensis goniomphalos*

Preliminary screening of molluscicidal effect of Bayluscide was determined the sublethal and lethal concentrations and also toxicity test were performed by following the procedures of Wangsomnuk, (2005). The snails both of adult and immature, 20 snails/each concentration (5 snails/cup, 4 replicas) were exposed to Bayluscide® (Niclosamide 70% wettable powder, Nanjing Essence Fine-Chemical Co., Ltd., Chaina) in 8 various concentrations of the active ingredient (two fold dilutions) of Bayluscide from 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625 and 0 (control) mg/l in plastic cup. Each cup contained 150 ml of Bayluscide solution (full

volume of cup) then covered with pore lid to prevent escaping of the snails. They were exposed for 24 hours at room temperature (range 26 - 30°C). After exposure, the snails were soaked in dechlorinated tap-water and kept for a period of 24 hours for observation their viability comparing to the control group. The quality of water using in experiment were measured such as temperature, dissolved oxygen, conductivity, salinity, turbidity and pH.

For detection viability of snails was done by needle stimulation, the alive snails would close their opercula. The death snails were protruded out the soft tissue without retraction or lost their opercula. The death snails would not move when leaving in a cup.

Definitive molluscicidal effects on *B. siamensis goniomphalos*

Definitive molluscicidal effects, the Bayluscide concentrations were calibrated due to baseline data of preliminary screening. The lethal (1 mg/l) and sublethal concentrations of Bayluscide were subdivided each interval into 12 concentrations as 1, 0.75, 0.5, 0.375, 0.25, 0.1875, 0.125, 0.09375, 0.062, 0.04685, 0.03125 and 0 (control) mg/l. The exposure of adult and immature snails was done as the procedure in preliminary screening. The mortality of the snails was investigated and recorded during the period of 1, 6, 12 and 24 h. The behavior and macroscopic visible reactions and mortality of the snails were observed and recorded. Lethal concentrations at 50 and 95% (LC_{50} and LC_{95}) were calculated by using Probit Analysis program (Sparks and Sparks, 1986).

Effective concentrations of Bayluscide on non-target aquatic animals

Non-target fresh water animals such as guppy fish (*Poecilia reticulata*), Pla Sew Kaosan (*Oryzias mekongensis*) and Hoi Kom (*Filopaludina martensi martensi*) were tested with 6 Bayluscide concentrations as 0.15 mg/l (sublethal concentration), 0.20 mg/l (LC_{25}), 0.28 mg/l (LC_{50}), 0.38 mg/l (LC_{75}), 0.60 mg/l (LC_{95}) and 0 mg/l (control) of Bayluscide solution (20 aquatic animals/each concentration) and observed the inactive, dead of those animals at 24, 48 and 72 h of exposure times and recorded as accumulative mortality.

Histological examination of the snails after exposure to Bayluscide

Tissue processing and sectioning

Snails were exposed to 6 Bayluscide concentrations as 0.15 mg/l (sublethal concentration), 0.20 mg/l (LC_{25}), 0.28 mg/l (LC_{50}), 0.38 mg/l (LC_{75}), 0.60 mg/l (LC_{95}) and 0 mg/l (control) same as the concentrations which tested to the non-target animals. Twenty four hours after exposure the snails from each group were soaked in dechlorinated tap-water then snails from each batch were removed the operculum and immediately fixed in Bouin's fixative for an hour. After that the soft tissues were removed form the shells by using needles and cut the muscle which tied head-foot portion to the shells. They were fixed overnight in the same fixative. Specimens were washed several times with distilled water then dehydrated with a graded ethanol (50, 70, 80, 95, and 100 % (twice) for an hour each) and clearing in xylene. They were infiltrated with paraffin-alcohol mixtures then embedded in paraffin wax.

The samples were sectioned for 5 _m thickness and stained with hematoxylin and eosin (Kim *et al.*, 2006).

Statistical Analysis

Number of death of adult and immature snails was analyzed for the relation to the concentrations of Bayluscide by using SPSS version 11.5.

Results

Preliminary screening of molluscicidal effects of Bayluscide on *B. siamensis goniomphalos*

Sublethal concentrations (the lowest concentration without death, 0%) for adult and immature snails were the same (0.03 mg/l) and also lethal concentration (the lowest concentration with all death, 100%) 1.00 mg/l (Table 1). The water quality using in the experiment were measured as following values; temperature 26 °C, dissolved oxygen 0.19 mg/l conductivity 600 µs/cm, salinity 222 ppm , turbidity 24 NTU and pH 7.2 .

Definitive molluscicidal effects on *B. siamensis goniomphalos*

The concentrations of Bayluscide for lethal effects of 50 and 95 % were analyzed from the data of definitive test by Probit Analysis program. For LC_{50} and LC_{95} of adult snails were 0.17 and 0.49 mg/l, respectively. LC_{50} and LC_{95} of immature snails were 0.28 and 0.60 mg/l, respectively (Figure 2). Immature *B. siamensis goniomphalos* was higher tolerant to Bayluscide than adult snails significantly ($P< 0.05$). The number of death snails was increased due to increasing of Bayluscide concentrations ($P< 0.05$).

Effects of Bayluscide on non-target aquatic animals

The effective concentration of Bayluscide on non-target fresh water animals such as guppy fish (*Poecilia reticulata*), Pla Sew Kaosan (*Oryzias mekongensis*), Hoi Kom (*Filopaludina martensi martensi*) were shown the results in Table 2 with ineffectiveness to Hoi Kom, slightly mortality effects to guppy fish and lethal effects to Pla Sew Kaosan.

Table 1 Preliminary screening for the effect of Bayluscide on B. siamensis goniomphalos adults and immature snails

Concentrations of Bayluscide (mg/l)	Number of death snails (%)	
	Adult (n=20)	Immature (n=20)
1	20 (100)	20(100)
0.5	17 (85)	14(70)
0.25	15(75)	10(50)
0.125	13(65)	4(20)
0.0625	2(10)	1(5)
0.03125	0	0
0.015625	0	0
control	0	0

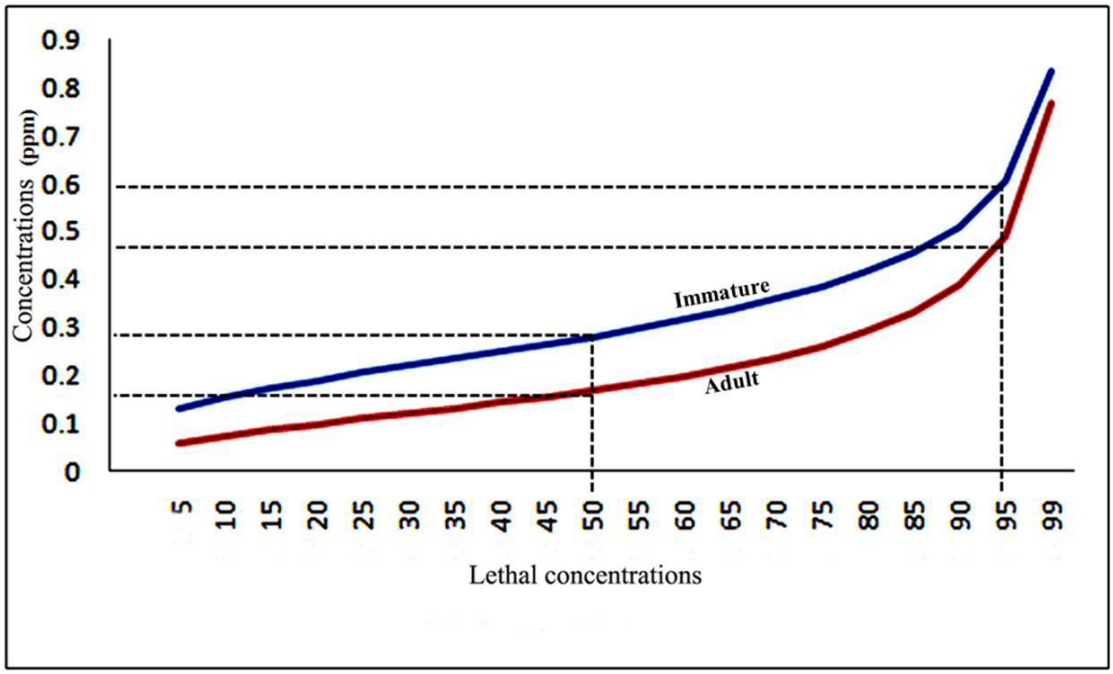

Figure 2 Graph of accumulative number of death of immature and adult of *B. siamensis goniomphalos* related to Bayluscide concentration obtained the data from Probit analysis.

Table 3 The effects of Bayluscide on three kinds of fresh water animals (20 animals/each concentration)

concentration of Bayluscide	Accumulative number of death (Observation at exposure time, h)											
	24				48				72			
	Hoi Kom	Pla Sew Koasan	Guppy fish		Hoi Kom	Pla Sew Koasan	Guppy fish		Hoi Kom	Pla Sew Koasan	Guppy fish	
			Male	Female			Male	Female			Male	Female
0.15 mg/l	0	20	3	0	0	20	3	0	0	20	3	0
0.20 mg/l	0	20	5	2	0	20	5	2	0	20	7	3
0.28 mg/l	0	20	1	0	0	20	1	0	0	20	1	1
0.38 mg/l	0	20	2	0	0	20	2	0	0	20	3	2
0.86 mg/l	0	20	10	12	0	20	20	20	0	20	20	20
		*	**	***								

* control group, 6 death

** control group, 2 death.

*** control group, 1 death.

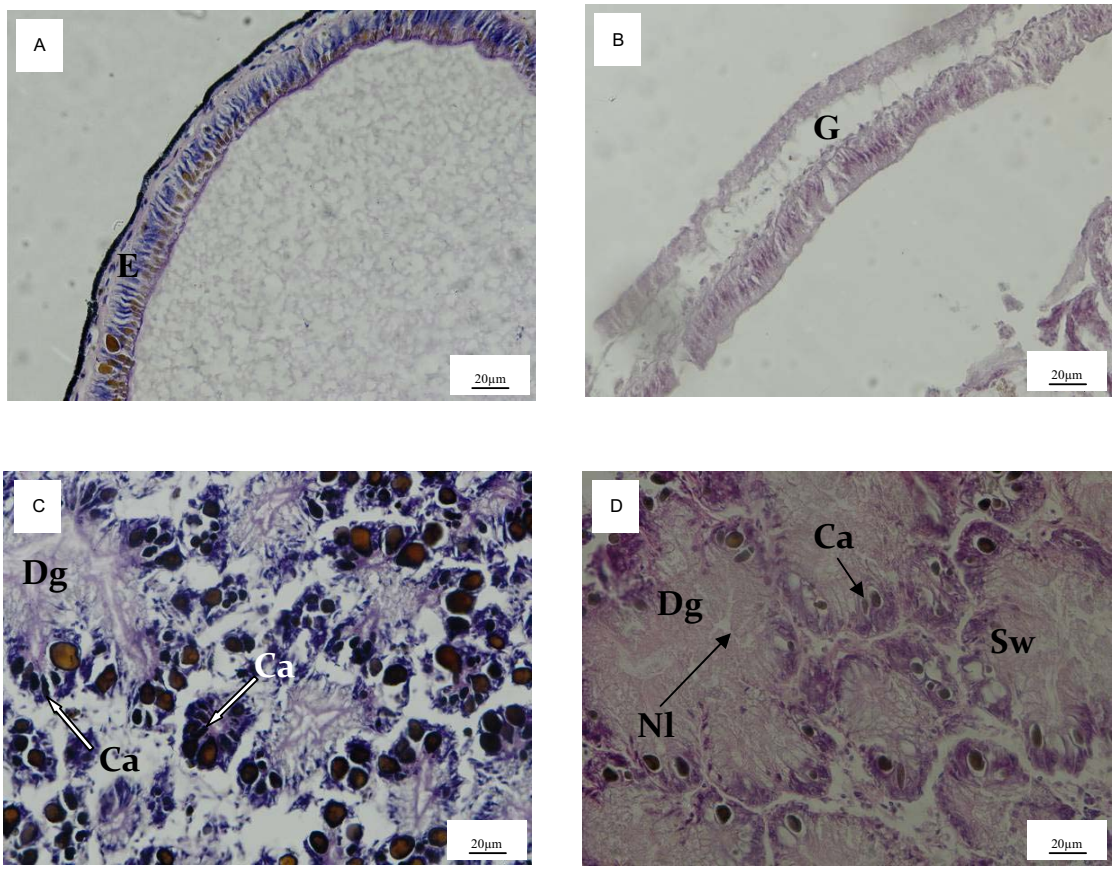

Figure 3 Paraffin -sections of snails stained with hematoxylin and eosin, A: Epithelial lining (E) of digestive tract of control group; B: Separation of epithelial lining from muscle layer showing gap (G) of digestive tract; C: digestive glands (Dg) of control group showing normal calcium cells (Ca); D: swelling of digestive gland (Sw), narrow lumen (Nl) of digestive glands and shrinkage of calcium cells (Ca) in treated snails.

Histological changes of the snails after exposed to molluscicide

Histological changes in tissue sections of snails were observed and taken photographs. In digestive tract, epithelial lining was separated from muscle layers showing long gaps with clearly defined in the treated groups (Figure 3 B).

Cells of digestive glands were swollen, shrinkage of calcium cells and their lumen become narrower (Figure 3 D), and no alteration was found in other tissues compared to the control group.

Discussion and Conclusions

Bayluscide is widely use for snail control for schistosomiasis in African and South American countries, in endemic areas (Giovanelli *et al.*, 2002; Greer *et al.*, 1996; Takougang *et al.*, 2006., 2007).

B. siamensis goniomphalos is operculate snails. It usually crawl on the surface of ground up to 3 meters deep (Suwannatrai et al., manuscript in preparation) or on water plants. Its habitat is more or less different from the snails of schistosome intermediate hosts, such as *Biomphalaria glabrata* for *Schistosoma mansoni* and *Bulinus* sp for *S. haematobium* which were pulmonate snails. They normally float on surface of water or on water plants. When application of molluscicide it was easily contact to those snails directly. The lethal concentration of those snails less than 0.5 mg/l (Greer *et al.*, 1996; Takougang *et al.*, 2006; Takougang *et al.*, 2007) or LC_{50} and LC_{90} (0.007 and 0.175 mg/l, respectively) (Giovanelli *et al.*, 2002) were also lower than *B. siamensis goniomphalos* (0.60 mg/l). Therefore it was different from *B. siamensis goniomphalos* which is operculate snails and lives in deep water and sometime hides under ground in cold season. Therefore the molluscicide may difficult to contact to *B. siamensis goniomphalos*. The size of snails may effect to concentration of Bayluscide such as Pomacea canaliculata, big size snails had higher concentrations of LC_{50} and LC_{90} (0.4 and 0.73 mg/l, respectively) (Wangsomnuk, 2005) than in *B. siamensis goniomphalos*. Adult of *B. siamensis goniomphalos* snails were death in greater number than immature snails in the same concentrations of Bayluscide, the same as results of the experiment study in fresh-water snails, the intermediate hosts of schistosomes. The number of death snails increased with the increasing of Bayluscide concentrations in both adult and immature snails (Ali, 2005). But it was different effects on snail host of *Schistosoma mansoni* which juvenile snails were more susceptible to chemical when compared with adult snails (Arijo *et al.*, 2007). The differences in this present study from previous ones were possibly due to the wide range of snails' size (8-15 mm) which may mix between mature and aged ones.

The effects of Bayluscide on non-target aquatic animals, with no lethal effect to Hoi Kom (*Filopaludina martensi martensi*), slightly effects to guppy fish but highly effected to Pla Sew Kaosan. In the field study, fish, frogs and tadpole were killed at minimal concentrations of 0.25 mg/l of Bayluscide and number of death was increased with the increasing of concentrations (Takougang *et al.*, 2006).

Histological appearance of *B. siamensis goniomphalos* exposed to Bayluscide, showed similar results to *B. glabrata* which showed the separation between epithelial lining and muscle layer. But in this study, there was no change in reproductive organs as in the study on *B. glabrata* which were reductions of spermatozoa and oocyte. Also its lumen of digestive gland of *B. glabrata* became wider (Zhou *et al.*, 1993) but inversed results on the present study.

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